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Unravelling the plant beneficial potential of *Bacillus nakamurai* BDI-IS1

François Nimbeshaho

COMMUNAUTÉ FRANÇAISE DE BELGIQUE
UNIVERSITÉ DE LIÈGE – GEMBLOUX AGRO-BIO TECH

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François NIMBESHAHO

Dissertation originale présentée en vue de l'obtention du grade de doctorat en sciences agronomiques et ingénierie biologique

Promoteur : Dr. Marc ONGENA
Année civile : 2025

Abstract

Agriculture in Burundi and many other tropical developing countries experiences severe challenges such as high disease and pest incidence, and the use of highly hazardous pesticides (more than 30% banned in Europe), posing serious threats to human health (food insecurity and intoxication) and environment. Bacterial biocontrol agents are safe and eco-friendly alternatives to the use of these chemicals and some *Bacillus* isolates belonging to species of the *B. subtilis* group are particularly interesting due to their strong antagonistic activity toward microbial phytopathogens mediated by bioactive secondary metabolites (BSMs) and to their capacity to form spores facilitating formulation of stable products. These species include *B. velezensis*, which is among the most successful commercialized biopesticides. Unfortunately, the exploitation of these bacteria is still a myth in Burundi and many other tropical African countries. Moreover, the efficacy of these products to control diseases in the field may be inconsistent and lower than expected, notably because the bacteria are not always well adapted to the quite specific abiotic conditions prevailing in some local agro-ecosystems as observed in Burundi.

The global objective of this work was to contribute to the development of biocontrol in Burundi by implementing the use of well-adapted bacilli to local agro-ecosystems. We sampled Burundian field soils at different locations and search for *Bacillus*-like isolates. Out of these samples, we selected one particular isolate named BDI-IS1 displaying strong and broad-spectrum antimicrobial activity via *in vitro* confrontation assays. Based on phenotypic and molecular traits, BDI-IS1 was identified as a new strain of the rare species *Bacillus nakamurai*. By coupling comparative genomics with metabolomics, we performed the first entire characterization of the secondary metabolome for a member of this species. BDI-IS1 has the potential to secrete two cyclic lipopeptides (surfactin and iturin A), one polyketide (dihydro)bacillaene, a siderophore bacillibactin, a dipeptide bacilysin and a diversity of lanthipeptides including plantazolicin, amylocyclicin, bacinapeptin and LCI. Many of these compounds are conserved across *B. nakamurai* strains and may serve as a chemical fingerprint for the species with plausible taxonomical relevance. We further generated multiple BDI-IS1 mutants and showed, through reverse genetics, the specific involvement of some BSMs in the observed antagonistic activities. Additionally, we unveiled for the first time the biocontrol potential of this species *B. nakamurai*. Indeed, greenhouse experiments showed that BDI-IS1 provides excellent protection of maize and tomato plants (up to 65% disease reduction) against northern corn leaf blight and tomato late blight, respectively. From a mechanistic point of view, our data indicate that BDI-IS1 may act either via direct antagonism or via the stimulation of systemic resistance in the host plant or a combination of both.

We next assessed the adaptability of BDI-IS1 to the stressful conditions of low pH and low temperature, prevailing often in Burundi and many tropical agro-ecosystems. It first revealed that BDI-IS1 has a remarkable tolerance to the acidic and mild-cold stress, up to pH 4.6 and 15°C. Moreover, these stresses have contrasted effects on the production of BSMs and the associated antagonistic activities against bacterial

pathogens. Except for surfactin production which is not affected by decreasing temperature and bacilysin secretion favored by low pH, the production of BSMs was negatively affected by these limiting conditions. Antibacterial activity was either preserved or suppressed at low temperature. Low pH (pH 5) led to either an increase (three-fold) or a decrease in antagonistic activity against two low pH-tolerant bacterial pathogens, *P. carotovorum* and *C. michiganensis*, respectively. This remodelling of established interspecies relationships may be explained by the abiotic stress-driven modulation of the production of known BSMs involved in inter-bacterial competition and other unknown BSMs or effectors.

In depth functional genomics showed also that BDI-IS1 is equipped with core genes related to the rhizospheric lifestyle and with additional abiotic stress resistance-related genes compared to other related *Bacillus* spp., suggesting an improved adaptation to environmental stress which would enable its successful root colonisation and persistence. In addition, we showed that it has the genetic ability to secrete plant growth hormones and molecules mediating plant stress tolerance, as well as the genetic potential to solubilize insoluble forms of phosphorus.

Interestingly, in many aspects, our data reveal that BDI-IS1 may be more promising than the star of the biocontrol market *B. velezensis* QST713 (commercialized as Serenade Aso®). It exhibited an equal or superior performance not only in terms of biocontrol potential but also considering adaptation to acidic and low temperature-related stresses.

In a more practical perspective, we also investigated cost-effective but efficient options for biomass production and formulation of BDI-IS1, according to the socio-economic context of smallholder farmers in Burundi. Solid-state fermentation using locally sourced lignocellulosic substrates derived from agricultural residues was found to be a promising way of multiplying the bacterium with a high spore content in the fermented substrate. This was further formulated as a dry mixture of residual substrate embedded with BDI-IS1 spores and secreted metabolites, and this product was found to be more effective than QST713 in protecting maize against northern leaf blight disease.

Prior to its registration and widespread dissemination to rural farmers, additional experiments are still needed to ensure BDI-IS1 overall efficacy upon application in the field at different locations in the country and to reduce disease incidence in other pathosystems. The innocuity of the bacterium towards other (beneficial) microorganisms, consistency of the efficacy of the bioproduct across different production batches and stability upon long-term storage must also be evaluated. Nevertheless, our work features this strain as a new very promising tool to foster a more productive agriculture that is respectful of human health and the environment, while also improving local organic waste management and thereby helping to achieve the UN's sustainable development goals.

Résumé

Au Burundi et dans de nombreux pays tropicaux en voie de développement, l'agriculture est confrontée à de graves défis tels que l'incidence élevée de maladies et de ravageurs, ainsi que l'utilisation de pesticides chimiques très nocifs (plus de 30 % sont interdits en Europe), ce qui constitue une menace sérieuse pour la santé des personnes (insécurité alimentaire et intoxication) et pour l'environnement. Certaines souches de *Bacillus* appartenant à des espèces du groupe *B. subtilis* sont particulièrement intéressantes en raison de leur forte activité antagoniste à l'égard des phytopathogènes microbiens, médiée par des métabolites secondaires bioactifs, et leur capacité à former des spores facilitant leur formulation en de produits stables. Ces espèces incluent *B. velezensis*, qui fait partie des meilleurs biopesticides rencontrés au marché. Malheureusement, l'exploitation de ces bactéries est encore un mythe au Burundi et dans de nombreux pays d'Afrique tropicale. En outre, l'efficacité de ces produits pour lutter contre les maladies dans les champs peut être limitée voire plus faible que prévu, notamment parce que les bactéries ne sont pas toujours bien adaptées aux conditions abiotiques très spécifiques prévalant dans certains éco-ecosystèmes locaux, comme c'est le cas au Burundi.

L'objectif global de ce travail était de contribuer au développement du biocontrôle au Burundi en mettant en œuvre l'utilisation des *Bacillus* bien adaptées aux agro-ecosystèmes locaux. A partir d'échantillons de sols collectés à différents endroits au Burundi, nous avons recherché des isolats ressemblant à *Bacillus* et avons sélectionné un isolat particulier baptisé BDI-IS1 présentant à la fois une forte et un large spectre d'activité antimicrobienne lors d'essais de confrontation *in vitro*. Sur base de traits phénotypiques et moléculaires, BDI-IS1 a été identifié comme une nouvelle souche de l'espèce rare *Bacillus nakamurai*. En couplant la génomique comparative et la métabolomique, nous avons réalisé la première caractérisation complète du métabolome secondaire d'un membre de cette espèce. BDI-IS1 est capable de sécréter deux lipopeptides cycliques (surfactine et iturine A), un polykétide (dihydro)bacillaène, un sidérophore (la bacillibactine), un dipeptide (la bacilysine), ainsi qu'une diversité de lanthipeptides dont la plantazolicine, l'amylocyclicine, la bacinapeptine et le LCI. Nombre de ces composés sont conservés dans les souches de *B. nakamurai* et peuvent servir d'empreinte chimique pour l'espèce, avec une possible pertinence taxonomique. Ensuite, nous avons généré une série de mutants BDI-IS1 et montré, par génétique inverse, l'implication spécifique de certains métabolites secondaires bioactifs dans les activités antagonistes de la souche. De plus, nous avons révelé, pour la première fois, le potentiel de biocontrôle de cette espèce *B. nakamurai*. En effet, des expériences en serre ont montré que BDI-IS1 fournit une excellente protection des plantes de maïs et de tomate (jusqu'à 65% de réduction de la maladie) contre les maladies de brûlure des feuilles et de mildiou, respectivement. D'un point de vue mécanistique, nos données indiquent que BDI-IS1 peut agir soit par antagonisme direct, soit par stimulation de la résistance systémique dans la plante hôte, soit par une combinaison des deux.

Par après, nous avons évalué l'adaptabilité de BDI-IS1 aux conditions stressantes de faible pH et de basse température qui prévalent souvent au Burundi et dans de nombreux agro-écosystèmes tropicaux. Il a d'abord été révélé que BDI-IS1 a une très bonne tolérance au stress acide et celui lié au froid, jusqu'à un pH de 4,6 et une température de 15°C. De plus, ces stress ont des effets contrastés sur la production de métabolites bioactifs, ainsi que sur les activités antagonistes de BDI-IS1 à l'égard des bactéries pathogènes. A l'exception de la production de surfactine qui n'est pas affectée par la diminution de la température et de la sécrétion de bacilysine qui est favorisée par un pH faible, la production des métabolites secondaires est négativement affectée par ces conditions de stress. L'activité antibactérienne a été soit préservée, soit supprimée sous l'effet de basse température. Un pH faible (pH 5) a entraîné soit une augmentation (trois fois), soit une diminution de l'activité antagoniste contre deux bactéries pathogènes tolérantes de ce pH bas, *P. carotovorum* et *C. michiganensis*, respectivement. Cette reconfiguration des relations inter-espèces peut être expliquée par la modulation par ces facteurs abiotiques de la production de certains composés bioactifs impliqués dans la compétition entre bactéries, mais également d'autres molécules ou effecteurs jusque-là inconnus.

Une analyse poussée, basée sur la génomique fonctionnelle, a également montré que BDI-IS1 est équipée de gènes importants liés au mode de vie dans la rhizosphère et possède des gènes supplémentaires liés à la résistance au stress abiotique par rapport à d'autres *Bacillus* spp. proches phylogénétiquement, ce qui suggère une meilleure adaptation au stress environnemental pouvant permettre sa meilleure colonisation racinaire et sa persistance. En plus, nous avons montré qu'il a la capacité génétique de sécréter des hormones de croissance des plantes et des molécules médiant la tolérance au stress chez les plantes, ainsi que le potentiel génétique de solubiliser des formes insolubles de phosphore.

Il est intéressant de noter que, sous de nombreux aspects, nos données révèlent que BDI-IS1 pourrait être plus prometteuse que la star du marché de biocontrôle *B. velezensis* QST713 (commercialisée sous le nom de Serenade Aso®). Il a montré une performance égale ou supérieure non seulement en termes de potentiel de biocontrôle, mais aussi en ce qui concerne l'adaptation aux stress liés à l'acidité et aux basses températures.

Dans une perspective plus pratique, nous avons également étudié des options moins coûteuses mais efficaces pour la production de biomasse et la formulation de BDI-IS1, en fonction du contexte socio-économique des petits exploitants agricoles au Burundi. La fermentation en milieu solide utilisant des substrats lignocellulosiques d'origine locale, issus de résidus agricoles, s'est avérée être un moyen prometteur de multiplier la bactérie avec une teneur élevée en spores dans le substrat fermenté. Ceci a ensuite été formulé sous la forme d'un mélange sec de substrat résiduel bourré de spores de BDI-IS1 et de métabolites sécrétés, et ce produit s'est avéré plus efficace que celui basé sur QST713 pour protéger le maïs contre la maladie de brûlure des feuilles.

Toutefois, avant son homologation et sa diffusion à grande échelle auprès des agriculteurs, des expériences supplémentaires sont encore nécessaires pour garantir

l'efficacité globale de la bactérie lors de son application en champs à différents endroits du pays, mais aussi celle de réduire l'incidence de maladie dans d'autres pathosystèmes. L'innocuité de la bactérie vis-à-vis d'autres microorganismes (bénéfiques), la consistance de l'efficacité du bioproduit entre les différents lots de production et la stabilité du produit lors du stockage à long terme doivent également être évaluées. Néanmoins, les vertus particulières de cette souche présentées dans notre travail font d'elle un nouvel outil très prometteur pour favoriser une agriculture plus productive et respectueuse de la santé humaine et de l'environnement, tout en améliorant la gestion locale des déchets organiques et en contribuant ainsi à la réalisation des objectifs de développement durable des Nations unies.

Remerciements

Ce présent travail marque l'étape ultime d'inlassables efforts personnels, mais aussi c'est un fruit d'une franche collaboration avec un bon nombre de personnes à compétences diverses et qui méritent toute ma reconnaissance. Avant tout, je tiens à exprimer ma profonde gratitude à mon promoteur Dr. Marc Ongena (Directeur de Recherches, FNRS) pour l'encadrement scientifique de qualité et la confiance indéfectible placée en moi, qui ont forgé le docteur et scientifique que je deviens. Merci également pour ton accompagnement moral tel d'un père à un fils, soutien sans lequel je ne serais parvenu à tenir jusqu'au bout. Mes sincères remerciements également à vous Pr. Venant Nihorimbere, co-concepteur avec Dr. Marc Ongena du projet PRD 2019 qui a sponsorisé ce travail et coordinateur sud dudit projet. Je vous serai toujours gré de la confiance placée depuis mon recrutement en tant que doctorant dans le projet, de ta disponibilité et ton écoute toujours attentive, ainsi que les riches discussions scientifiques et éthiques tenues en cadre formel ou informel chez vous.

Mes remerciements sont adressés également aux membres du comité d'accompagnement, aussi membres du jury de ma thèse, Pr. Philippe Jacques, Pr. Franck Delvigne, Pr. Stéphane Declerck. Merci pour votre disponibilité et vos enrichissantes discussions, lors de nos réunions annuelles, qui ont façonné ce travail et permis qu'il aboutisse à terme. Avec Pr. Monica Höfte et Pr. Sébastien Massart, merci d'avoir accepté de consacrer votre temps à l'évaluation de ce travail et pour vos pertinentes remarques soulevées lors de la défense privée qui ont permis l'amélioration de ce manuscrit.

Je tiens également à remercier Pr. Godefroid Gahungu, mon professeur de Chimie à l'Université du Burundi, dont son mentorat m'a conduit vers les sentiers du projet PRD 2019. Vous vous souvenez bien, sans votre accueil académique adéquat et encouragement dans ma première année d'Université, cette étape ne serait qu'illusoire. Avec Pr. Théophile Ndikumana, vous n'avez cessé de croire en moi et m'avez toujours soutenu tout au long de mes études. Je vous serai toujours reconnaissant.

Merci à toi cher Gaspard Nihorimbere, compagnon de cette route de doctorat. Notre collaboration a été le moteur de l'aboutissement de nos travaux. Dès la collecte d'échantillons aux fins fonds du Burundi, la conduite de nos expériences respectives, le rélevèment des divers défis administratifs à l'Université du Burundi et à l'ISABU, la rédaction de nos manuscrits d'articles et durant les congrès internationaux, nous étions toujours ensemble et notre complémentarité a résonné plus loin et forgé même l'amitié de nos familles respectives. Merci à toi aussi Virginie Korangi Alleluia avec qui, Gaspard et moi, avons partagé le labeur de notre article review en rapport avec la problématique de protection de cultures et évaluation de possibilités d'implémentation d'alternatives biologiques à l'utilisation de pesticides chimiques dans la région des grands lacs d'Afrique centrale.

Ce travail de thèse n'aurait pas abouti à sa fin sans le concours des anciens et nouveaux membres du personnel du MiPI (Microbial Processes and Interactions) et je tiens à les remercier vivement. Je remercie plus spécifiquement toute l'équipe de Marc

d'antan (Anthony, Greg, Thibaut, Sofja, Jelena, Adrien, Augustin, Farah, Sébastien) et certaines personnes comme Papa et Olivia, pour l'accueil chaleureux me réservé dès mon arrivée, les soirées bien arrosées, les balades, les discussions matinales autour d'un café, ... Adrien, merci pour ton encadrement durant mes premiers jours au laboratoire. Cher Anthony, merci pour ta formation scientifique, ton soutien moral et ta franchise de parole lors de nos échanges qui ravivait mon cœur parfois aigri, découragé. Reste toujours cette bonne personne qui tu es et un ami sur qui je peux compter. Cher Sébastien, merci pour ta formation de qualité en biologie moléculaire (faite avec beaucoup de passion) et pour l'ami qui tu es. Chers Augustin et Guillaume, merci pour vos coups de main dans les anlyses au q-TOF, vos riches discussions, votre compagnie dans les courses de midi (vous m'avez forgé en coureur régulier hhh), bref pour votre amitié. Mes sentiments de gratitude vont aussi à l'endroit de Marina, moins qu'une secrétaire mais un monument autour duquel tout le MiPI tourne, toujours accueillante et souriante, et toujours prête à l'écoute. Merci à vous Cathy, Margue, Danielle, Sam, Andrew et Stéphanie pour votre assistance technique, ainsi qu'à Maria qui prend soin de notre de travail à tous. Grâce à vous, la vie au laboratoire devient plus aisée qu'elle ne semble l'être. Cher Thomas, tu es le dernier de la liste mais pas the least, merci pour la bonne compagnie et la chouette ambiance créée dès ton arrivée, qui ont ranimé mon quotidien ces derniers mois de rédaction de thèse.

Deux femmes très spéciales, mon épouse Ornella Gateka et notre fille Guénola Franella Bwitonzi, ont bien souffert de mon absence durant ces années de thèse. Merci mes chéries pour le sacrifice consenti, merci pour les encouragements, les appels videos, les video notes remplis de bizous (notre fille), … après mes longues journées de travail. Votre abnégation a été le ciment de mes nuits blanches (passées parfois au laboratoire ou derrière mon ordinateur). Je tiens également à remercier mes parents et mon oncle (tuteur dès mes 6 ans), mes frères et sœurs, cousins et cousines, et la famille élargie pour leurs encouragements, leur amour et soutien indéfectible à ma famille, surtout en cette période de mon absence bien jalonnée de pas mal d'épreuves. Je remercie également la communauté burundaise de Gembloux, la communauté de Maison Nord-Sud et mes amis vivant en Belgique, vos encouragements et moments de qualité partagés ensemble ont allégé le poids de l'éloignement de ma famille. Toute ma gratitude également au Royaume de Belgique à travers l'ARES qui a financé le projet PRD 2019 dont émanait ma bourse, mais aussi à ses mécanismes sociaux à savoir le CPAS de Gembloux qui m'a apporté de l'aide au moment opportun, me permettant ainsi de vivre dignement.

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GLCCA: Great Lakes Countries of Central Africa
GDP: Gross Domestic Product
NRPS : Non-Ribosomal Peptide Synthase
PKS : PolyKetide Synthase
NRP : Non-ribosomal peptides
sfp: Phosphopantheinic acid transferase
RiPP: Ribosomally produced and post-translationally modified peptide
BSMs: Bioactive Secondary Metabolites
ATP: Adenosine Triphosphate
BGC: Biosynthetic gene cluster
AT domain: Acyltransferase Domain
A domain : Adenylation domain
TE domain : Thioesterase domain
PCP/ACP (T) domain : Peptidyl Carrier Protein/ Acyl Carrier Protein
CoA : Coenzyme A
MT domain : Methyltransferase domain
E domain : Epimerase domain
MCT domain : Malonyl-CoA-Transacylase
DH domain : DeHydratase domain
KR domain: KetoReductase domain
ER domain: Enoyl Reductase domain
DHB: 2,3-DiHydroxylated Benzoyl
Lan: Lanthionine
LAP: Linear Azol(In)E-containing Peptide
UPLC-ESI-qTOF-MS: Ultra Performance Liquid Chromatography-ElectroSpray Ionisation-quadrupole Time Of Flight-Mass spectrometry
MeOxz: Methoxazole
Oxz: Oxazole
MeLan: Methyl-Lanthionine
Dha: Dehydroalanine
Dhb: Dehydrobutyrine
PTM: Post-Translational Modification
PCR : Polymerase Chain Reaction
2H-bae : Dihydrobacillaene
DwF : Down Forward
DwR : Down Reverse
CFU: Colony Forming Unit
ANOVA: Analysis of Variance
LBA : Luria-Bertani Agar medium
BDI-IS1: **1**st isolate from **IS**ARE-**B**URUN**DI**
OD_{600nm}: Optic Densisty at 600 nm
psi: pound square per inch
EDTA: EthyleneDiamine Tetraacetic Acid
EIC: Extracted Ion Chromatogram
TIC: Total Ion Chromatogram
QS: Quorum Sensing

QQ: Quorum Quenching
SynCom: Synthetic Community
MS:MS: Tandem Mass Spectrometry
UpF: Up Forward
UpR: Up Reverse
VerifF: Verification Forward
MMG: Minimum Medium with Glucose and Glutamic-acid
CFS: Cell-Free Supernatant
mL: milliliter
Ppm: Part Per Million
IPM : Integrated Pest Management
MINAGRI: Minstry in charge of Agriculture
ISABU: Institut des Sciences Agronomiques du Burundi
Da: Dalton
VOC: Volatile Organic Compounds
ISR: Induced Systemic Resistance
WT : Wild Type
DNA : Deoxyribonucleic acid
RNA : Ribonucleic acid
AOS: Allene oxide synthases
PAL: Phenylalanine ammonia lyase

Chapter 1

General introduction

The three first sections of this chapter are adapted from:

Gaspard Nihorimbere, Virginie Korangi Alleluia, François Nimbeshaho, Venant Nihorimbere, Anne Legrève and Marc Ongena (2024). *Bacillus*-based biocontrol beyond chemical control in central Africa: the challenge of turning myth into reality. *Front. Plant Sci.* 15:1349357. doi:10.3389/fpls.2024.1349357.

In this review paper, which highlights the agricultural problems prevailing in Burundi, Rwanda and Democratic Republic of the Congo, as well as the status of pesticides use and the possibility of implementing biocontrol in the region, Gaspard Nihorimbere, Virginie Korangi Alleluia and I contributed equally from the project conceptualisation to the writing of the manuscript and addressing reviewers' comments in the final version, and are thus co-first authors.

The remaining sections (4-6) have not yet been published and are specific to this thesis.

1. Context

The importance of agriculture for the development of societies has been demonstrated for several centuries and it employs about 43% of the world's working population (Roser, 2023). In the Great Lakes Countries of Central Africa (GLCCA), referred to in this report as Burundi, Rwanda, and the Democratic Republic of Congo (DRC), agriculture is the main activity employing more than 94%, 46% and 60% of the working population, while contributing to 40%, 30% and 36% of gross domestic product (GDP) in Burundi, Rwanda, and DRC, respectively (FAAPA, 2021; Lokuruka, 2021; PND, 2018). However, this sector faces several constraints including low soil fertility and high incidence of diseases and pests leading to food insecurity (Bjornlund et al., 2020). For example, up to 52%, 43% and 38% of children under five years in Burundi, DRC and Rwanda, respectively, are malnourished (FAO, 2023a; Lokuruka, 2021; WFP, 2021). In the GLCCA, plant diseases and pests are generally controlled by chemical pesticides, especially those affecting cash crops such as coffee, cotton, tomatoes, potatoes, vegetables, and fruits (Muliele et al., 2018; Niyongere et al., 2015; Okonya, Petsakos, et al., 2019). However, some bioaggressors are inefficiently managed by traditional practices, such as the use of plant extracts, while others are not controlled (Korangi Alleluya et al., 2021; MINAGRI-Burundi, 2018; Rutikanga, 2015).

Globally available alternatives inspired by the integrated pest management (IPM), including good agricultural practices and biological control, are not widely used or, in some cases, non-existent. Biocontrol products are the most promoted tools within the IPM framework, as they are generally recognized as safe (GRAS) compared to chemical pesticides (Raveau et al., 2020). Biocontrol products include biochemicals (semiochemicals, plant extracts, plant growth regulators and organic acids), macroorganisms (insects, mites, and nematodes) and microorganisms (beneficial bacteria, fungi, protozoa, viruses, yeasts) and their derivatives (cyclic lipopeptides, enzymes, chitosan oligopolysaccharides, etc.) (DunhamTrimmer, 2023), as well as the recently developed new biocontrol product, double strand RNA (dsRNA) (Septiani et al., 2025; Zarrabian et al., 2025). *Bacillus*-based products dominate the biocontrol market compared to their microbial counterparts (DunhamTrimmer, 2023; Helepciuc & Todor, 2023) due to their ability to secrete an arsenal of bioactive secondary metabolites (BSMs) and to form resistant endospores, allowing stable formulations and ensuring long-term survival in the environment, especially in the current climate change context (Miljaković et al., 2020; Radhakrishnan et al., 2017). Unfortunately, although some preliminary studies have demonstrated efficacy in controlling some important local pathogens, these *Bacillus*-based products are still a “myth” for local farmers. Efforts should be directed towards promoting the use of already available *Bacillus*-based products against endemic pathogens devasting crops in local farming systems. However, the adaptability of these foreign strains is not totally guaranteed into local agricultural systems. Henceforth, bioprospecting for indigenous and well-adapted *Bacillus* strains to local ecological conditions, with biocontrol and plant growth promotion properties should be promoted to ensure sustainable plant disease

management in the region. The great potential of indigenous *Bacillus* strains over the commercial ones has been shown, for example in the management of grapevine trunk disease (Langa-Lomba et al., 2023) and tropical fruit diseases (Reyes-Estebanez et al., 2020). Owing to the poverty prevailing in these countries, cost-effective large-scale production and formulation procedures of the isolated locally-adapted bioagents should be set up for a wide adoption of this innovative bio-sourced techniques and ensure a more productive agriculture, good health and safe environment.

2. High plant disease pressure in Central Africa and pesticides use related-flaws

2.1. Main plant diseases in the region and their mitigation strategies

We performed a comprehensive survey of the major diseases and causal agents affecting crop production in the GLCCA region by compiling data from published studies (articles and books) with those obtained from governmental and non-governmental agencies in the form of reports, newspapers, or online database. It revealed that agricultural crops, either in the field or in storage, are mainly affected by microbial pathogens consisting of fungi, oomycetes, bacteria, and viruses (Table 1-1). Among the most important fungal diseases are late blight of potato and tomato caused by *Phytophthora infestans*, angular leaf spot of bean caused by *Pseudocercospora griseola*, early blight of tomato caused by *Alternaria solani*, stem rot caused by *Sclerotium rolfsii*, late and early leaf spot caused by *Nothopassalora personata* and *Cercospora arachidicola* on peanut. These pathogens are most commonly controlled with the chemical mancozeb or its derivatives. Metalaxyl, benomyl, iprobenfos, copper (II) chloride and metalaxyl are other important fungicides used in the region. One of the most common bacterial diseases prevailing in the region is *Xanthomonas* wilt of banana caused by *X. campestris* pv. *musacearum*, but unfortunately there is no effective pesticide available to control this pathogen (Table 1-1). Viral plant pathogens responsible for cassava mosaic disease, cassava brown streak disease, banana bunchy top disease and maize lethal necrosis, also pose serious threat to food security in the region but here again, no efficient chemical control is available. For example, the outbreak of cassava mosaic disease in 2004-2005 led to severe food shortages that threatened many families in the north-eastern provinces of Kirundo and Muyinga in Burundi. As a result, 100 famine-related deaths were reported (Legg et al., 2006).

Insects, nematodes, and weeds also cause severe yield losses. Important insect pests affecting major crops in the region include fall armyworm (*Spodoptera frugiperda*), tomato leafminer (*Tuta absoluta*), coffee bugs (*Antestiopsis orbitalis ghesquierei*), whitefly (*Bemisia tabaci*), banana aphid (*Pentalonia nigronervosa*), and coton aphid (*Aphis gossypii*) (Belga, 2020; Cokola et al., 2021; Dushimirimana et al., 2016; Fiaboe et al., 2021; MINAGRI-Burundi, 2018; Niassy et al., 2021; Niyibizi et al., 2019; Nyabyenda, 2005). Control of these insects relies heavily on the use of synthetic

products such as acephate against *S. frugiperda* and *T. absoluta*, chlorpyrifos-ethyl against *A. orbitalis ghesquierei* and imidacloprid against aphids (MINAGRI-Burundi, 2018; ARECO-Rwanda Nziza, 2020; GUCE, 2022). Several species of nematodes also damage plants, including *Meloidogyne javanica* on various crops, *Pratylenchus goodeyi* and *Helicotylenchus multicinctus* on banana plants, and *Ditylenchus* spp. on potatoes (Coyne et al., 2018) and are managed by using chemicals such as dazomet and terbufos. Parasitic plants or weeds that compete with crops such as sugarcane, rice, sorghum, and maize are also problematic and include among others *Striga* spp., *Cyperus* spp. and *Echinochloa* spp. Their control involves chemical herbicides like glyphosate, atrazine and dalapon (Nyabyenda, 2005; Rodenburg et al., 2016; Runo & Kuria, 2018).

Table 1-1: Microbial pathogens affecting crop production in the GLCCA region and chemical pesticides used for their control

Pathogens and pests	Species	Host plant	Impact[a]	Pesticides active ingredients	References
Bacteria	*Xanthomonas campestris* pv *musacearum*	Banana (*Musa* spp.)	***	NI	(Ndayihanzamaso et al., 2016; Ndungo et al., 2008; Nkuba et al., 2015; Nyabyenda, 2006; Rietveld et al., 2020)
	Pseudomonas syringae pv. *phaseolicola, P. syringae* pv. *syringae*	Bean (*Phaseolus vulgaris*)	***	Streptomycin sulphate	(Nyabyenda, 2005)
	Xanthomonas campestris pv. *phaseoli*		***	Streptomycin sulphate	(Nyabyenda, 2005)
	Xanthomonas campestris pv manihotis	Cassava (*Manihot esculenta*)	NI	NI	(Nyabyenda, 2005)
	Ralstonia solanacearum	Pepper (Chilli and sweet) (*Capsicum* sp.)	NI	NI	(Minengu et al., 2018 ; Nyabyenda, 2006)
	Pseudomonas solanacearum	Potato (*Solanum tuberosum*)	***	NI	(Nyabyenda, 2005)
	Ralstonia solanacearum		NI	NI	(Harahagazwe et al., 2007; Munyuli et al., 2017; Okonya, Ocimati, et al., 2019; K. Sharma et al., 2021)
	Pseudomonas fuscovaginae	Rice (*Oryza sativa*)	***	Formol	(Nyabyenda, 2005)

	Clavibacter xyli	Sugar cane (*Saccharum officinarum*)	***	Methoxy-ethyl, mercury chloride	(Nyabyenda, 2006)
	Xanthomonas vasculorum		***	Methoxy-ethyl, mercury chloride	(Nyabyenda, 2006)
	Xanthomonas albilineans		*	NI	(Nyabyenda, 2006)
	Ralstonia solanacearum	Tomato (*Lycopersicum esculentum*)	***	Formol	(Nyabyenda, 2006)
	Pseudomonas solanacearum		***	NI	(Nyabyenda, 2006)
	Corynebacterium michiganense		***		(Nyabyenda, 2006)
Fungi	*Fusarium oxysporum* var f. sp. *cubense*	*Banana* (*Musa* spp.)	***	NI	(Ndayihanzamaso et al., 2020; Nyabyenda, 2006)
	Armillaria mellea, Cordona musae		*	NI	(Nyabyenda, 2006)
	Cladosporium musae		*	NI	(Nyabyenda, 2006)
	Gloesporium musarum		*	NI	(Nyabyenda, 2006)
	Helminthosporium tolurosum		*	NI	(Nyabyenda, 2006)
	Mycosphaerella musicola		*	NI	(Nyabyenda, 2006)
	Stachylidium theobrome		*	NI	(Nyabyenda, 2006)
	Ascochyta phaseolarum	Bean	***	Mancozeb, benomyl, benlate	(Nyabyenda, 2005; Ruraduma et al., 2012)

Colletotrichum lindemuthianum	(*Phaseolus vulgaris*)	***	Benomyl, mancozeb, thiophanate-methyl, benlate	(Nyabyenda, 2005; Ruraduma et al., 2012)
Fusarium solani f. sp. *phaseoli*		***	NI	(Nyabyenda, 2005)
Mycovellosiella phaseoli		***	Benomyl, mancozeb, thiophanate-methyl, benlate	(Nyabyenda, 2005)
Pseudocercospora griseola		***	Benomyl, thiophanate-methyl, mancozeb	(Busogoro et al., 1999; Farrow & Muthoni-Andriatsitohaina, 2020; Kijana et al., 2017; Nyabyenda, 2005; Ruraduma et al., 2012)
Pythium spp.		***	NI	(Nyabyenda, 2005)
Rhizoctonia solani		***	NI	(Nyabyenda, 2005)
Sclerotinia sclerotiorum		***	NI	(Nyabyenda, 2005)
Thielaviopsis basicola		***	NI	(Nyabyenda, 2005)
Uromyces appendiculatus		***	Mancozeb, benlate	(Farrow & Muthoni-Andriatsitohaina, 2020; Nyabyenda, 2005)
Alternaria spp.	Cabbage (*Brassica* spp.)	***	NI	(Nyabyenda, 2006)
Botrytis cinerea		***	NI	(Nyabyenda, 2006)
Peronospora spp.		***	Mancozeb	(Nyabyenda, 2006)
Sclerotinia spp.	Carrot (*Daucus carota*)	***	NI	(Nyabyenda, 2006)
Cercospora hemingsii	Cassava	NI	NI	(Nyabyenda, 2005)

Glomerella manihotis	(*Manihot esculenta*)	NI	NI	(Nyabyenda, 2005)
Hemileia vastatrix	Coffea (*Coffea* spp.)	***	Copper oxychloride 50%, dithianon, triadimefon	(Nyabyenda, 2006)
Ascochyta sp., *Phoma* sp.		NI	NI	(MINAGRI-Burundi, 2016; Nyabyenda, 2006)
Cercospora coffeicola		NI	NI	(MINAGRI-Burundi, 2016; Nyabyenda, 2006)
Colletotrichum coffeanum		NI	Copper oxychloride 50%, dithianon, captafol, chlorotalonil	(MINAGRI-Burundi, 2016; Nyabyenda, 2006)
Rhizoctonia solani		NI	NI	(MINAGRI-Burundi, 2016; Nyabyenda, 2006)
Golovinomcyces spp.	Eggplant (*Solanum melongena*)	***	NI	(Nyabyenda, 2006)
Leveillura spp.		***	NI	(Nyabyenda, 2006)
Peronospora spp.		***	NI	(Nyabyenda, 2006)
Exserohilum turcicum, Helminthosporium. maydis	Maize (*Zea mays*)	**	Thiram, thioral (thiram + mancozeb), benomyl	(Nyabyenda, 2005)
Puccina polysora		*	NI	(Nyabyenda, 2005)
Ustilago zeae, Sphacelotheca reilina		*	NI	(Nyabyenda, 2005)
Puccinia sp.	Morella (*Solanum aethiopicum*)	NI	NI	(Nyabyenda, 2005)
Alternaria spp.	Onion (*Allium cepa*)	***	Maneb, mancozeb	(Nyabyenda, 2005)
Peronospora spp.		***	Maneb, mancozeb	(Nyabyenda, 2006)

Puccinia spp.		***	Maneb, mancozeb	(Nyabyenda, 2006)
Nothopassalora personata (Syn. Cercospora personata), Cercospora arachidicola	Peanut (*Arachis hypogaea*)	***	NI	(Nyabyenda, 2005)
Macrophomina phaseolina		**	NI	(Nyabyenda, 2005)
Puccinia arachidis		**	NI	(Nyabyenda, 2005)
Sclerotium rolfsii		**	NI	(Nyabyenda, 2005)
Colletotrichum nigrum, C. capsici	Pepper (Chilli and sweet) (*Capsicum* sp.)	***	NI	(Nyabyenda, 2005)
Rhizoctonia solani	Potato (*Solanum tuberosum*)	***	NI	(Harahagazwe et al., 2007; Nyabyenda, 2005)
Pyricularia oryzae	Rice (*Oryza sativa*)	***	Benomyl, thiram, iprobenfos, isoprothiolane	(Kanyange et al., 2019; Liboga et al., 2020; Nabahungu & Visser, 2013; REMA, 2011)
Cercospora oryzae		**	NI	(Nyabyenda, 2005)
Pythium bebaryanum		**	NI	(Nyabyenda, 2005)
Rhizoctonia solani		**	NI	(Nyabyenda, 2005)
Sclerotium rolfsii		**	NI	(Nyabyenda, 2005)
Gerlachia oryzae		NI	NI	(Nyabyenda, 2005)
Helminthosporium oryzae		NI	NI	(Nyabyenda, 2005)
Sarocladium oryzae		NI	NI	(Nyabyenda, 2005)

Peronosclerospora sorghii	Sorghum (*Sorghum bicolor*)	***	NI	(Nyabyenda, 2005)
Colletotrichum graminicola		NI	NI	(Nyabyenda, 2005)
Exserohilum turcicum		NI	NI	(Nyabyenda, 2005)
Puccina purpurea		NI	NI	(Nyabyenda, 2005)
Sphacelotheca cruenta, S. reiliana, S. sorghi		NI	NI	(Nyabyenda, 2005)
Puccinia sp.	Spinach (*Basella alba*)	NI	Mancozeb	(Nyabyenda, 2006)
Colletotrichum falactum	Sugar cane (*Saccharum officinarum*)	***	NI	(Nyabyenda, 2006)
Fusarium moniliforme		***	NI	(Nyabyenda, 2006)
Ustilago scitaminea		***	NI	(Nyabyenda, 2006)
Puccinia melanocephala		*	NI	(Nyabyenda, 2006)
Sclerospora sacchari		*	NI	(Nyabyenda, 2006)
Alternaria solani	Sweet potato (*Ipomoea batatas*)	***	NI	(Nyabyenda, 2006)
Armillaria mellea	Tea (*Camellia sinensis*)	***	NI	(Nyabyenda, 2006)
Rosellinia arcuate		***	NI	(Nyabyenda, 2006)
Colletotrichum camelliae		*	NI	(Nyabyenda, 2006)
Corticium salmonicolor		*	NI	(Nyabyenda, 2006)
Pestalotiopsis theae		*	NI	(Nyabyenda, 2006)
Fusarium oxysporum f. sp. *lycopersici*	Tomato (*Lycopersicon esculentum*)	***	NI	(Nyabyenda, 2006)
Cladosporium fulvum		***	NI	(Nyabyenda, 2006)

	Alternaria solani		***	Mancozeb, metalaxyl	(Nyabyenda, 2006)
Oomyceta	*Phytophthora infestans*	Potato (*Solanum tuberosum*)	***	Mancozeb and metalaxyl	(Bararyenya et al., 2018; Biruma et al., 2007; Harahagazwe et al., 2007; Okonya, Ocimati, et al., 2019)
	Phytophthora infestans	Tomato (*Lycopersicon esculentum*)	***	Mancozeb and metalaxyl	(Nyabyenda, 2006)
	Pythium spp.		***	NI	(Nyabyenda, 2006)
Virus (and vector)	African cassava mosaic virus "ACMV", East African cassava mosaic virus "EACMV", East African cassava mosaic virus-Uganda "EACMV-UG" (Vector: *Bemisia tabaci*)	Cassava (*Manihot esculenta*)	***	NI	(Bigirimana et al., 2004; Nyabyenda, 2005; Thresh & Cooter, 2005)
	Cassava brown streak virus "CBSV", Ugandan cassava brown streak virus "UCBSV", Cassava root necrosis disease "CRND" (Vector: *Bemisia tabaci*)		***	NI	(Bigirimana et al., 2011; Maruthi et al., 2017; Muhindo et al., 2020; Munganyinka et al., 2018; Nyabyenda, 2005; Okonya, Ocimati, et al., 2019)
	Banana bunchy top virus "BBTV" (Vector: *Pentalonia nigronervosa*)	Banana (*Musa* spp.)	***	NI	(Boloy et al., 2014; Gaidashova et al., 2010; Mukwa et al., 2014; Niyongere et al., 2013; Okonya, Ocimati, et al., 2019; Raut & Ranade, 2004)

Sweet potato chlorotic stunt virus "SPCSV" *(Vector: Bemisia tabaci),* Sweet potato feathery mottle virus "SPFMV" (Vector: *Myzus persicae* and *Aphis gossypii*)	Sweet potato (*Ipomoea batatas*)	***	NI	(Nyabyenda, 2005; Sheffield, 1957)
Bean common mosaic virus "BCMV" (Vector: Aphid)	Bean (*Phaseolus vulgaris*)	***	Dimethoate	(Nyabyenda, 2005 ; Ruraduma et al., 2012)
Virus A, Virus X, Virus S, Virus Y *(Vector: Aphid)*	Potato (*Solanum tuberosum*)	***	NI	(Nyabyenda, 2005)
Groundnut rosette virus "GRV" (Vector: *Aphis craccivora*)	Peanut (*Arachis hypogaea*)	***	NI	(Nyabyenda, 2005)
Maize chlorotic mottle virus "MCMV" (Vectors: Thrips, root worms and leaf beetles), Maize streak virus "MSV" (Vector: *Cicadulina* spp.) Maize lethal necrosis "MLN" Sugarcane mosaic virus	Maize (*Zea mays*)	***	NI	(Adams et al., 2014; Casinga et al., 2021; Isabirye & Rwomushana, 2016; Lukanda et al., 2014; Mahuku et al., 2015; Nyabyenda, 2005; Redinbaugh & Stewart, 2018)
Pepper mild mottle virus (Vector: Thrips)	Pepper (Chilli and sweet) (*Capsicum* spp.)	***	NI	(Nyabyenda, 2006)

[a]Microbial pathogens are ranked from slightly to high impact per crop; *: Slightly high; **: moderate high; ***: high; NI: No Information

The analysis of the respective lists of approved pesticides for use (Table S1) in the three countries shows that fungicides and insecticides are the mostly consumed. Accordingly, more than 80% of the bioagressors observed in the GLCCA are fungi and insects (Nyabyenda, 2005, 2006). Globally, mancozeb is the main fungicide active ingredient used in the region, while the main marketed insecticide products include dimethoate, imidacloprid, lambda-cyhalothrin, cypermethrin and chlorpyrifos-ethyl (ARECO-Rwanda Nziza, 2020; GUCE, 2022; MINAGRI-Burundi, 2018). The compiled data on total quantities of pesticides imported between 2017 and 2021 (FAO, 2023) in the three countries (Table 1-2) harnesses this evidence of reliance on chemical pesticides. Fungicides are relatively highly consumed in Burundi and Rwanda, while insecticides are mostly consumed in the DRC.

Table 1-2: Cumulative quantity (in tons) of pesticides imported in GLCCA between 2017-2021.

Country	Cultivated land (km^2)[a]	Insecticides	Herbicides	Fungicides	Country of origin
Burundi	20,330	1,361	682	2,492	China, Belgium, Kenya, Indonesia
Rwanda	18,117	6,178	4,430	20,216	India, Switzerland, Belgium, China, South Africa, Kenya, Spain, Indonesia, France, and United Kingdom
DRC	335,720	7,133	1,406	872	China, France, South Africa, United Kingdom, Belgium, Netherlands, Spain, USA, Rwanda, and Uganda

[a] Data on cultivated land were retrieved from the website of the World Bank group (Groupe de la Banque mondiale, 2023)

2.2. Pesticide management

Pesticides are any substance or mixture of substances composed of chemical or biological components intended to repel, kill, or inhibit the growth of any harmful organism (vectors of human and animal diseases, plant pathogens) or substances intended to regulate plant growth (FAO & WHO, 2014). Pesticides management is the responsibility of the Ministry of Agriculture in each of the three countries. Technically, the registration and the control of pesticides utilization are regulated by a multidisciplinary national advisory council in Rwanda and DRC, and by the

Veterinary Products, Pesticides and Food Regulation Board in Burundi. Although this council is provided for in the legal texts, it has never been established in the DRC, where full control of pesticides is ensured by a special Bureau for the Certification and Registration of Phytosanitary Products (BCHPP). These regulatory bodies are chaired by the Registrar in Rwanda (Ministerial Order n°002/11. 30 of 14/07/2016 establishing the regulations on agrochemicals in Rwanda), the Secretary General of the Ministry of agriculture in the DRC (circular note n°014/SG/AGRIPEL/2018 of 27/12/2018 on the procedure for the registration and distribution of phytosanitary products in the Democratic Republic of Congo) and the Minister of agriculture in Burundi (Decree Law n°1/04 of 11/02/2021 on the control and management of pesticides in Burundi).

Pesticides' distributors (importers or retailers) in the three countries must obtain a license from the competent authorities. The registration process includes the acquisition and submission of the application form (dossier) upon payment of the required fees, the evaluation of the dossier, the assessment of the quality and efficacy of the pesticides by experts at the applicant's own expense, and the final decision by the competent authorities. This would lead either to registration of the product or to rejection or to temporary registration if further information is needed in the dossier or if the product is still undergoing trials or if there is an emergency requiring the use of the pesticide.

2.3. Flaws in the pesticides management sector and associated risks

Pesticide on the market must be regularly reconsidered by the relevant registration authorities and subsequently included in a list of approved or banned products. The aim is to ensure the quality, efficacy and safety of the pesticides used. However, there are some specific features of the management of pesticide in the region that deserve special attention.

First, many chemical pesticides banned in the European Union (EU Food Safety, 2023) are still officially registered in the GLCCA countries, representing up to 30% (Table 1-3) of the total number of products legally distributed (Table S1). It includes the highly hazardous but most commonly used fungicides mancozeb and thiram, the insecticides imidacloprid, acephate, dimethoate and dichlorvos, and the herbicide paraquat (Lewis et al., 2016). Furthermore, some ingredients such as endosulfan and dichlorodiphenyltrichloroethane (DDT), which have been officially discarded by governments in the GLCCA region, are still available in local markets (Bassily, 2008; Ngweme et al., 2019). Unfortunately, this situation is also prevailing in neighbouring countries of GLCCA like Kenya and Tanzania, where most of these dangerous products are also registered and in use (Table 1-3).

Second, malpractices in the handling of chemical pesticides are common, either among traders or end-users such as farmers or industrial workers (Figure 1-1). These malpractices include the sale of adulterated products, failure to use personal protective equipment, incorrect dosage and selection of products, and application at

inappropriate times (Balasha et al., 2023; Okonya, Petsakos, et al., 2019). One of the consequences is that residues of pesticides have been found at toxic levels in crops and vegetables such as tomato and amaranth (Kavatsurwa et al., 2014; Ngweme et al., 2021; Ndisanze et al., 2022). Relevant concentrations of pesticide residues have also been detected in human urine, blood, and serum (Bayebila et al., 2021), while some traces were found in fishes from Lake Tanganyika (P. Manirakiza et al., 2002).

Figure 1-1: Malpractices in chemical pesticides (CPs) use. (A) Whitish traces of mancozeb on tomatoes sold at COTEBU market, Bujumbura Burundi (Photo provided by authors, 2021). (B) Handling of CPs without personal protection equipment (PPE) and repackaging of CPs (liquid form) into small bottles for sale at CECOMAF (Centre de Commercialisation des produits Maraîchers et Fruitiers), Kinshasa, DRC (Photo provided by authors, 2022). (C) Small quantities of CPs (powder form) repackaged in plastic bags for sale at CECOMAF (Photo provided by authors, 2022). (D) A farmer applying CPs without PPE in Rwanda (Photo provided by Ishimwe Yvonne, 2023).

Consequently, some acute symptoms and even deaths of humans and animals due to pesticide poisoning have been reported in the region, as well as severe symptoms such as reddened eyes, itchy skin, teary eyes, burning eyes, runny nose, headache, difficult breathing, and heavy sweating (Balasha et al., 2023; Ndayambaje et al., 2019; Okonya, Petsakos, et al., 2019). Chronic effects of pesticide exposure, including cancer, infertility, and neurological problems (I. Mahmood et al., 2016), may be a reality in the region or may occur in the near or distant future, although no such studies have been conducted till to date. Several reasons encompassing poverty, ignorance, and illiteracy among users (Niyongere et al., 2015; Muliele et al., 2018; Ndayambaje et al., 2019; Balasha et al., 2023), inadequate governance in the pesticide sector, and possible socio-economic influence from agrochemical companies on officials (Gaberell & Viret, 2020), may explain this chaotic situation in the region.

Table 1-3: Status of some EU banned chemical pesticides in GLCCA.

Class of pesticides	Active ingredients banned in EU	Chemical family	Regulation status				
			Burundi	DRC	Rwanda	Kenya	Tanzania
Insecticides	Acephate	Organophosphorus	R	NI	NI	R	R
	Alpha-cypermethrin	Pyrethroid	R	NI	R	R	R
	Benfuracarb	Carbamate	R	NI	NI	NI	R
	Beta Cyfluthrin	Pyrethroid	R	NI	NI	R	R
	Carbosulfan	Carbamates	R	NI	NI	R	R
	Chlorfenapyr	Pyrrole	NI	NI	R	R	R
	Chlorpyrifos	Organophosphorus	R	R	R	R	R
	Diafenthiuron	Thioureas	NI	NI	R	R	R
	Diazinon	Organophosphorus	R	B	B	R	R
	Dichlorvos	Organophosphorus	R	BU	B	R	R
	Dimethoate	Organophosphorus	R	R	B	R	R
	Endosulfan	Organochlorine	B	BU	B	B	R
	Fenbutatin oxide	Organometallics	NI	NI	R	NI	NI
	Fenitrothion	Organophosphorus	R	R	NI	R	R
	Fenthion	Organophosphorus	R	NI	B	R	R
	Fenvalerate	Pyrethroids	R	NI	NI	R	R
	Fipronil	Phenylpyrazole	NI	R	R	R	R
	Flufenoxuron	Benzoylureas	NI	NI	R	NI	R
	Hydramethylnon	Trifluoromethyl aminohydrazone	NI	R	R	R	NI
	Imidacloprid	Neonicotinoid	R	R	R	R	R
	Isoxathion	Organophosphorus	R	NI	NI	NI	NI
	Omethoate	Organophosphorus	R	NI	NI	R	R
	Oxydemeton-methyl	Organophosphorus	R	NI	NI	R	NI
	Permethrin	Pyrethroids	R	NI	R	R	R
	Pirimiphos-methyl	Organophosphorus	R	NI	NI	R	NI
	Profenofos	Organophosphorus	R	NI	R	R	R
	Pymetrozine	Triazine	NI	NI	R	NI	NI

	Tetradifon	Organochlorine	NI	NI	R	R	R
	Tetramethrin	Pyrethroids	NI	R	NI	NI	R
	Thiacloprid	Neonicotinoid	NI	NI	R	R	NI
	Thiamethoxam	Neonicotinoid	NI	NI	R	R	R
	Tralomethrin	Pyrethroids	R	NI	NI	NI	R
	Triazophos	Organophosphorus	R	NI	NI	NI	R
Herbicides	Atrazine	Triazine	R	NI	B	R	R
	Dalapon	Organochlorine	R	NI	R	NI	NI
	Hexazinone	Triazine	R	NI	NI	R	R
	Imazapyr	Imidazolinone	NI	NI	R	R	R
	Linuron	Methylureas	NI	NI	R	R	NI
	Oxadiazon	Oxadiazole	NI	R	NI	NI	R
	Paraquat	Pyridine	R	BU	B	R	R
	Propanil	Anilide	NI	NI	R	R	R
	Terbutryn	Triazine	NI	NI	R	NI	NI
Fungicides	Benomyl	Carbamate	R	NI	R	BU	R
	Bitertanol	Triazole	NI	NI	R	R	R
	Carbendazim	Carbamate	NI	BU	R	R	R
	Chlorothalonil	Organochlorine	R	BU	R	R	R
	Cyproconazole	Triazole	NI	NI	R	R	R
	Epoxiconazole	Triazole	NI	NI	R	R	R
	Fenamidone	Imidazolinone	NI	NI	R	R	NI
	Fenarimol	Pyrimidine	NI	NI	R	NI	NI
	Iprobenfos	Organophosphorus	R	NI	NI	NI	NI
	Iprodione	Dicarboximides	R	NI	R	R	R
	Mancozeb	Thiocarbamates	R	R	R	R	R
	Pencycuron	Phenylureas	NI	NI	R	R	R
	Propineb	Carbamates	NI	NI	R	R	R
	Triadimenol	Triazole	NI	R	NI	R	R
	Thiram	Thiocarbamate	R	R	R	BU	R
Rodenticides	Bromadiolone	Coumarin	NI	R	NI	NI	R
	Brodifacoum	Coumarin	R	BU	R	NI	R
	Chlorophacinone	Indandione	R	NI	NI	NI	R
	Coumatetralyl	Coumarin	R	NI	R	NI	R

	Diphacinone	Indandione	R	NI	R	NI	R
Insecticide-nematicides	Carbofuran	Carbamates	R	NI	R	B	R

3. Biocontrol as a promising sustainable alternative to the use of chemicals

3.1. Existing non-biological alternative methods to chemicals

The adverse effects of chemical pesticides have prompted the worldwide scientific community and policy makers to search for alternatives and promote the so-called IPM defined as a holistic approach that aims to control plant pests and diseases by mobilizing all existing methods while reducing reliance on chemicals (Stenberg, 2017). IPM promotes the development of non-biological strategies such as prevention, monitoring, and rational use of chemical phytoprotectants products, and the implementation of biological control methods, which will be discussed later.

Good agricultural practices are preventive measures consisting, for example, of the use of certified varieties, crop rotation, field sanitation and the use of balanced fertilization. These practices have been adopted to some extent in the GLCCA region and promising results have been obtained. For example, cassava mosaic disease and maize streak disease have been controlled using resistant varieties (Legg et al., 2006; Nkurunziza et al., 2012). The banana bunchy top disease has recently been reduced via macro- and micropropagation of healthy suckers (Niyongere et al., 2013; Tchatchambe et al., 2019; Paka et al., 2021). The incidence of banana *Xanthomonas* wilt and potato bacterial wilt caused by *R. solani* has been limited via crop rotation, field sanitation, sterilization of farm tools, and avoidance of their exchange (Harahagazwe et al., 2007; Ndayihanzamaso et al., 2016; Uwamahoro et al., 2020). However, despite many attempts involving certified varieties or other agricultural practices, no substantial results have been achieved to control fungal diseases in the region. In addition, implementation of good agricultural practices is hampered by several factors, including demographic pressure on arable land, the relative time required to develop new varieties, the lack of durability of resistance in developed varieties, and the consequences of climate change on the local agro-ecosystems (Pandit et al., 2022).

Phytosanitary products must be used in a rational way, considering the epidemiological factors of the specific pathogens, which are usually obtained from field monitoring. Where chemicals are irreplaceable, careful consideration must be given to the dose applied and the less toxic products must be favored. For example, flupyradifurone has been proposed to replace the insecticide imidacloprid (Maloney et al., 2020) and copper hydroxide in substitution of mancozeb (FPS Health, 2023) in

EU. Unfortunately, this practice is less widespread in the region, where the choice of pesticide is made at random without prior information on the disease status and recommended product for optimal efficiency and less harzardous effect on human health and environment.

3.2. Biological control methods in local agricultural systems

Biological control is an approach to plant protection based on the use of living organisms and/or their derivatives. These include macroorganisms (predatory insects, mites, and nematodes), microbials (beneficial bacteria, fungi, protozoa, viruses, yeasts) and biochemicals (semiochemicals, plant extracts, plant growth regulators and organic acids) (DunhamTrimmer, 2023), as well as the recently promoted exogenous application of double strand RNA (dsRNA) acting via the RNA interference (RNAi) mechanism (Septiani et al., 2025; Zarrabian et al., 2025). Biocontrol products market is gradually increasing, owing to their general eco-friendly and host specificity characteristics (Essiedu et al., 2020). The biocontrol market accounts for 10% of the global pesticide market, with North America leading the way in promoting BCPs, while African share is very insignificant and led by countries like Nigeria, South Africa, and Egypt (Marrone, 2024; Mordor Intelligence, 2025). Biological control is a new paradigm in GLCCA and only seven biocontrol products (3%) are registered up to now (Table S1). While some of the types of biocontrol agents are totally absent like the dsRNA-based products, others have been utilised in the three countries either proposed by officials or derived on the general community-based knowledge.

3.2.1. Macroorganisms

Macroorganisms are either predators or parasitoids of living organisms (insects, mites, or nematodes) used in the form of eggs, larvae, pupae, or adults to kill or parasitize the target pest (Ramalakshmi et al., 2020; DunhamTrimmer, 2023). Insect predators have been successfully used in the region to control some pests in the region. For instance, *Epidinocarpis lopezi* introduced from South America, has been used to control the cassava mealy bug (*Phenacoccus Manihoti*) in the region (Neuenschwander, 2001; Nyabyenda, 2005), while *Gyranusoidea tebygi* and *Anagyrus mangicola* have been used to control the devastating mango mealy bug in Burundi (FAO, 2022). Other common predators such as the green lacewing (*Chrysoperla carnea*), Malaysian ladybird beetle (*Chilocorus nigritus*) and mealybug ladybird (*Cryptolaemus montrouzieri*) have been reported to be effective in controlling the cotton aphid jassid, peanut thrips; sugarcane scales; coffee and mango mealybugs, respectively (Ramalakshmi et al., 2020) and could be introduced locally to control these pests.

3.2.2. Biochemicals

Biochemicals, in the form of plant extracts appear to be the most widely used biocontrol agents in the region, but their use is rudimentary and based on traditional and community-based knowledge. They are derived from various plant species such as *Azadirachta indica*, *Capsicum* spp., *Allium sativum*, *Tephrosia* spp., *Tithonia diversifolia*, *Ricinus communis*, etc. and are used as insecticides to control various pests such as *S. frugiperda* on maize, *Tuta absoluta* on tomato, *Ophiomya phaseoli*,

Aphis fabae on beans, etc. (Korangi Alleluya et al., 2021; MINAGRI-Burundi, 2016; Rutikanga, 2015). In addition, two plant molecules azadirachtin and pyrethrin, and spinosad from actinomycetes have been registered as insecticides in Rwanda (Table S1). However, plant extracts as biopesticides require more space to grow, which would create competition with other staple and cash crops for agricultural land.

3.2.3. Microorganisms

Several microorganisms encompassing bacteria, fungi, and yeasts are commercialized as biocontrol agents. Fungal based agents mainly include the genera *Trichoderma*, *Metarhizium* and *Beauveria*; while yeasts include the genera *Pichia*, *Yarrowia* and *Saccharomyces*. The largest group of worldwide marketed microorganisms are bacteria (up to 75%), dominated by plant growth-promoting rhizobacteria (PGPR) of the genera *Bacillus*, *Pseudomonas* and *Streptomyces* (Saeed et al., 2021; Bonaterra et al., 2022; Dimkić et al., 2022). However, these globally adopted microbial-based alternatives are quite absent in the GLCCA region. The bioinsecticide *B. thuringiensis* is registered in the three countries, while *B. bassiana* and *T. harzanium* are only found in Rwanda.

3.3. Global fate of *Bacillus* over their PGPR counterparts in biocontrol

Plant growth-promoting rhizobacteria are a group of bacteria found mainly in the vicinity of plant roots, the rhizosphere. The plant provides them with nutrients in the form of root exudates, while these bacteria protect the host and promote its growth. They include several species belonging to different genera, namely *Rhizobium*, *Azotobacter*, *Streptomyces*, *Enterobacter*, *Klebsiella*, *Rhodococcus*, *Paenibacillus*, *Variovorax*, *Azosprillum*, *Bulkholderia*, *Serratia*, *Pseudomonas* and *Bacillus* (Caulier et al., 2018). Bacterial strains of the genera *Streptomyces*, *Paenibacillus*, *Pseudomonas* and *Bacillus* have been shown to be effective biocontrol agents against various plant pathogens and pests, with *Bacillus*-based products leading the market (Wang et al., 2021).

Commercialized *Bacillus*-based biocontrol products are largely dominated by the bioinsecticide *B. thuringiensis* (J. Kumar et al., 2021), but there is a growing interest in other *Bacillus* spp. strains with antagonistic potential against plant pathogens and/or with plant growth promotion properties (Etesami et al., 2023a) for a more sustainable and productive agriculture. This attractiveness is also remarkably fueled by their intrinsic ability to form resistant endospores and relative rapid growth on a variety of substrates which facilitate greatly the formulation process (Ajuna et al., 2024). In addition, these Gram-positive bacteria have evolved a remarkable ability to secrete a vast array of bioactive secondary metabolites (BSMs), and their biosynthesis recruits up to 12% of their DNA genomic sequence (Etesami et al., 2023a). *Bacillus*-based biocontrol formulations registered worldwide for the fight of plant diseases and growth stimulation include, but not exhaustively, *B. licheniformis* SB3086 (Ecoguard®, Novozymes®, Biofungicide®, Green Relief®), *B. pumilus* GB34 (GB34®, Concentrated Biological Fungicide®, Ballad®), *B. velezensis* MBI600 (Subtilex®, Histick N/T®), *B. velezensis* GBO3 (Kodiak®, Companion®), *B.*

velezensis QST713 (Serenade®, Rhapsody®) and *B. velezensis* FZB42 (Taegro®, Rhizovital®) (Lahlali et al., 2022; Yadav et al., 2022).

3.4. Possibility of implementing *Bacillus*-based biocontrol in Central Africa

The market for biocontrol agents based on strains of the *B. subtilis* group for the control of plant diseases is growing rapidly around the world but no product based on this bacterium is registered in the region. However, some promising preliminary studies using *B. velezensis* strains to control fungal diseases in Burundi and DRC have demonstrated the potential of these bacteria to to be effective in field conditions (Kulimushi et al., 2018; Nihorimbere et al., 2010), which could pave the way for their possible future dissemination and adoption by local farmers. For instance, *Bacillus velezensis* S499, isolated in Ituri/DRC in 1950s (Delcambe & Devignat, 1957), is effective against *Fusarium* sp. on tomato (65-70% of disease reduction) under field conditions in Burundi (Nihorimbere et al., 2010) and was found active against *Rhizopus stolonifer, Penicillium variable, Fusarium verticillioides* with disease severity reduction of 39-67% (Kulimushi et al., 2018). Another study involving *B. velezensis* GA1 have shown that this bacterium was able to control peanut stem rot disease caused by *Athelia rolfsii*, up to 60% of protection (Korangi Alleluya et al., 2023).

Furthermore, a growing number of studies demonstrates the efficacy of these bacteria in controlling a range of important and widespread phytopathogens reported in Burundi and other tropical countries. For example, *B. altitudinis* and *B. velezensis* were shown to control rice blast caused by *P. oryzae* (15% and 25% of disease reduction, respectively) (Lam et al., 2021), while *B. amyloliquefaciens* NJN-6 was effective to inhibit the mycelial growth of the banana pathogen *F. oxysporum* f.sp. *cubense*, 30-40% (J. Yuan et al., 2012). *B. subtilis* AUBB20 inhibited the growth of the coffee pathogen *F. xylarioides* (Muleta et al., 2007). Antagonistic activities of *B. subtilis* strains have also been reported against several fungi and oomycetes, including *Alternaria linariae* (da Silva Junior et al., 2023), *R. solani* (Al-Mutar et al., 2023), and *P. infestans* (Zhang J. et al., 2023b). These strains are also reported to manage bacterial diseases. For instance, *B. subtilis* and *B. methylotrophicus* DR-08 were found to be effective against *R. solanacearum* (Elazouni et al., 2019; Im et al., 2020). *B. megaterium* USB2103 efficiently controlled common bean bacterial wilt caused by *Xanthomonas axonopodis* pv. *phaseoli* (Giorgio et al., 2016) and the sheath brown rot of rice caused by *P. fuscovaginae* was successfully controlled (76.6%) by *B. amyloliquefaciens* Bk7 (Kakar et al., 2014). Other examples of *Bacillus* spp. with potential applications in the control of fungal and bacterial diseases for crop protection in GLCCA are shown in Table 1-4.

In addition to their efficacy against phytopathogenic fungi and bacteria, *Bacillus* spp. are also recognized for their potential to control nematodes, insects and weeds (Mnif & Ghribi, 2015). For instance, surfactins from *B. velezensis* S499 showed insecticidal activity against the fruit fly *Drosophila melanogaster* (Assié et al., 2002). The wheat rhizosphere *B. altitudinis* D30202 was reported to have putatively

bioherbicidal activity against the grass *Avena fatua* L. owing its genetic ability to secrete bioherbicidal metabolites (Ma et al., 2023). Several *Bacillus* strains such as *B. altitudinis*, *B. pumilis*, *B. velezensis*, *B. mojavensis*, etc. have been reported to exhibit nematicidal activity against two threatening agricultural nematodes including *Meloidogyne incognita* and *Heterodera glycines* (Dalvan do Nascimento et al., 2022; Guimarães Pacifico et al., 2021; Ye et al., 2022). Cell-free culture supernatants and volatiles of *B. amyloliquefaciens* BV03, PTA4838 and *B. velezensis* MBI600 killed more than 85% of *Helicotylenchus dihystera* infesting soybean (Camatti et al., 2023). So even if very few products have been developed to the market scale so far, the results already available are promising for the design of bionematicides, bioinsecticides and bioherbicides based on *Bacillus*.

Table 1-4: Some global success stories of strains of the *Bacillus subtilis* clade in biocontrol of important crop pathogens reported in the GLCCA.

***Bacillus* strains**	**Plant**	**Pathogen**	**Experiment condition**	**Biocontrol potential**	**References**
B. velezensis NJN-6	Banana	*Fusarium oxysporum* f.sp. *cubense*	*In vitro*	30-40% of *Foc* inhibition compared to control	(Yuan et al., 2012)
B. velezensis EB1			*In vitro, in greenhouse*	75.43% inhibition rate	(Xiang et al., 2023)
B. amyloliquefaciens W19			*In vitro, in greenhouse*	21% inihibition rate	(Wang et al., 2013)
B. siamensis Gxun-6			*In vitro, in greenhouse*	>68.8% inhibition rate; >88.26% biocontrol efficacy	(N. Shen et al., 2022)
B. pumilus CCIBP-C5		*Mycosphaerella. fijiensis*	*In vitro, in greenhouse*	45% inhibition rate; 33.6% biocontrol efficacy	(Di Francesco et al., 2017)
B. licheniformis, B. siamensis, B. subtilis subsp. *Inaquosorum*			*In greenhouse*	ns	(Marcano et al., 2016)
B. velezensis QST713	Bean	*Xanthomonas axonopodis* pv. *phaseoli*	*In vivo*	52.26% disease incidence reduction	(Belete et al., 2021)
B. pumilus		*Sclerotium rolfsii*	*In greenhouse*	26% disease incidence reduction	(Pleban et al., 1995)

B. velezensis MBI600, *B. velezensis* FZB42, *B. velezensis* QST713		*Colletotrichum lindemuthianum*	*In greenhouse*	21.9%,15.2%, 10.9% diseased plants respectively	(Tinivella et al., 2009)
B. velezensis GBO3	Cassava	*F. solani*	*In vitro, in greenhouse*	Approx. 10mm inhibition zone; 0% disease incidence (asymptomatic plants)	(Freitas et al., 2019)
B. subtilis ME9		*X. phaseoli pv. manihotis*	*In vitro*	Approx. 14 mm inhibition zone diameter	(Y. Feng et al., 2023)
B. subtilis AUBB20	Coffea	*F. xylarioides*	*In vitro*	Approx. 30-70 mm inhibition zone	(Muleta et al., 2007)
B. pumilus	Cotton	*Rhizoctonia solani*	*In greenhouse*	56% disease incidence reduction	(Pleban et al., 1995)
B. velezensis SQR9	Cucumber	*F. oxysporum* f.sp. *cucumerinum*	*In vitro, in greenhouse*	49-61% disease reduction compared to control	(Y. Cao et al., 2011)
*B. subtilis Y*B-04			*In vitro, in greenhouse*	>90% control efficacy	(W. Xu et al., 2022)
B. subtilis TM4	Maize	*F. verticolloïdes*	*In field*	<9% disease incidence	(Mirsam et al., 2021)
B. licheniformis	Mango	*C. gloeosporioides*	*In vivo* (on harvested fruits)	30-40% disease incidence	(Govender et al., 2005)

B. safensis C3	Mung bean	*X. axonopodis P. syringae*	*In vitro*	ns	(Romero-Severson et al., 2021)
B. subtilis BsW4, Bs76, *B. amyloliquefaciens* Ba100	Pea	*Ascochyta pinodes*	*In vitro, in greenhouse* and *in field*	>65% biocontrol efficacy	(N. Liu et al., 2016)
B. velezensis TN-TB4, *B. amyloliquefaciens* TN-TB6	Peanut	*Cercospora arachidicola*	*In vitro*	>55% inhibition rate	(Thanh & Yen, 2023)
B. subtilis G1		*Macrophomina phaseolina*	*In greenhouse, in field*	15-20% disease incidence	(Shifa et al., 2018)
B. velezensis LHSB1		*S. rolfsii*	*In vitro, in greenhouse*	93.8% inhibition rate, 62.6–70.8% biocontrol efficacy	(Chen L. et al., 2019)
B. velezensis 6-5	Potato	*Phytophtora infestans*	*In vitro*	>90% inhibition rate	(Zhang J. et al., 2023a)
B. amyloliquefaciens Bk7	Rice	*P. fuscovaginae*	*In vitro, in greenhouse*	93% inhibition rate, 76.6% biocontrol efficacy	(Kakar et al., 2014)
B. subtilis PTS-394	Pepper	*F. solani*	*In vitro, in greenhouse* and *in field*	69.63% biocontrol efficacy; 74.43% biocontrol efficacy	(Qiao et al., 2023)
B. subtilis 168		*Ralstonia solanacearum*	*In vitro*	ns	(Yi et al., 2016a)

B. subtilis HSY21	Soybean	*F. oxysporum*	*In vitro, in greenhouse* and *in field*	81.30 % inhibition rate; 63.83% control effect; 57.07% control effect	(Han S. et al., 2021)
B. amyloliquefaciens Q-426	Spinach	*F. oxysporum* f.sp *spinaciae*	*In vitro*	Approx. 28 mm inhibition zone diameter	(P. Zhao et al., 2014)
B. subtilis CAS15	Sweet pepper	*F. oxysporum* f.sp *capsici*	*In greenhouse*	Reduction of the disease incidence by 12.5 to 56.9%	(X. Yu et al., 2011)
B. megaterium TRS-4	Tea	*Fomes lamaoensis, Sphaerostilbe repens, Poria hypobrumea, S. rolfsii*	*In vitro, in greenhouse*	55–84% inhibition rate	(U. Chakraborty et al., 2006)
B. amyloliquefaciens XJ5	Tomato	*Alternaria solani*	*In vitro*	82.5% inhibition rate	(Mu et al., 2023)
B. velezensis K01		*Botrytis cinerea*	*In vitro , in vivo* (on detached leaves and harvested fruits)	84.1% inhibition rate, >80% disease reduction, >78% disease reduction	(Y. Xue et al., 2023)
B. subtilis EPC016		*F. oxysporum* f.sp. *lycopersici*	*In vitro , in greenhouse*	46.04% to 60.78% inhibition rate	(Ramyabharathi and Raguchander, 2014)

B. velezensis UQ9000N		*F. oxysporum* f.sp. *lycopersici*, *M. phaseolina*	*In vitro*	52 % to 56% inhibition rate	(Arkhipov et al., 2023)
B. velezensis FJAT-46737		*R. solanacearum*	*In vitro, in greenhouse*	Approx. 82.0% biocontrol efficiency	(Chen et al., 2020))
Bacillus amyloliquefaciens FJAT-2349			*In greenhouse*	97.6% biocontrol efficiency	(Chen M. et al., 2019)
B. amyloliquefaciens PKM16		*S. sclerotiorum*	*In vitro, in greenhouse*	40.27% inhibition rate	(Do Prado Mattos et al., 2023)
B. subtilis IJ10	Turmeric	*F. solani, Pythium aphanidermatum*	*In vitro, in greenhouse*	>45% inhibition rate, >60% biocontrol efficacy	(Kharshandi and Kayang, 2023)

ns : not specified

4. Mechanisms of *Bacillus*-mediated biocontrol of plant diseases

4.1. *Bacillus* phylogeny

Bacillus is a rod-shaped, Gram-positive and spore-forming bacterium that is widespread in a variety of ecological niches (Q. J. Yin et al., 2023). This genus belonging to the phylum Bacillota, class Bacilli and the family Bacillaceae was first established in 1872 by Cohn and now comprises more than 110 species with validly published names (Oren, 2024; Parte et al., 2020). Phylogenetically, *Bacillus* species are divided into two clades, namely the *Bacillus cereus* clade and *Bacillus subtilis* clade. *B. cereus* clade includes the well-known and most commercialized bioinsecticide *B. thuringiensis*, the human and animal pathogen *B. anthracis* and the food spoiler, opportunistic pathogen *B. cereus*. On the other side, *B. subtilis* clade comprises most of *Bacillus* strains marketed for the biocontrol of microbial pathogens i.e. *B. subtilis*, *B. pumilus*, *B. licheniformis*, *B. nakamurai, B. siamensis, B. amyloliquefaciens*, *B. velezensis*, etc. (Dunlap, 2019; Nikolaidis et al., 2022). Thanks to the advancements of molecular-based techniques and discovery of new marker genes (gyrase A, gyrase B, RNA polymerase subunit B) providing better discrimination between closely related species than the classic 16S rRNA sequencing, species belonging of the *B. subtilis* clade have been clustered into different operational groups. These include the *B. subtilis* group, *B. licheniformis* group, *B. pumilus* group and *B. amyloliquefaciens* group; and the latter gathers the majority of commercial biocontrol agents against phytopathogens i.e. *B. amyloliquefaciens*, *B. velezensis*, *B. nakamurai* and *B. siamensis* (Dobrzyński et al., 2022; Ngalimat et al., 2021; S.-Y. Wang et al., 2022; X. Xu & Kovács, 2024).

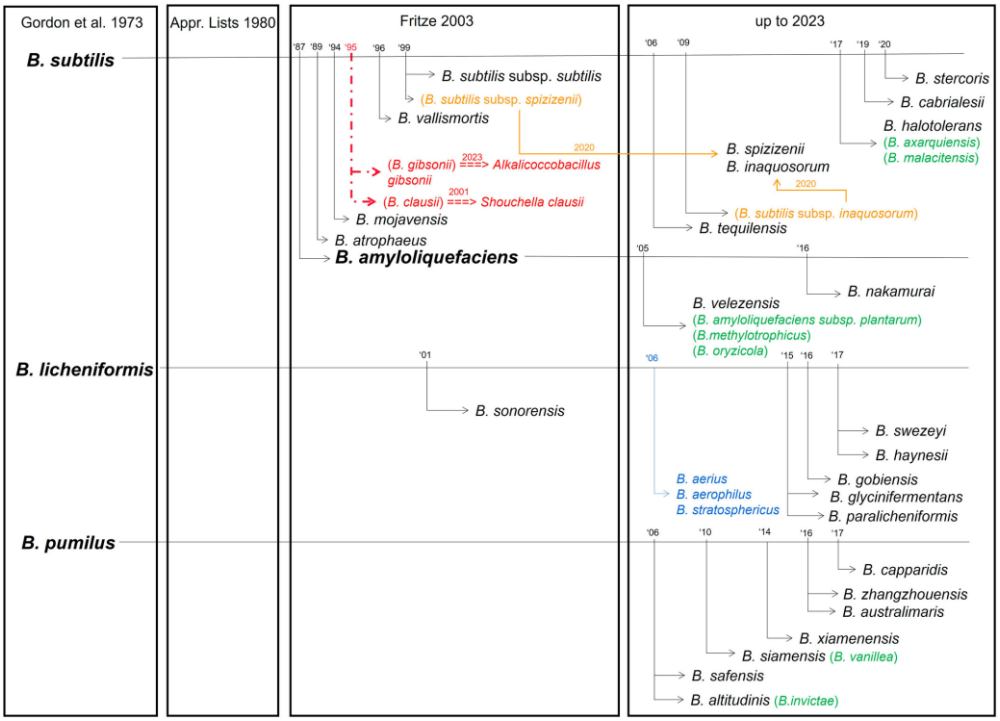

Figure 1-2: Phylogenetic evolution of strains within the *B. subtilis* clade. Strains are classified into sub-groups following their closely relatedness and listed all along the timeline of their publication date. Blue, green, red, orange and black colours denote respectively rejected species names, heterotypic synonyms, re-classified species to other genera, promoted strains to species status and accepted species names by the International Code of Nomenclature of Prokaryotes (ICNP) (X. Xu & Kovács, 2024).

4.2. Biochemical-based weaponry of *Bacillus* into its ecological niche

4.2.1. Hydrolytic enzymes

Strains of the *B. subtilis* complex produce a vast array of lytic enzymes such as glucanases (*bglACHPS*, *yckE)*, cellulases (*licABCHR*), chitosanases (*csn*), amylase (*amyE*), xylanases (*xynABR*, *xylABC*), pectinases (*pel*, *pelB*), lipases (*lip*, *lipA*, *lipB*, *lipC*, *lipM*), proteases (*aprE*, *bpr, epr*, *vpr*, *nprE*) (Ajuna et al., 2023; Zalila-Kolsi et al., 2023). In batch conditions, they are mostly produced in the late exponential phase and during the stationary phase (Contesini et al., 2018; Kierul et al., 2015). These catabolic enzymes are generally secreted upon the presence of their specific substrates in the growth medium and when the preferred nutrient source is being exhausted. These latter two conditions will define the induction or repression of any of those enzymes. Correlatively, this complex regulatory system involves different master and pleitropic regulators (Table 1-5) mostly expressed in the late log stage and during the maturation stage of bacterial growth, and they include SigA, SigK, SigR, CcpA, TnrA,

DegU, CodY, AbrB, ScoC, ComA, KipR, GerA, Spo0A (Freyre-González et al., 2013; Pedreira et al., 2022). These enzymes are not only essential for the metabolism of various nutrient sources which are often present as complex polymers (Stülke & Hillen, 2000), but also for the global mechanism of self-defence against its natural enemies (fungi, insect, nematodes, etc.) by breaking down their cell wall, proteins and DNA (Panicker & Sayyed, 2022; Saberi Riseh et al., 2024). They are also involved in mutualistic relationships that bacilli establish in its ecological niche, in degrading the plant root pectin for example which guarantees a successful plant root colonisation and intimate cohabitation (Boubsi et al., 2023). Besides, hydrolytic enzymes from *B. subtilis* strains revert many biotechnological applications including in pharmaceutics, agriculture, food industry, bioenergy, cosmetics, textile, paper industry, environmental depollution, etc. owing to their remarkable stability to pH, heat and organic solvents (Danilova & Sharipova, 2020; Fasim et al., 2021; Stülke et al., 2023).

Table 1-5: Different types of hydrolytic exoenzymes from strains of the *B. subtilis* clade and their genetic regulation.

Types enzymes	Enzyme (gene symbol)	Amino acid sequence length	Function	Regulation
Glucanases	6-phospho-β-glucosidase (*bglA*)	479 aa	β-glucoside utilisation	
	endo-1,4-β-glucanase *(bglC*)	499 aa	β-1,4-glucan degradation	*SigA*
	Phospho-β-glucosidase *(bglH*)	469 aa	Salicin utilisation	*SigA*, *CcpA* and *licT*
	β-glucoside permease of the phosphorus transferase system (*bglP*)	609 aa	β-glucoside uptake and phosphorylation, control of LicT activity	*SigA*, *CcpA* and *licT*
	Aryl-phospho-β-glucosidase (*yckE*)	477 aa	Utilization of aryl-glucosides	*SigA*, *CcpA* and *licT*
Cellulases	PTS lichenan transporter subunit IIA (*licA*)	110 aa	lichenan uptake and phosphorylation	*SigA*, *CcpA*, *licR*
	PTS lichenan transporter subunit IIB (*licB*)	102 aa	Lichenan uptake and phosphorylation, control of LicR activity	*SigA*, *CcpA*, *licR*
	PTS lichenan transporter subunit IIC (*licC*)	452 aa	lichenan uptake and phosphorylation	*SigA*, *CcpA*, *licR*

	6-phospho-β-glucosidase *(licH)*	442 aa	Lichenan utilisation	*SigA*, *CcpA*, *licR*
	endo-β-1,3-1,4 glucanase (*bglS*)	242 aa	Lichenan degradation	*SigA*, *CcpA* and *licT*
	Transcriptional activator of *licABCH* (*licR*)	641 aa	Regulation of lichenan utilization	*SigA*
	Transcriptional antiterminator of the *bglP-bglH-yxiE* operon and *bglS* (*licT*)	277 aa	Control of β -glucan and β-glucoside utilisation	*SigA*
Xylanases	Endo-1,4-β-xylanase (*xynA*)	213 aa	Xylan degradation	*SigA*
	xylan β-1,4-xylosidase (*xynB*)	533 aa	Xylan degradation	*SigA*, *CcpA* and *XylR*
	Endo-xylanase (*xynC*)	422 aa	Xylan degradation	*AbrB*
	Arabinoxylan arabinofuranohydrolase (*xynD*)	513 aa	Arabinoxylan degradation	*AbrB*
	β-xyloside permease (*xynP*)	463 aa	Xylan utilisation	*SigA*, *CcpA* and *XylR*
	Xylose isomerase (*xylA*)	445 aa	Utilisation of xylan and xylose	*SigA*, *CcpA* and *XylR*
	Xylulokinase (*xylB*)	499 aa	Utilisation of xylan and xylose	*SigA*, *CcpA* and *XylR*
	Transcriptional repressor of the *xylA-xylB* and *xynP-xynB* operons (*xylR*)	350 aa	Regulation of xylan and xylose utilization	
Amylases	α-amylase (*amyE*)	660 aa	Starch degradation	*SigA*, *CcpA* and *AbrB*
	pullulanase (debranching enzyme) (*amyX*)	718 aa	Starch degradation	*SigD*
Pectinases	Pectate lyase C (*pel*)	420 aa	Degradation of polygalacturonic acid	*TnrA*, *CcpA* and *ComA*
	Pectate lyase (*pelB*)	345 aa	Degradation of polygalacturonic acid	

Lipases	Triacylglycerol lipase (*lip*)	212 aa	Lipid degradation	*AbrB*
	Lipoyl synthase (*lipA*)	298 aa	Synthesis of lipoic acid	
	Extracellular esterase, lipase (*lipB*)	210 aa	Lipid degradation	
	Spore coat phospholipase B (*lipC*)	213 aa	Spore germination	*SigK*, *TnrA*, *KipR*, *GerE*
	Octanoyltransferase (*lipM*)	278 aa	Biosynthesis of lipoic acid	
Proteases	Major extracellular alkaline serine protease (*aprE*)	381 aa	Protein degradation	*SinR*, *ScoC*, *AbrB*, *DegU*, *CodY*, *SigA*
	Bacillopeptidase F (*bpr*)	1433 aa	Protein degradation	*DegU*
	Minor extracellular serine protease (*epr*)	645 aa	Protein degradation	*SinR*, *ScoC*, *Spo0A*, *SigD*, DegU
	Extracellular neutral protease B (*nprE*)	521 aa	Degradation of proteins	ScoC, AbrB, *CodY*
	Extracellular metalloprotease (*mpr*)	313 aa	Protein degradation	*CodY*

4.2.2. Biofilm matrix polymers

Matrix polymers are biosynthesised by all biofilm-forming microorganisms and consist, in the case of *B. subtilis*, mainly in the exopolysaccharides Eps (*epsABCDEFGHIJKLMNO* operon), the protein fibers TasA (*tapA-sipW-tasA*), the hydrophobin-like protein BslA (*bslA* gene), and also extracellular DNA (eDNA), poly-γ-glutamic acids, lipids and some mineral deposits like calcium (Arnaouteli et al., 2021; Pomerleau et al., 2024). This interconnected, tri-dimensional polymer network is a prerequisite for biofilm development, where it sticks together and encloses in a shelter-like way the complex multicellular community constituting the biofilm (Berlanga-Clavero et al., 2022; Nordgaard et al., 2022). Within this complex structure, bacterial population evolves on a spatio-temporal basis into different subpopulations with specific tasks i.e. extracellular matrix producing cells, surfactin producing cells, protease producing cells, flagellated motile cells, competent cells, sporulating cells and those involved in cannibalism (Azulay et al., 2022; Yannarell et al., 2023).

The biosynthesis of these matrix polymers is mediated by a synergistic network of regulatory proteins including Spo0A, AbrB, AbbA, SinR, SinI, SlrR and RemA, governing hence the transition from motile planktonic cells to biofilm sessile cells through two main schemes (Milton & Cavanagh, 2023). The main route of synthesis

of matrix polymers and thus the biofilm is initiated by the external signal-triggered phosphorylation of the global regulator Spo0A which, in turn, represses the transcriptional regulator genes AbrB and SinR, while upregulating their anti-repressors genes AbbA and sinI (Figure 1-3). The inactivation of AbrB and SinR by the formation of the complexes AbbA:AbrB and SinI:SinR results in the activation of the proteins RemA and SlrR; which further activate the matrix genes (Figure 1-3). SinR and AbrB are normally the repressors of biofilm matrix genes in motile planktonic cells (Pomerleau et al., 2024; R. Wu et al., 2024). Another route with moderate impact on matrix genes is initiated by an unknown signal which inhibits the protein YwcC with a natural repressive activity of the SlrA, leading to the turning on of the latter protein. SlrA has mild affinity to bind to SinR, resulting in the formation of the complex SlrA:SinR. This inhibition of SinR by the SlrA (SlrA:SinR) will hence permit the increase of SlrR pool and thus the activation of matrix polymers genes (Figure 1-3), while also repressing the expression of cell chaining and motility genes (Bremer et al., 2022; Newman & Lewis, 2013).

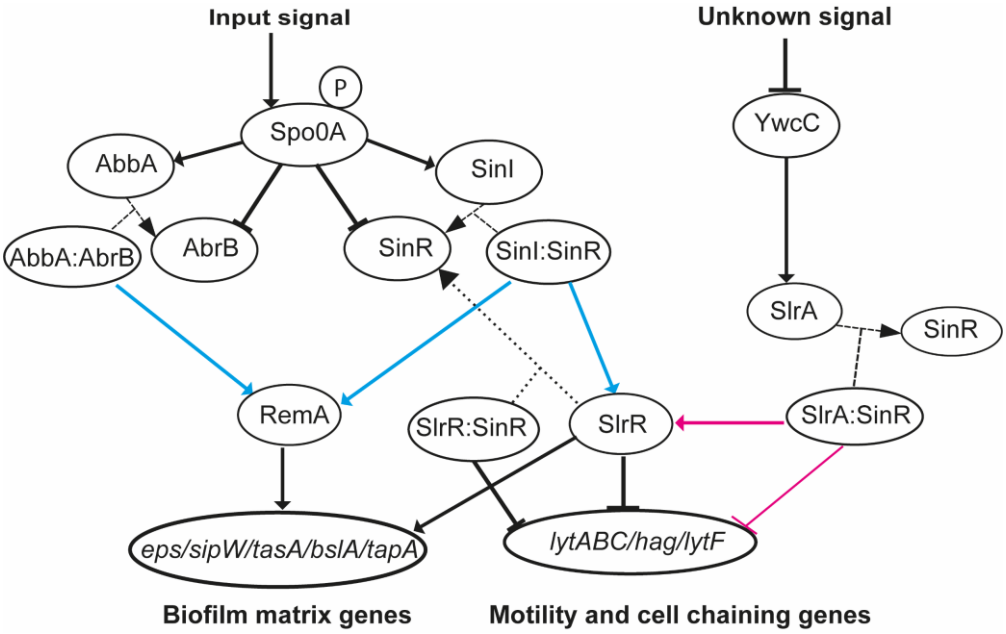

Figure 1-3: Regulation of the biofilm matrix genes in *B. subtilis* (adapted from Milton & Cavanagh, 2023). Ovals denote a protein or a complex of proteins, while the P in circle adjacent to Spo0A imply its phosphorylation. Direct activation of the protein is illustrated by black arrows, while repression is indicated by a T-bar. Blue arrows show an indirect induction of protein expression, while the pink ones represent the secondary and not consistent (with moderate intensity) route of regulation of matrix genes and motility/cell chaining genes. Dashed arrows indicate the direction of sequestration leading to the formation of inactive protein complex, which is represented aside linked by a dashed bar.

The different components of the matrix have developed their own structure organisation that is beneficial at end to the overall structure of the biofilm. For instance, Eps are responsible of the hydrogel-like aspect of the matrix, BslA for creating hydrophobicity, whereas TasA aggregates into strong fibres (Y. Xue et al., 2024). The cation Ca^{2+} participates in structure stability reinforcement in conjunction with Eps and the remaining components lipids and nucleic acids intervene in biofilm biosynthesis processes, adhesion, structural integrity and other biological functions (Keren-Paz et al., 2022; Panlilio & Rice, 2021; Y. Xue et al., 2024). The differentiation in molecular expression of the genes involved in matrix production in *B. subtilis* has also a direct impact on the biofilm architecture (Azulay et al., 2022; S. Liu et al., 2022). *B. subtilis* mutant strain depleted in matrix production was not only unable to form wrinkles that are known as highway facilitating water and nutrients transport but also were more susceptible to antibiotics (Trejo et al., 2013).

4.2.3. Bioactive secondary metabolites

Strains of the *B. subtilis* clade, especially those of the *B. amyloliquefaciens* complex are excellent factories of an arsenal of BSMs, for which secretion involves up to 12% of their entire genomic DNA sequences (Fan et al., 2018). The biosynthesis of these compounds is a complex task executed via two main biosynthesis pathways. First, some are secreted via the non-ribosomal route by the conjunction or not of two giant multi-modular enzyme assemblies i.e. non-ribosomal peptide synthetases (NRPS) and polyketide synthases (PKS) yielding the non-ribosomal peptides (NRPs). Secondly, other metabolites are produced ribosomally and followed by enzymatic post-translational modifications giving rise to a class of biomolecules called ribosomally produced and post-translationally modified peptides (RiPPs) (S. Iqbal et al., 2023). In addition, *B. subtilis* strains secrete a large panoply of bioactive volatile organic compounds, which expand its chemical warfare and thus, the spectrum of its ecological functions (Kai, 2020; Poulaki & Tjamos, 2023).

4.2.3.1. Non-ribosomal peptides

The building machinery of the synthesis of NRPs rely on the multi-modular complex enzymes NRPS and/or PKS, themselves organized into multiple domains, following the colinearity rule (X.-H. Chen et al., 2009). These mega-enzymes necessitate, for their proper functioning, the services of the 4′-phosphopantetheinyl transferase (sfp) that converts the peptidyl carrier protein (PCP) or acyl carrier protein (ACP) from inactive apo-form to the active holo-form by transferring the 4′-phosphopantetheine (prosthetic group) from coenzyme A to the highly conserved serine residue in both PCP or ACP of each module (Figure 1-4) (Aleti et al., 2015; Mofid et al., 2004; J. Wang et al., 2024).

Each elongation cycle (i.e. per module) for NRPS suggests the coordination of three main domains (Figure 1-4) consisting (1) adenylation domain (A domain) that binds selectively to the appropriate amino acid and activates it in the form of adenylated aminoacyl, (2) holo-PCP domain (T domain) to which the adenylated aminoacyl is transferred and linked through a thioester bond, (3) the condensation domain (C

domain) that incorporates the new building block (amino acid) to the growing peptide via a peptidyl bond. For PKSs, each module is equipped with (1) acyl transferase domain (AT domain) responsible for activating the initial building block malonyl-CoA and then transferring it to the next domain, (2) the sfp-activated acyl carrier protein domain (ACP domain) to which binds the activated malonyl-CoA, (3) the ketosynthase (KT) domain responsible for the decarboxylation and condensation reactions between the ACP-linked molonates. After the elongation of the amino acid chain or acyl chain is ended, the thioesterase domain (TE) of the last module releases the mature peptide from the enzymatic complex and is responsible for the possible structure cyclisation (Pedersen et al., 2022; Théatre et al., 2022). Many additional domains interplay occasionally in the biosynthesis of non-ribosomal peptides modifying their core structures and these include the dehydratase (DH), ketoreductase (KR), methyltransferase (MT), enoylreductase (ER), and epimerisation (E) domains responsible for the generation of double bonds, hydroxyl groups, for methyl substitution on carbon skeleton, for the double bond reduction and for the conversion of L-isomer into D- isomer, respectively (Aleti et al., 2015; Mohan et al., 2024).

Non ribosomal metabolites can be classified based on the biosynthesis pathway and they encompass the three families of cyclic lipopeptides enzymatically produced by the NRPS (surfactin and fengycin) or the hybrid NRPS/PKS (iturin), the three families of polyketides enzymatically engineered either by the sole PKS (difficidin and macrolactin) or the hybrid PKS/NRPS (bacillaene) and the siderophore bacillibactin produced via the NRPS complex (Puan et al., 2023; Timofeeva et al., 2022; R. Yang et al., 2020). Beside these strictly non-ribosomally produced peptides, *B. subtilis* strains secrete a dipeptide antibiotic called bacilysin (with its chlorinated form chlorotetain) via a sfp-independent non-ribosomal pathway (Nannan et al., 2021; Özcengiz & Öğülür, 2015).

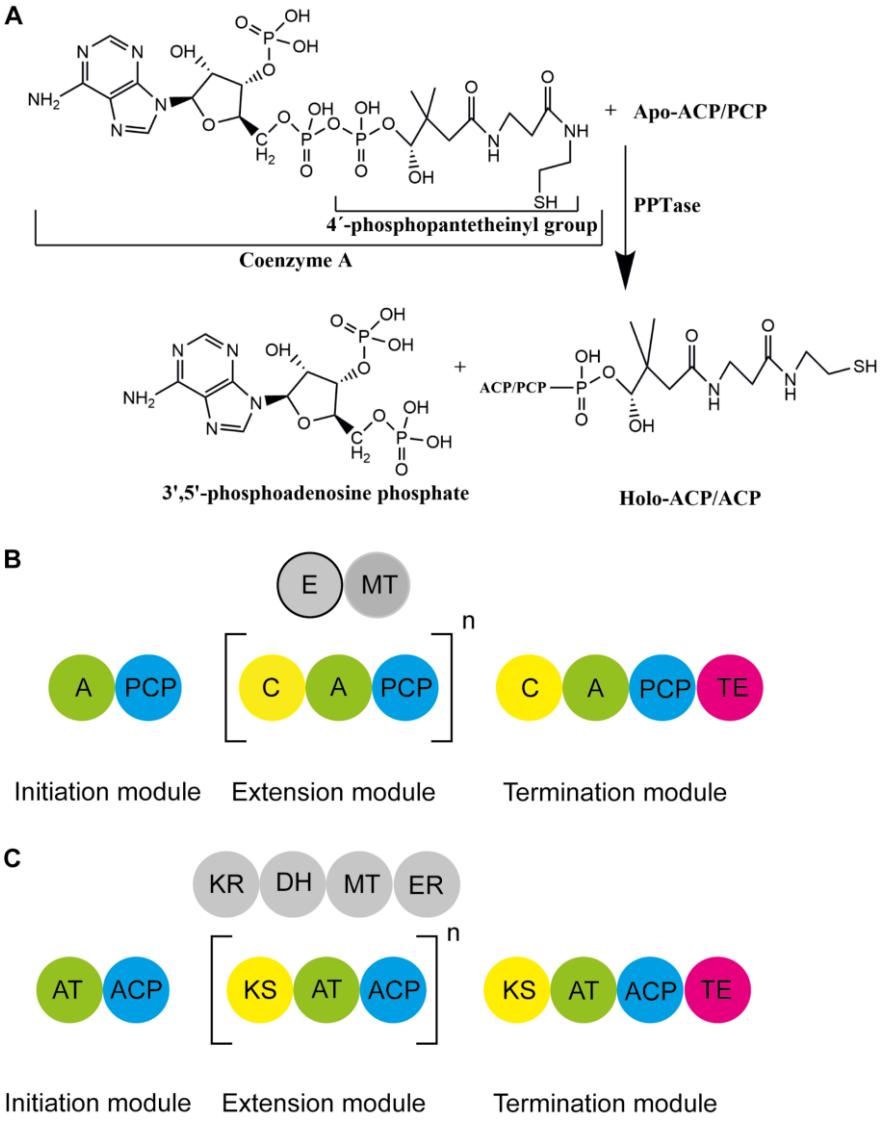

Figure 1-4: Organization of the NRPS and PKS enzymatic machinery for *B. subtilis*. **A.** Phosphopantetheinyl transferase (PPtase)-mediated activation of the thiolation domain (ACP or PCP). **B.** NRPS enzymatic assembly: Core domains-Adenylation (A), Peptidyl Carrier Protein (PCP), Condensation (C) and Thioesterase (TE) domains, and auxiliary domains-Epimerase (E) and Methyl Transferase (MT) domains. **C.** PKS enzymatic assembly. Core domains-acyltransferase (AT), acyl carrier peptide (ACP), and ketosynthase (KS), thioesterase (TE) and auxiliary domains- ketoreductase (KR), dehydratase (KR), methyltransferase (MT) and enoyl reductase (ER). The letter n in superscript for the elongation module implies that this process is repeated several times depending on the type of metabolite (Aleti et al., 2015; Mohan et al., 2024; Pedersen et al., 2022).

4.2.3.1.1. The siderophore bacillibactin

Bacillibactin is a catecholate siderophore produced by most *B. subtilis* strains under iron (Fe III) starvation conditions. In optimal conditions of *Bacillus* growth (pH 7), Fe (III) is insoluble, and these bacteria evolved an adaptation enabling its uptake by secreting bacillibactin with excellent chelation ability (A. Khan et al., 2016). This *fur*-regulated metabolite is a cyclic trimeric ester consisting in three linked units of glycine-threonine-2,3-dihydroxybenzoate (DHB) (Pi & Helmann, 2017). The iron chelation property of bacillibactin relies on its DHB unit with a high iron affinity ($K_f = 10^{47.6}$) (Dertz et al., 2006). Bacillibactin is a by-product of the multi-modular NRPS enzymatic complex, that is encoded by the *dhbABCEF* operon (Figure 1-5). *dhbC* encodes for isochorismate synthase, responsible for the conversion of chorismate to isochorismate, *dhbB* directs the synthesis of isochorismate lyase that transforms isochorismate into 2,3-dihydro-2,3-DHB, *dhbA* gene is for 2,3-dihydro-2,3-DHB dehydrogenase that yields 2,3-DHB by reduction. The *dhbE* encodes for the 2,3-DHB-AMP ligase which activates the 2,3-DHB by adenylation and *dhbF* ORF stands for the NRPS bacillibactin synthetase with two modules, in which the activated adenylated 2,3-DHB binds to the sfp-activated PCP followed by the sequential reactions leading to the synthesis of glycine and threonine. It also supports the trimerization of the three motifs and the cyclization of the whole structure (Dunyashev et al., 2021; A. Khan et al., 2016).

The biosynthesis of this catecholate-siderophore is under the pleiotropic regulator *fur* and the uptake and internalization of Fe (III)-bacillibactin involves the specific FeuABC membrane-bound transporter, assisted by the complex YusV/ATPase for energy supply. A special protein trilactone hydrolase, YuiL (*basA*), liberates the Fe (III) by hydrolysis and reduce it to Fe (II) form, assimilable by the cell (Miethke et al., 2006; Pi & Helmann, 2017). Besides this *Fur*-regulated ferric sequestrating compound, an alternative *Fur*-independent pathway of iron acquisition under iron depleted conditions does exist and involves the elemental Fe (II/III) acquisition factor (EfeUOB) (M. E. Roy & Griffith, 2017).

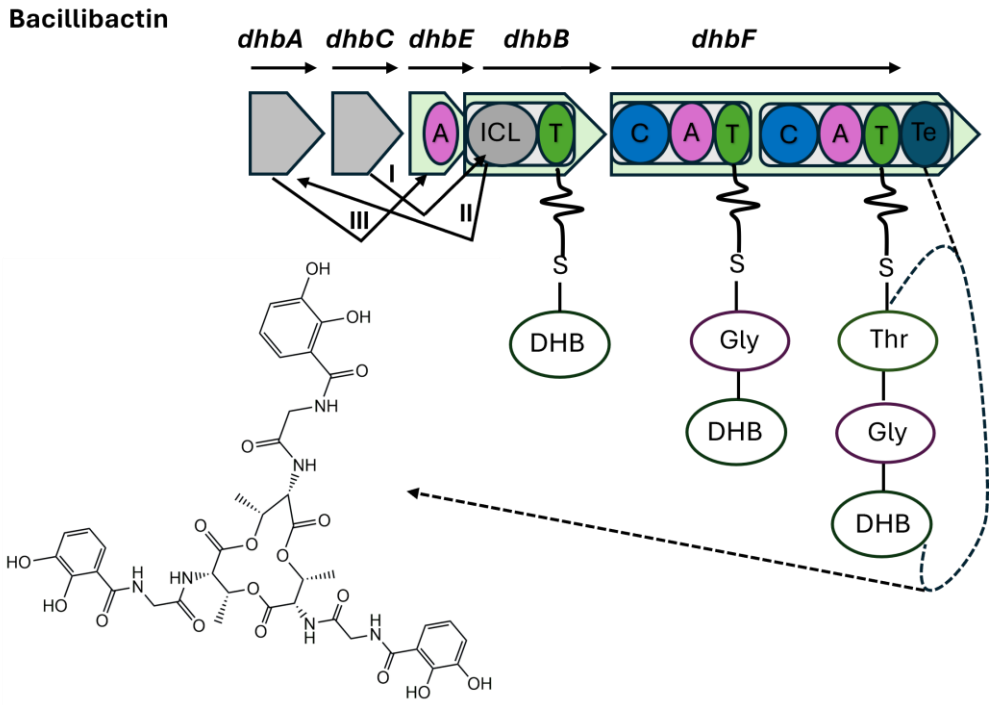

Figure 1-5: Biosynthetic pathway of bacillibactin. A, C, T and Te denote respectively the adenylation, condensation, PCP and thioesterase domains, while ICL and DHB stand for isochorismatase lyase and 2,3-dihydroxybenzoate, respectively. I, II and III indicate the order of initial enzymatic reactions leading to the synthesis of 2,3-DHB, respectively.

4.2.3.1.2. Cyclic lipopeptides

Cyclic lipopeptides are the most studied BSMs produced by the strains of the *B. subtilis* clade and these encompass surfactin, iturin and fengycin families (Balleux et al., 2024; Yin et al., 2023). Their co-secretion is mainly restricted to the species *B. velezensis*, while other species of the *B. subtilis* clade will co-produce one or two of these interesting bioactive metabolites (Q. J. Yin et al., 2023). These biomolecules revert an amphiphilic nature, which make them great biosurfactants able to interact with biological matrixes, like cytoplasmic membranes. Indeed, their chemical structure is composed by a hydrophobic region made of fatty acid chain and a hydrophilic region consisting in a peptidyl moiety (N. Ali et al., 2022; Penha et al., 2020). The differences between these chemical families reside in the length of their hydrocarbon chain and the composition of the amino acid sequences of their respective peptide region. In each family, a diversity of canonical structural variants occurs following the variable length of their fatty acid chain or a possible isomerisation (*iso-* or *anteiso-*). Structural variants can result also from the substitution of amino acids within their respective sequences (Z. Wang et al., 2024).

The secretion of these great antibiotics is regulated differentially, but the main master regulators implicated are *codY*, *comA*, *degU* and Spo0A (J. Sun et al., 2021). However, each metabolite biosynthesis maybe influenced by various other regulators such as *comQXP*, *rapC*, *sodA*, *degQ*, *comK*, *abrB*, *rok*, *phorR*/*phoP* for surfactin, while the fengycin production involves *sigA*, *comP*, *degQ*, *phoR*/*phoP* and the polynucleotide phoshorylase (PNPase) and *sigA*, *degQ* for iturin (R. Yang et al., 2020).

(1) Surfactin biosynthesis:

The first family and mostly conserved within the *B. subtilis* clade is surfactin family. Structurally, it is composed by a β-hydroxy fatty acid chain varying from 12 carbons (C_{12}) to 17 carbons (C_{17}) bound to a cyclized peptidyl chain made of seven amino acids consisting in $^{L}Glu_1$-$^{L}Leu_2$-$^{D}Leu_3$-$^{L}Val_4$-$^{L}Asp_5$-$^{D}Leu_6$-$^{L}Leu_7$. The fatty acid part links to the first amino acid Glu by an amide bond, whereas it tethers to the 7th amino acid through a lactone bond. This biosurfactant with implication in cell motility, space colonization and biofilm formation, as well as with anticancer, hemolytic and tension surface lowering properties, is synthetised by the multi-modular NRPS enzymes and is encoded by the *srfA-ABCD* operon (Figure 1-6). The first three successive modules ($^{L}Glu_1$-$^{L}Leu_2$-$^{D}Leu_3$-) are encoded by *srfAA*, while *srfAB* stands for the next three modules (-$^{L}Val_4$-$^{L}Asp_5$-$^{D}Leu_6$-). The gene *srfAC* encodes for the last module (-$^{L}Leu_7$), whilst the last ORF *srfAD* directs the synthesis of a subsidiary thioesterases domain (Figure 1-6).

In addition to the canonical variants (C_{12} to C_{17}) and some isoforms (*iso*- or *anteiso*- of the fatty acid chain), the last amino acid Leu_7 can be substituted by val_7 due to NRPS low specificity and media composition. Surfactin is structurally linked to other cyclic lipopeptides produced by strains of the *B. licheniformis* group and *B. pumilus* group, lichenysin and pumilacidin, respectively. Lichenysin ($^{L}Gln_1$-$^{L}Leu_2$-$^{D}Leu_3$-$^{L}Val_4$-$^{L}Asp_5$-$^{D}Leu_6$-$^{L}Ileu_7$) differs from surfactin by the substitution of $^{L}Glu_1$ by $^{L}Gln_1$ and $^{L}Leu_7$ by $^{L}Ileu_7$ in their amino acid sequences, whereas pumilacidin amino acid sequence contains Leu_4 and Ile_7 instead of val_4 and Leu_7 for surfactin (Figure 1-6).

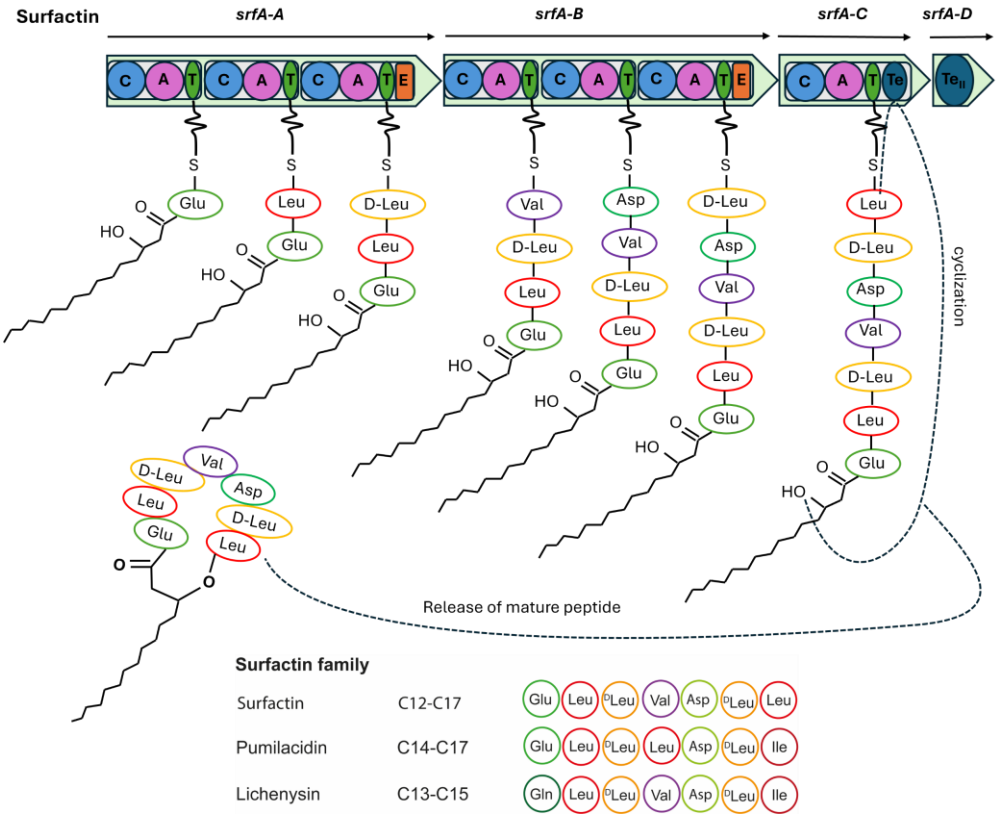

Figure 1-6: Surfactin biosynthesis pathway and its homologues within *B. subtilis* clade. A, C, T, E and Te represent the adenylation, condensation, PCP, epimerase and thioesterase domains respectively.

(2) Fengycin biosynthesis

This second family of cyclic lipopeptide comprises a decapeptide ($^{L}Glu_1$-$^{D}Orn_2$-$^{D}Tyr_3$-$^{D}Thr_4$-$^{L}Glu_5$-$^{D}Ala_6$-$^{L}Pro_7$-$^{L}Gln_8$-$^{D}Tyr_9$-$^{L}Ile_{10}$) amino acid part linked to a β-hydroxy fatty acid chain of varying length (from 14 to 19 carbons), at the first amino acid $^{L}Glu_1$. The cyclization of this NRPS product, known for its great antifungal activity, occurs between the last amino acid $^{L}Ile_{10}$ and the third amino acid $^{D}Tyr_3$ through a lactone bond which results in an octapeptide ring. This cyclic lipopeptide is encoded by the *fenABCDE* biosynthetic gene cluster (BGC), where *fenC*, *fenD* and *fenE* harbour each two modules that control the synthesis of Glu_1-Orn_2, Tyr_3-Thr_4, and Glu_5-Ala_6, respectively. The three next modules (Pro_7, Gln_8, Tyr_9) are encoded by *fenA*, while the last module (Ile_{10}) is encoded by *fenB* (Figure 1-7). Fengycin possesses structural homologues including plipastatin A & B, fengycin A and B and agrastatin (Y. Yin et al., 2024).

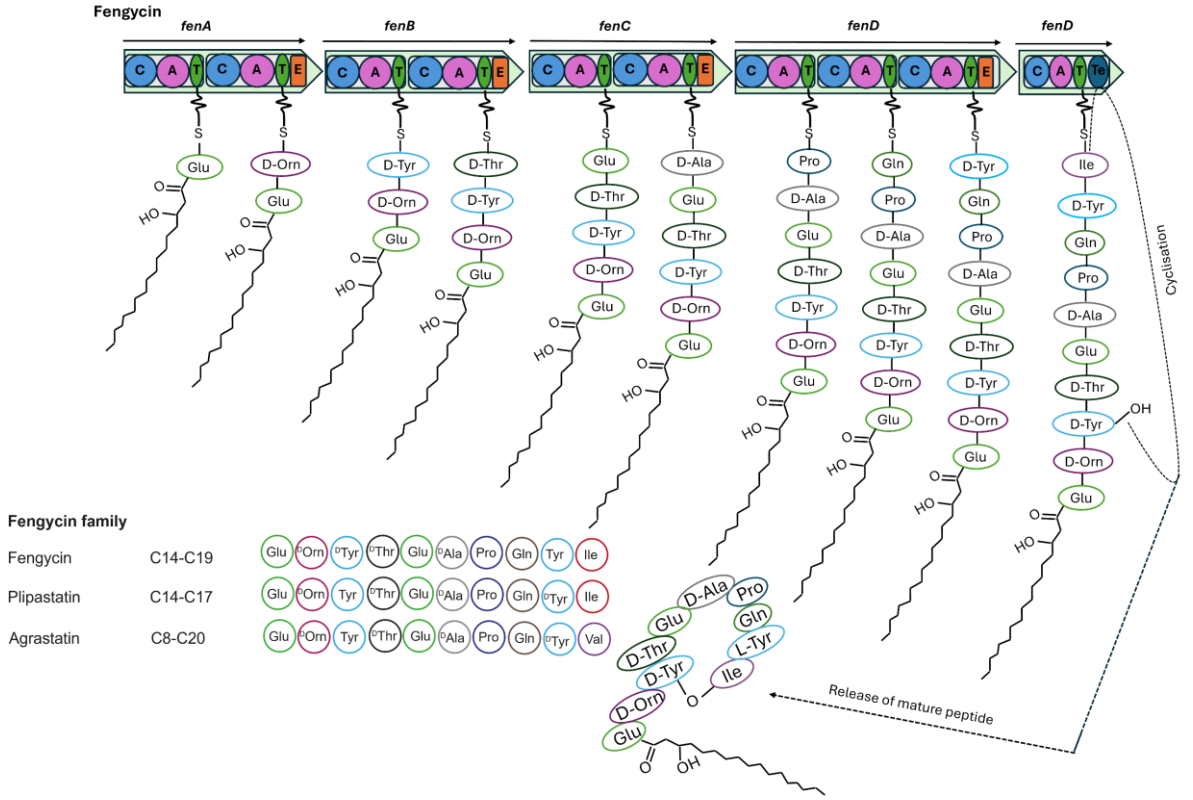

Figure 1-7: Fengycin biosynthesis and the diversity of its homologues within the *B. subtilis* clade. A, C, T, E and Te represent the adenylation, condensation, PCP, epimerase and thioesterase domains respectively

(3) Iturin biosynthesis

The third family of *Bacillus* cyclic lipopeptides, iturins, is a heptapeptide amino acid moiety ($^{L}Asn_1$-$^{D}Tyr_2$-$^{D}Asn_3$-$^{L}Gln_4$-$^{L}Pro_5$-$^{D}Asn_6$-$^{L}Ser_7$) fused to β-amino fatty acid of variable carbon chain (C_{13} to C_{17}) (C. Wan et al., 2022). The macrocyclization of the structure of this hybrid NRPS/PKS bio-product is through two amide bonds between the carbonyl group of the fatty acid and the first amino acid, and between the carbonyl group of the last amino acid and the β-amine group of the fatty acid. The seven modules catalysing the biosynthesis of the different amino acids are encoded by *ituABCD* operon (Figure 1-8). The genes *ituD* and *ituA* encodes PKS modules responsible of lipoinitiation, extension, amination of the fatty acid and attachment to the first amino acid Asn_1 (Figure 1-8), whereas *ituB* dictates the synthesis of Tyr_2, Asn_3, Gln_4 and Pro_5 and *ituC* the biosynthesis of Asn_6 and the last amino acid (Ser_7).

Iturin A, well studied for its antifungal activity, has several structural variants beyond the canonical homologues. Mycosubtilin, produced by some strains with the *B. subtilis* clade, is an isoform of iturin A differing only by a simple inversion between the last two amino acids giving rise -Ser_6-Asn_7. Other variants encompass iturin C, majovensin A and bacillomycin where the amino acid sequence is affected by substitution (Figure 1-8). For instance, the amino acid sequence of bacillomycin, which is produced by the plant-root associated bacteria model *B. velezensis* FZB42, contains four substitutions compared to iturin A, where the last four amino acids are replaced by Pro_4-Glu_5-Ser_6-Thr_7 (Figure 1-8).

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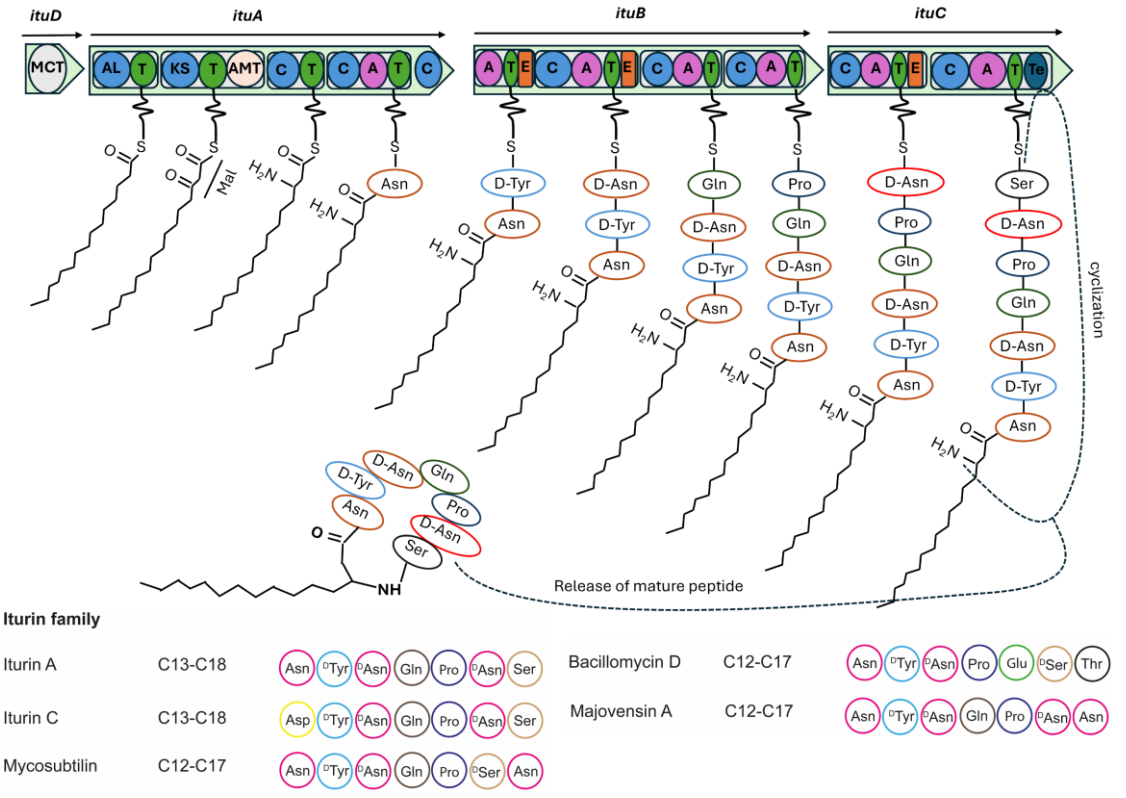


Figure 1-8: Iturin biosynthesis pathway and the diversity of iturinic compounds within the *B. subtilis* group. A, C, T, E and Te represent the adenylation, condensation, PCP, epimerase and thioesterase domains respectively. MCT, AL, AMT and KS stand for malonyl-CoA transacylase, acyl ligase, amino transferase and ketosynthase domains respectively.

4.2.3.1.3. Polyketides

This family of non-ribosomally produced peptides consists in three main chemical families including bacillaene, macrolactin and difficidin. Their biosynthesis is non-iteratively sustained only by the multi-modular *trans*-acyltransferase (AT) PKS enzyme, except the bacillaene which results from the action of the hybrid *trans*-AT PKS/NRPS (Kaspar et al., 2019; Miao et al., 2023).

(1) Bacillaene

Bacillaene is a polyene antibiotic of which synthesis is dictated by the *baeBCDEGHIJLMNRS* and *acpK* operon in *B. velezensis* strains (Fan et al., 2019). This BGC is composed by a set of genes encoding standalone enzymes and multi-modular organized enzymes. These orphan genes include *baeC*, *baeD* and *baeE* encoding for three separate AT (*trans*-malonyltransferase) domains, *acpK* for acyl carrier protein, *baeB and baeG* encoding for hydroxyacylglutathione zinc-dependent hydrolase and 3-hydroxymethylglytaryl-CoA synthase, respectively. *baeH* and *baeI* encode for biosynthesis of enoyl-CoA hydratase, and *baeS* for cytochrome P450, an oxido-reductase converting dihydrobacillaene into bacillaene. Multi-modular NRPS/PKS enzymes involved in the synthesis of bacillaene are encoded by the successive genes *baeJ-baeR* (Figure 1-9), where *baeJ* is for the hybrid NRPS/PKS modules, *baeL* for PKS modules, *baeM* for PKS modules, *baeN* for hybrid NRPS/PKS modules and *baeR* for PKS modules. The nascent bacillaene structure is aminated in the NRPS modules, the first module of *baeJ* and the 12th of *baeN* for the incorporation of glycine and alanine, respectively (Figure 1-9). For the model strain *B. subtilis* 168, there is one additional gene *pksA* (pks is equivalent to bae in *B. velezensis* FZB42) for which the role has not yet clearly understood, though believed to act as TetR transcription regulator (Miao et al., 2023). The transcription regulation of the production of this metabolite is too complex, involving the master regulators *Spo0A*, *AbrB*, *CodY*, *DegU*, *ComA* and *ScoC* (Vargas-Bautista et al., 2014).

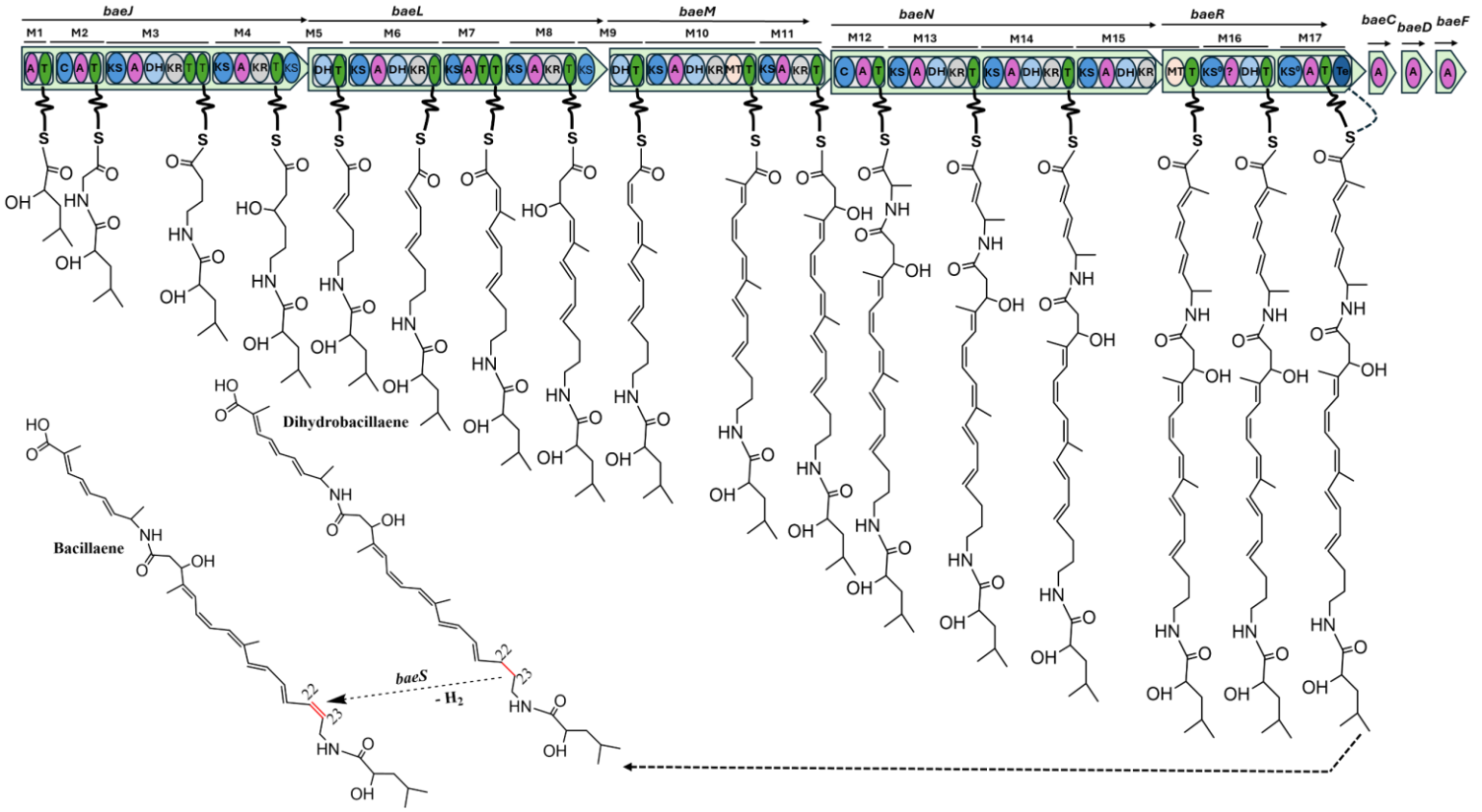


Figure 1-9: Bacillaene biosynthesis pathway. The two first modules M1 and M2 of *baeJ*, and module M12 of *baeN* encode for glycine and alanine amino acids. A, C, KS, T, DH, KR, MT and Te represent the adenylation, condensation (NRPS), ketosynthase, ACP (PKS) or PCP (NRPS), dehydratase, ketoreductase, methyltransferase and thioesterase domains respectively.

(2) Macrolactin

Macrolactin is another family of *Bacillus* polyketides but, which together with difficidin, are products of the exclusive PKS enzymes. The biosynthesis of this 24-membered lactone ring (with three diene group) is genetically dictated by the *mlnABCDEFGHI* operon (Figure 1-10), with the first gene *mlnA* encoding for a discrete *trans*-AT enzyme responsible of further iterative elongation processes that load malonyl-CoA onto the different PKS multi-domain modules *mlnBCDEFGH* (Schneider et al., 2007; T. Wu et al., 2021). The cyclisation of this macrolactin A chain is ensured by the α,β-fold hydrolase, β-ketoacyl synthase and keto-reductase modules encoded in *mlnH* gene. The gene *mlnI* encodes for a β-lactamase responsible of succinylation or malonylation of the core structure at position 7-OH, resulting into 7-O-succinyl-macrolactin A and 7-O-malonyl-macrolactin A variants, respectively (Fan et al., 2019; Mukherjee et al., 2022).

Macrolactin A can undergo other modifications like the glycosylation (catalysed by a separate enzyme GlycT) at position 7-OH, forming the macrolactin B. This glycosylated macrolactin can receive a succinyl group at the sugar moiety, giving birth to macrolactin D (Mukherjee et al., 2022). This antibiotic is active against many pathogenic bacteria by inhibiting protein synthesis (Vasilchenko et al., 2025), as well as against some pathogenic fungi and plant pests (C. Pandey et al., 2023; K. Zhao et al., 2024).

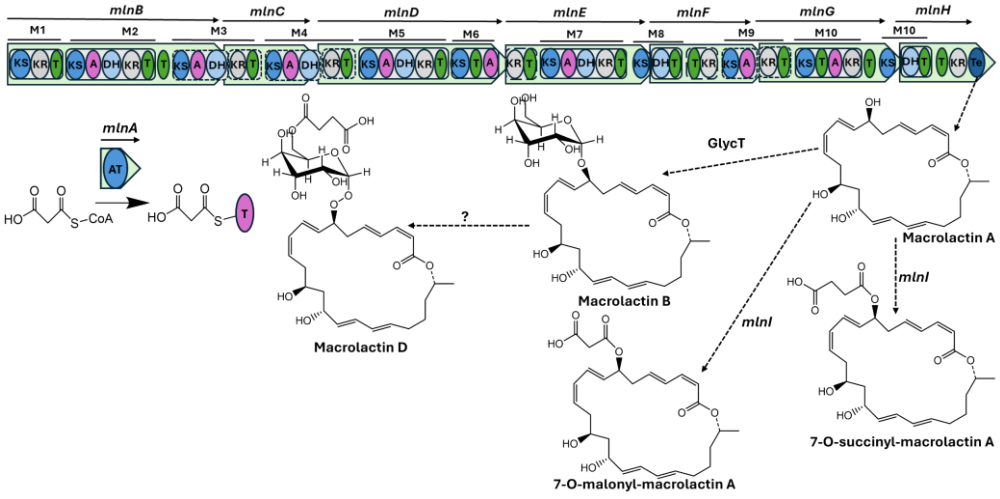


Figure 1-10: Macrolactin biosynthesis pathway. A, KS, T, DH, KR and Te represent the adenylation, ketosynthase, ACP (PKS), dehydratase, ketoreductase and thioesterase domains respectively.

(3) Difficidin

Difficidin is the third family of polyketides secreted by the strains of the *B. subtilis* clade and its biosynthesis is accomplished by the unique complex enzymatic machinery *trans*-AT PKS. This Spo0A regulated-macrocyclic polyene is genetically encoded by the *dfnABCDEFGHIJKLMXY* operon (N. Liu et al., 2023), where the set of genes *dfnDEFGHIJ* encode the multi-domain modular PKS and the remaining are standalone genes responsible for the biosynthesis of enzymes catalysing various initial and post-tailoring reactions. Indeed, *dfnA*, *dfnB*, *dfnX, dfnC, dfnY, dfnL, dfnK and dfnM* encodes modules implicated in the synthesis of ACP *trans*-malonyltransferase, acyl-CoA synthetase, the free-standing ACP, β-ketoacyl-ACP reductase, a D-fructose-6-phosphate amidotransferase, hydroxymethylglutaryl-CoA synthase (HMGS), enoyl-CoA hydratase (EH) and oxydo-redox unit cytochrome P450, respectively (Fan et al., 2019). The *dfnABX* govern the initiation steps involving substrate recognition, activation and incorporation to the ACP domain (*dfnX*) that will interact with the ketosynthase (KS) of the first module (*dfnD*), and thus the growth of the chain by iterative subsequent reactions catalysed by the PKS enzymes (*dfnDEFGHIJ*) (Figure 1-11).

The intermediate reactions ensuring the conversion of a keto function to exomethylene group prior the cyclization of the structure by the TE domain, are catalysed by the HMGS and EH encoded in *dfnL* and *dfnK*, respectively. *dfnY* gene is believed to encode a particular kinase responsible of the phosphorylation of the nascent macrocycle at position-15 to give rise to the mature difficidin. Furthermore, the *dfnM* gene encoding the cytochrome P450 will hydroxylate the difficidin at position-5 to form the oxydifficidin variant (K. Chakraborty et al., 2021; X. H. Chen et al., 2006).This excellent antibiotic active against many pathogenic bacteria, is under the control of the transcriptional regulator Spo0A (K. Chakraborty et al., 2021; N. Liu et al., 2023).

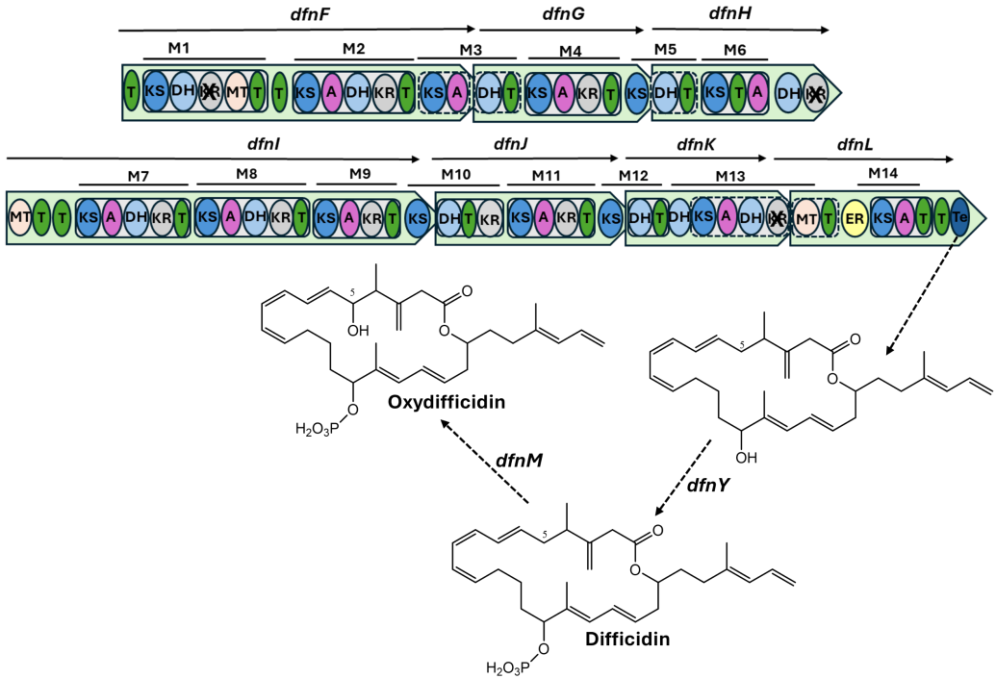


Figure 1-11: Difficidin biosynthesis pathway. A, KS, T, DH, KR, MT, ER and Te represent the adenylation, ketosynthase, ACP, dehydratase, ketoreductase, methyltransferase, enoyl reductase and thioesterase domains, respectively.

4.2.3.1.4. The oligopeptide bacilysin

Bacilysin, along with its chlorinated form chlorotetain, is a small dipeptide ($C_{12}H_{18}N_2O_5$, 270 Da) composed with the fusion of L-alanine at its C-terminus to the N-terminus of the non-proteinogenic amino acid L-anticapsin (Figure 1-12) (Özcengiz & Öğülür, 2015). This oligopeptide is produced by most of the strains within the *B. subtilis* clade in their late maturation stage and death stage, and its biosynthesis is through a particular sfp-independent non-ribosomal pathway (Nannan et al., 2021). The different enzymes involved in its synthesis are encoded by *bacABCDEFG* operon. The initial substrate is the prephenate which is recognized and decarboxylated by *bacA* (decarboxylase) into 7R-*en*-H_2HPP. *bacB* encodes an oxidase and isomerase converting 7R-*en*-H_2HPP into 3E-*ex*-H_2HPP in an equimolar proportion with 3Z-*ex*-H_2HPP. These isomers undergo then an epoxidation, for which the gene is not yet known, to give rise epoxy-3E-*ex*-H_2HPP/epoxy-3Z-*ex*-H_2HPP. An NADH/NAD^+-dependent reductase encoded in *bacG* reduces those stereoisomers to epoxy-4S-H_4HPP, after which a transaminase encoded in *bacF* aminates the epoxy-4S-H_4HPP to yield dihydroanticapsin. *BacC* oxidase the structure by dehydrogenation to bear the anticapsin. A ligase

encoded in *bacD* fuses the L-alanine and L-anticapsin to give rise to mature bacilysin (Figure 1-12). The unique *bacE* not involved in bacilysin synthesis, is a permease that prevent self-suicide by effluxing bacilysin outside the cell (Ertekin et al., 2020; Parker & Walsh, 2013).

This broad antagonistic antibiotic against fungi and bacteria reverts interesting physico-chemical properties, such as the excellent heat-tolerance (15 min at 100°C) and great stability at both acid and alkali pH range (1.4-12, 4h at 20°C) (Nannan et al., 2021; Özcengiz & Alaeddinoglu, 1991). The biosynthesis gene clusters governing the secretion of bacilysin are upon quorum-sensing mediated regulators. The regulators ComQ/ComX, ComP/ComA, *degU* and *Spo0A*, as well as PhrC, *srfA* and *lutR* influence positively the expression of *bac* operon, whereas the pleiotropic regulators *abrB*, *SocC*, *CodY* have a negative impact on *bac* BGC expression (Islam et al., 2022; Özcengiz & Öğülür, 2015). Nevertheless, bacilysin can, in turn, act as a broad signal molecule impacting some cellular processes like colony pigmentation and spore quality. In fact, it was shown that the spore constituent dipicolinic acid was reduced into the spores of bacilysin-mutant strains, resulting in a decrease of spore resistance and germination (Ertekin et al., 2020; Özcengiz & Öğülür, 2015). A recent work pinpoints that bacilysin control the sporulation through the downregulation of CcpA, and that some key metabolic pathways related to quorum sensing, citrate cycle and secondary metabolite synthesis were perturbed in bacilysin-depleted strain (Kutnu et al., 2022).

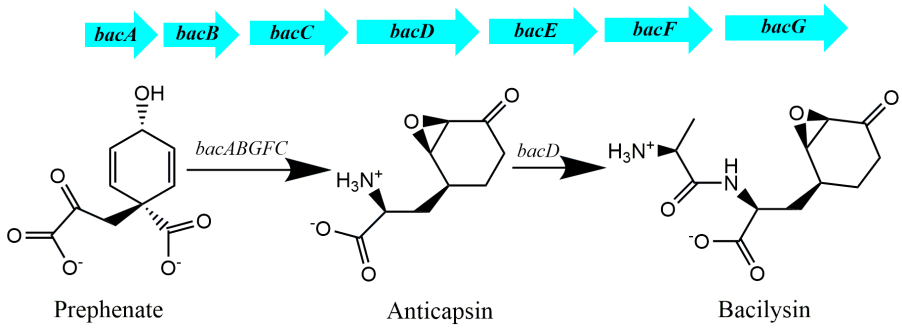


Figure 1-12: Overview of bacilysin biosynthesis. The set of genes *bacABGFC* act successively to yield anticapsin, which is fused later to L-alanine to form bacilysin.

4.2.3.2. Ribosomally produced and post-translationally modified peptides (RiPPs)

This category of bioactive secondary metabolites encompasses ribosomally produced peptides with diverse biological and/or ecological functions, though the initial essence of their production is to antagonize the proliferation of

similar or closely related bacteria strains. Bacteriocins are subdivided into three classes owing mainly to their structural features like the post-translational modifications of the core peptide, N-terminal sequence homology, and/or their physico-chemical properties such as molecular weight, heat and pH stability (Darbandi et al., 2022; Negash & Tsehai, 2020). The first class of bacteriocins include small heat-stable peptides (<5 kDa) which undergo post-translational modifications (PTMs), and they are hence termed RiPPs. The second class comprises heat-stable ribosomal peptides with <10 kDa, which don't contain lanthionine moieties and no post-tailoring enzymatic reactions. Bacteriocin class III includes high molecular weight peptides (>30 kDa) without modifications and are less resistant to heat. Besides these three classes mostly produced by Gram-positive bacteria, Gram-negative bacteria produce also bacteriocins that were categorized into two groups i.e. high molecular weight bacteriocins (30-80 kDa) like colicins produced by *E. coli* and low molecular mass proteins (1-10 kDa) such as the heat, extreme pH and proteases resistant-macrocins secreted by enteric bacteria (Mercado & Olmos, 2022; Negash & Tsehai, 2020). Additionally, other bacteriocins-like substances (BLIS) have been described, but it is still unclear whether they are produced ribosomally or not (Caulier et al., 2019).

Most of the described bacteriocins in the *B. subtilis* clade are the ribosomally produced and post-translationally modified peptides (RiPPs) of the class I. However, the recent reclassification of this complex group, that have taken into consideration the diversity of biosynthetic enzymes supporting the PTM reactions, have expanded this group of RiPPs to novel compounds previously belonging to other class of bacteriocins or not (Montalbán-López et al., 2021; Ongpipattanakul, Desormeaux, Dicaprio, et al., 2022). Noteworthy, assignment of such group of RiPPs only to a particular species or genus of bacteria could be a complicated task since their distribution uncovers Firmicutes, Proteobacteria, Actinobacteria, etc. Henceforth, our focus will be regarded on RiPPs that have been described from *B. subtilis* clade.

The class of RiPPs is an interesting and highly expanding class of compounds which is biosynthesized by the conjunction of multiple enzymes adjacent to the precursor peptide. Notably, this precursor peptide is composed of core peptide on which occur the PTMs and leader and/or follower peptide recognizing and activating PTM enzymes for a better binding affinity, as well as dictating the order of PTM reactions. After the maturation of intended peptide is terminated, the leader and/or follower peptide is cleaved by proteases to liberate the peptide (Eslami & van der Donk, 2024). Actually, RiPPs from the *B. subtilis* clade can be classified into three main large classes including the lanthipeptides and the *Ycao* superfamily enzymes-derived RiPPs, circular RiPPs (head-to-tail cyclized bacteriocins, including some

sactipeptides). However, other RiPPs belonging to other groups have been reported such as the glycocin (glycosylated RiPP), sublancin 168 (Biswas et al., 2021; Paik et al., 1998), an unnamed lasso peptide from *B. licheniformis* (Raphel & Halami, 2024), the epipeptide EpeX (radical S-adenosylmethionine enzyme-dependent) from *B. subtilis* (Kalamara et al., 2023), sublichenin (subtilin-like) from *B. licheniformis* MCC 2512 (Halami, 2019) and the signaling peptide ComX characterized by an indole cyclization and prenylation (Ongpipattanakul, Desormeaux, Dicaprio, et al., 2022).

In addition, some ribosomally produced RiPP-like or bacteriocin-like bioactive compounds with uncommon structures are occasionally reported in *B. subtilis* strains. These include, not exhaustively, LCI from *B. subtilis* active against *X. campestris* and *P. solanacearum* (Gong et al., 2011; Saikia et al., 2019), APC2 from *B. amyloliquefaciens* FS6 with activity against *F. solani* (R. Wang et al., 2021), the antilisterial metabolite BacBS2 from *B. velezensis* (Perumal et al., 2019) and the lactococcin Lcn972 (originally found in *Lactococcus* spp.) from *B. velezensis* HN-Q-8 with antagonistic effect against *Streptomyces scabies* (J. Zhao et al., 2022).

4.2.3.2.1. Lanthipeptides

Lanthipeptides (peptides containing lanthionine moiety) consist in polycyclic thiolated structures composed of two cross-bound particular amino acids lanthionine (Lan) and methyllanthionine (MetLan). These β-thioether cross-linked particular amino acids evolve from the thio-mediated tethering of cysteine and the dehydrated form of serine (dehydroalanine, Dha) or the dehydrated form of threonine (dehydrobutyrine, Dhb) generating lanthionine and methyllanthionine, respectively (C. Li et al., 2021; van der Donk & Nair, 2014).

Actually, lanthipeptides are divided into five classes (I-V) characterized by (1) an aminoacyl-tRNA dependent dehydratase LanB and a separate cyclase LanC for class I, (2) an ATP-dependent bifunctional LanB synthetase (dehydration and cyclization) for class II, (3) a NTP-dependent trifunctional LanKC synthetase (lyase, kinase, cyclase) for class III, (4) a NTP-dependent trifunctional LanL synthetase (lyase, kinase and cyclase) for class IV, while (5) the recently proposed class V (called lanthidins) is not well understood, apart from containing the Dhb residues common to lanthipeptides (Montalbán-López et al., 2021). The cyclase domain of the bifunctional Lan B of class II is similar to Lan C of class I. Lanthipeptides from class III differ from class IV by the absence of zinc-binding motifs in their cyclase domain and the presence of a labionin ring in their structures (Hegemann & Süssmuth, 2020; Hernandez Garcia & Nair, 2023; Repka et al., 2017).

However, many of the already discovered lanthipeptides derived from strains of the *B. subtilis* clade belong to the three first subclasses. The

compounds of the class I include subtilin, balucin, ericin A and ericin S both isolated from *B. subtilis* strains (Y. Fu et al., 2023; Stein, Borchert, Conrad, et al., 2002; Q. Zhang et al., 2022). Class II lanthipeptides covers bioactive metabolites such as amylolysin and mersacidin from *B. velezensis* strains, the two-peptides forming lanthipeptides lichenicidin (from *B. licheniformis*), haloduracin (from *B. halodurans*) and amyloliquecidin GF610 (from *B. velezensis* GF610) (Arguelles Arias et al., 2013; Dischinger et al., 2009; Gerst et al., 2022; Herzner et al., 2011; Lawton et al., 2007). Bacinapeptin (from *B. nakamurai* NRRL B-41092) is one of the recently described metabolites of class III lanthipeptides (D. Xue et al., 2022). The biosynthesis pathways and structures of subtilin, lichenicidin and bacinapeptin (Figure 1-13) of class I-III, respectively, will be discussed hereafter as examples of the large and growing number of lanthipeptides secreted by strains of the *B. subtilis* clade.

(1) Subtilin

Subtilin ($C_{148}H_{227}N_{39}O_{38}S_5$) was the first reported lanthipeptide to be produced by *Bacillus* strains and was originally isolated from *B. subtilis* ATCC 6633 cultures in 1944 (Q. Zhang et al., 2022). Its precursor peptide contains 56 amino acids residues with 32 residues of the core peptide (Banerjee & Hansen, 1988). The biosynthesis machinery of this peptide is encoded by *spaBTCSIFEGRK* gene cluster with *spaS* devoted for the precursor peptide, *spaBTC* for modification and export, *spaIFEG* for immunity and *spaRK* for regulation (Stein, Borchert, Kiesau, et al., 2002; Q. Zhang et al., 2022). The extracellular serine proteases *AprE*, *WprA* and *Vpr* secreted by the same strain are responsible of the cleavage of the leader peptide and liberation of mature and bioactive subtilin (Corvey et al., 2003; Van Tilburg et al., 2020).

Produced at maximum concentration during the stationary phase, this nisin-like antibiotic is produced under the control of self-regulator *spaRK* and the transition state pleiotropic regulon AbrB whose action is conveyed through *SigH*. At the end of log phase and during the maturation phase, AbrB is repressed followed by the dowregulation of sigH, which creates an upregulation of *spaRK* and then overexpression of core, modifying, transport and immunity genes (Heinzmann et al., 2006; Kleerebezem et al., 2004; Stein et al., 2003). Moreover, subtilin acts as a pheromone inducing its secretion in the producer-strain (Dey et al., 2023; Kleerebezem, 2004).

(2) Lichenicidin

Lichenicidin is a class II lantibiotic isolated from *B. licheniformis* strains with a paired peptide composition (Lchα or Bliα, and Lchβ or Bliβ) whose structures may vary from one strain to another (Antoshina et al., 2024; Barbosa et al., 2022; Begley et al., 2009; Caetano et al., 2011; Shenkarev et

al., 2010). This bi-peptide constituted lantibiotic contains 31 amino acids residues per peptide linked together by four intra-thioether bonds, and the N-terminal 2-oxobutyryl group (Figure 1-13). Lichenidicin biosynthesis is encoded by two structural genes *lchA1* and *lchA2* corresponding to Lchα and Lchβ peptides, and they are modified by dedicated specific enzymes encoded by *licM1* and *licM2* (Caetano et al., 2014; Dischinger et al., 2009). The export genes (*licTP*) encode two serine proteases that act iteratively, *lchT* removes the leader peptide of *lchA1* and the large part of leader peptide of *licA2*, except the hexapeptide NDVNPE which is further recognized by *licP* and permits the release of Licβ. However the precise role of the remaining genes putatively involved into regulation (*licXRY*) and immunity (*licFGEHI*) have not yet been understood (Barbosa et al., 2015, 2022; Repka et al., 2017).

Compared to other monopeptide class II lanthipeptides, the first peptide Lchα shares the same structural identity with amylolysin (Arguelles Arias et al., 2013) and mersacidin (Chatterjee et al., 1992) found in other *Bacillus* strains. As mode of action, lichenicidin targets the lipid II, a precursor of cell wall peptidoglycan either by inhibiting its synthesis or inducing the formation of pores leading to cytosol leakage (Barbosa et al., 2022).

(3) Bacinapeptin

Bacinapeptin belongs to the emerging class III lanthipeptides, which are mainly characterized by the presence of a labionin ring in their structural backbone (Figure 1-13) (D. Xue et al., 2022). The Bacillota (formerly Firmicutes) constitutes the second largest group within the Bacteria domain, after the Actinobacteria, to harbour the characteristic lanKC BGC of class III lanthipeptides (D. Xue et al., 2023). These include, not exhaustively, bacinapeptin from *B. nakamurai* NRRL B-41092, andalusicin from *B. thurigiensis* sv. *andalousiensis* (Grigoreva et al., 2021), amylopeptin from rat gut-isolated *B. amyloliquefaciens* (Y. Zhang et al., 2022), paenithopeptin from *Paenibacillus thiaminolyticus* NRRL B-4156 (D. Xue et al., 2022).

The biosynthetic pathway of bacinapeptin is encoded by *bcn* BGC, which includes *bcnA1* and *bcnA2* for two precursor peptides A1 and A2, respectively, *bcnKC* for modification enzymes, *bcnMT* for methyltransferase, *bcnT1* and *bcnT2* for transport (Figure 1-13). However, the excision of the leader peptide was found to be executed by two separate and individual metalloproteases *bcn-gP1* and *bcn-gP2*, located far from the *bcn* operon (D. Xue et al., 2022). Due to their recent discovery, studies on the general transcriptional regulation of their biosynthesis are still lacking.

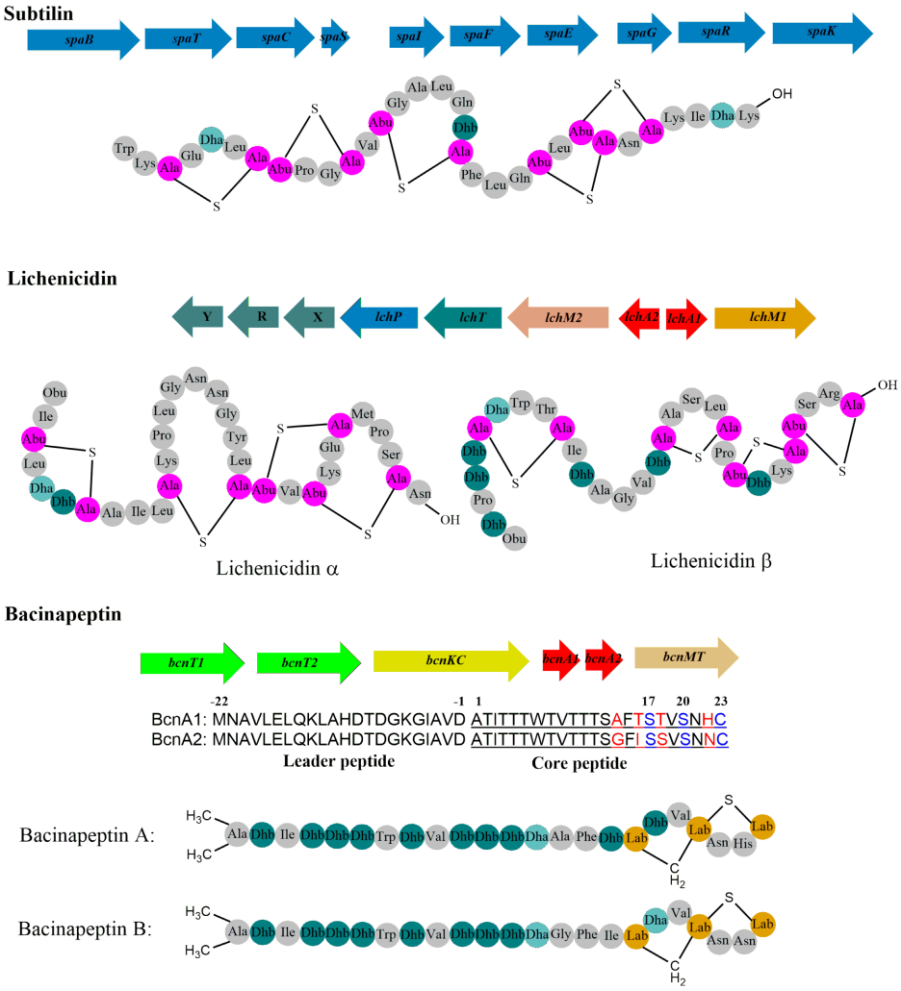


Figure 1-13: Schematic organisation of the BGCs and amino acid sequence-based structures of subtilin, lichenicidin and bacinapeptin. Dha, Dhb, Obu and Abu represent dehydroalanine (dehydrated Ser), dehydrobutyrine (dehydrated Thr), 2-oxobutyryl (modified Ala) and aminobutyric acid (modified Ala) residues, respectively. Lab represent labionin ring, which is characteristic of class III lantibiotic and involves either Ser or Cys linked together by a methylene group on one part and a thiol group to another part. The hydroxyl group (-OH) on the last amino acid of subtilin and lichenicidin indicates the C-terminal of the whole amino acid sequence.

4.2.3.2.2. Ycao superfamily enzymes-derived RiPPs

This class of RiPPs encompasses a large and still growing number of compounds characterized by modification enzymes of the Ycao superfamily.

These enzymes catalyze an ATP-dependent O-phosphorylation coupled to a nucleophilic attack (intra- or intermolecular) on the carbonyl group of the preceding amide, which opens up the door to further tailoring reactions. The outcome of these tailoring reactions depends on the nature of the nucleophile and will generate either the azolines (e.g. linear azoline/azole-containing peptides (LAP)) if the intramolecular attack is performed by the side chains of Ser/Thr/Cys or the bottromycins if the intramolecular attack is performed by the N-terminal amine, or thioamitides if an intermolecular nucleophilic attack is performed by an external sulfur donor (Dunbar et al., 2012; Ongpipattanakul, Desormeaux, van der Donk, et al., 2022). So far, the Ycao enzymes-derived RiPPs found in the *B. subtilis* clade uncovered the LAPs plantazolicin, first detected in *B. velezensis* FZB42 (Scholz et al., 2011), and sonorensin, detected in *B. sonorensis* cultures (Chopra et al., 2015).

(1) Plantazolicin

Plantazolicin is a LAP compound described for the first time in 2011 in *B. velezensis FZB42* (Scholz et al., 2011) and is marked by the presence of two thiazoles (Thz), three methyl-oxazoles (MeOxz), four oxazoles (Oxz), one methyl-oxazolidine (MeOxH), and two adjacent Ile bridging the two clusters of five heterocycles (Figure 1-14). Its biosynthetic machinery is encoded by *pznABCDEFGHIJKL* operon, with *pznA* encoding for the precursor peptide. The heterocycle formation is ensured by *pznBCDJ* where the Ser/Thr/Cys residues are cyclodehydrated by PznC induced by the docking/scaffolding enzyme PznD (otherwise described as Ycao enzyme), whereas *pznB* encodes a FMN-dependent dehydrogenase oxidizing the formed azoles and *pznJ* for maturation. The excision of the leader peptide is performed by *pznE* encoding a transmembrane zinc protease, while *pznFGHK* play the roles related to transport (*pznGH*), to immune function (*pznF*) and possible negative regulator (*pznK*). Whereas *pznI* encodes an unknown peptide till now, *pznL* is responsible for the N^{α},N^{α}-dimethylation of N-terminal Arg yielding plantazolicin A from its unmethylated variant and precursor plantazolicin B (Ongpipattanakul, Desormeaux, DiCaprio, et al., 2022; Scholz et al., 2011). This interesting antibiotic is not only restrained in *B. velezensis* FZB42, it was also reported in *B. pumilus* (Molohon et al., 2011; Scholz et al., 2011), implying that this metabolite may possibly be conserved in many species within the *B. subtilis* clade.

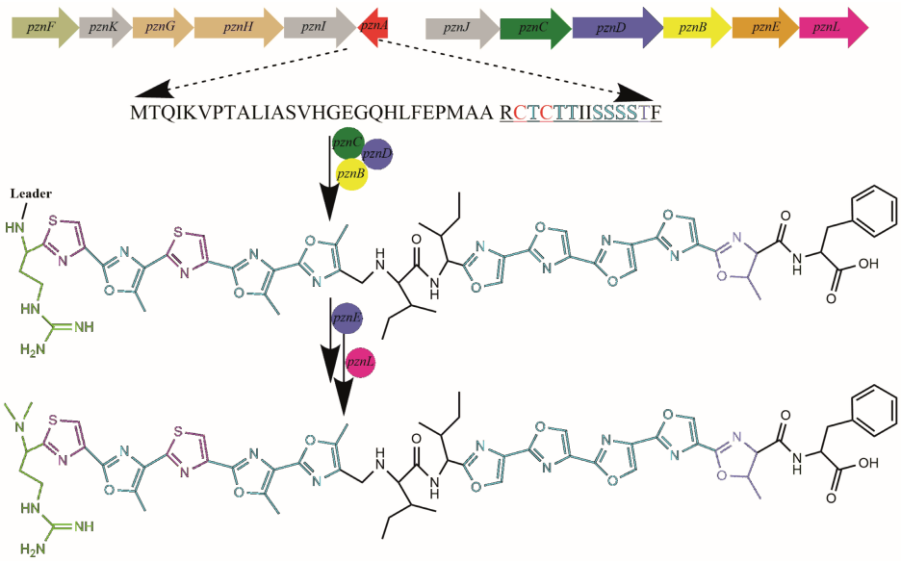


Figure 1-14: Overview of the biosynthetic pathway of plantazolicin. Methyl-oxazolidine (MeOxH) is colored in violet, oxazoles (Oxz) and methyl-oxazoles (MeOxz) are in light blue colour, while the two thiazoles (Thz) are represented in pink. The first amino acid Arg that undergoes dimethylation is marked in green.

4.2.3.2.3. Circular RiPPS

This group encompasses ribosomally and post-translationally modified compounds whose tailoring reactions lead to head-to-tail cyclization, implying fusion of N- and C-termini. Known head-to-tail cyclized RiPPs from the *B. subtilis* clade include amylocyclicin, first described in *B. velezensis* FZB42 (Scholz et al., 2014), and pumilarin, first described in *B. pumilus* (van Heel et al., 2017). In addition, other metabolites classified as sactipeptides are included in this group after their subsequent head-to-tail cyclization following the formation of the sactionine bridge. They are defined by an intramolecular thioether bridge (sactionine) between cysteine S- and α-carbon of another amino acid, a reaction catalyzed by a radical s-adenosylmethionine (rSAM) enzymes. These include subtilosin A and sporulating killing factor (skf), which have been reported in *B. subtilis* strains (Alajlani, 2022; Babasaki et al., 1985; Flühe et al., 2013; González-Pastor et al., 2003). Other sactipeptides, but non-circular, have been reported from the *B. cereus* clade, such as thuricin CD, thuricin Z/huazacin, and thurincin H from *B. thuringiensis* strains (Y. Chen et al., 2021).

(1) Amylocyclicin

Amylocyclicin (6,381 Da) is a circular peptide consisting in 64 amino acids and was first described in *B. velezensis* FZB42 in 2014. Its precursor peptide is encoded by *acnA*, while *acnC* is responsible for the maturation function including the leader peptide cleavage and head-to-tail cyclization (Figure 1-15). Although, *acnB* encodes a membrane protein whose exact function is not yet known, its depletion in a constructed mutant leads to loss of activity, suggesting that it plays a critical role. The *acnDEF* genes are associated with putative transport and immunity functions (Scholz et al., 2014).

(2) Subtilosin A

Subtilosin A is a head to-tail cyclized sactipeptide first reported from *B. subtilis* 168 in 1985 (Babasaki et al., 1985; Ongpipattanakul, Desormeaux, van der Donk, et al., 2022). The mature peptide consists of 35 amino acids and contains three thioether cross-links formed between the sulfur group of Cys_{13}, Cys_{7} and Cys_{4} and the α-C of Phe_{22}, Thr_{28} and Phe_{31} respectively, followed by the intramolecular fusion of the terminal C- of Gly_{35} and the terminal N- of Asn_{1} (Figure 1-15). Its biosynthetic machinery is encoded by *sboAX-albABCDEFG*, where *sboA* encodes the precursor peptide and *albA* the modification enzyme radical s-adenosylmethionine (rSAM), which is responsible for the formation of sactionine bonds. The cleavage of the leader peptide and macrocyclization are carried out by *albEF* genes. The transport of the mature peptide is performed by *AlbCD* genes. The function assigned to *albBG* genes is still not clear, although they have been taught to intervene in subtilosin immunity. The recently discovered subgene *sboX* encodes for an unidentified bacteriocin-like product. In addition, the pair *sbo-alb* genes are under the transcriptional control of the master regulators Spo0A and AbrB (Ishida et al., 2022; Kawulka et al., 2004; Stein, 2020; G. Zheng et al., 2000).

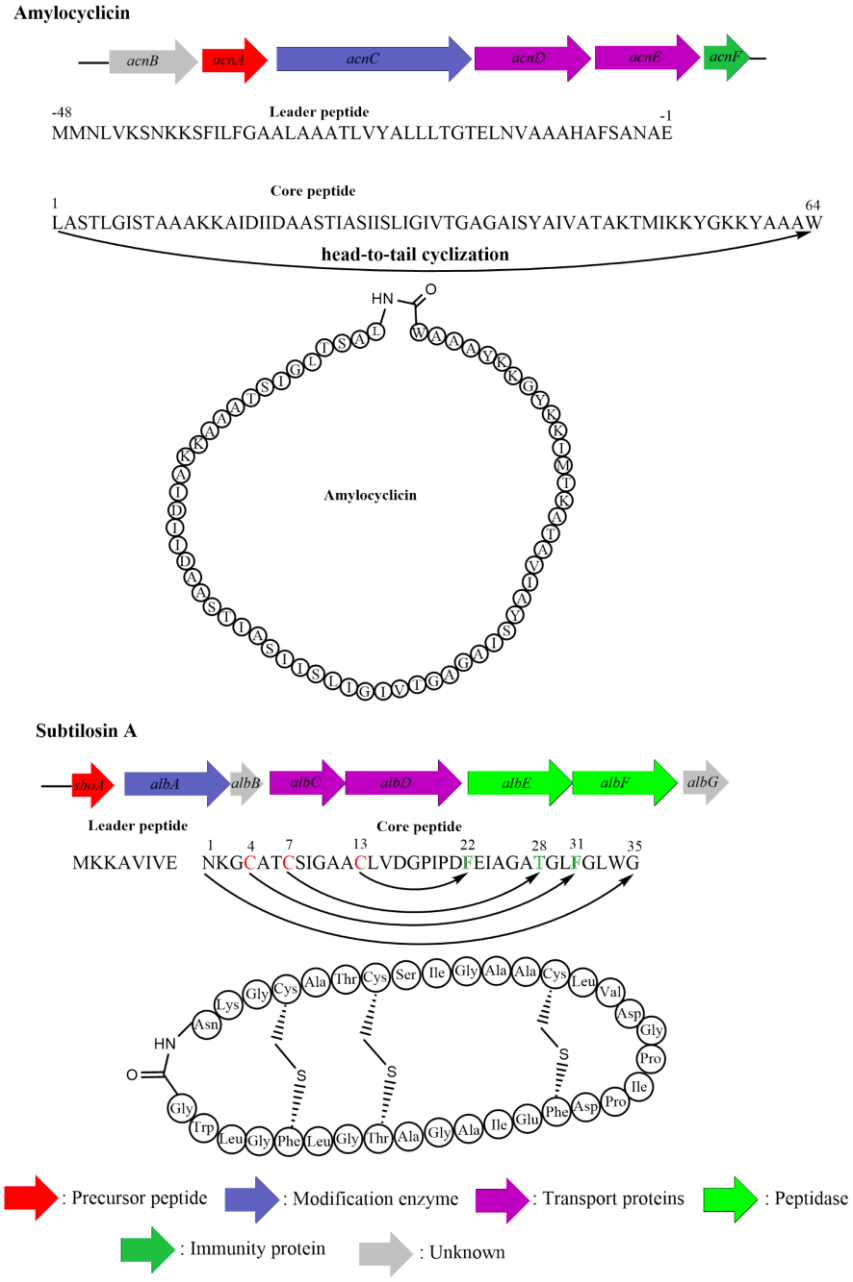


Figure 1-15: Biosynthetic route of head-to-tail cyclized amylocyclicin and subtilosin A (also a sactipeptide).

4.2.3.3. Organic volatiles compounds

The final group of chemicals secreted by strains of *B. subtilis* clade are volatiles, commonly referred to as volatile organic compounds (VOCs). They are mostly products of the primary metabolism (glycolysis, proteolysis and lipolysis) as derivatives of the pyruvate and acetyl-CoA intermediates. They also evolve as by-products of the tricarboxylic acid cycle (TCA cycle), which is initially devoted to energy metabolism (Figure 1-16). These VOCs are generally characterized by low vapour pressure, high lipophilicity, and low molecular mass (not more than 300 Da) (Cellini et al., 2021; Poulaki & Tjamos, 2023; Veselova et al., 2019). This class of compounds consists of hydrocarbons, ketones, alcohols, aldehydes, terpenes, esters, aromatics, nitrogen-containing compounds, and acids (Figure 1-16) (Kai, 2020) and plays an important role as chemical signals within intraspecific, interspecific and interkingdom relationships and processes (Y. Chen et al., 2015; Farag et al., 2017; Netzker et al., 2020).

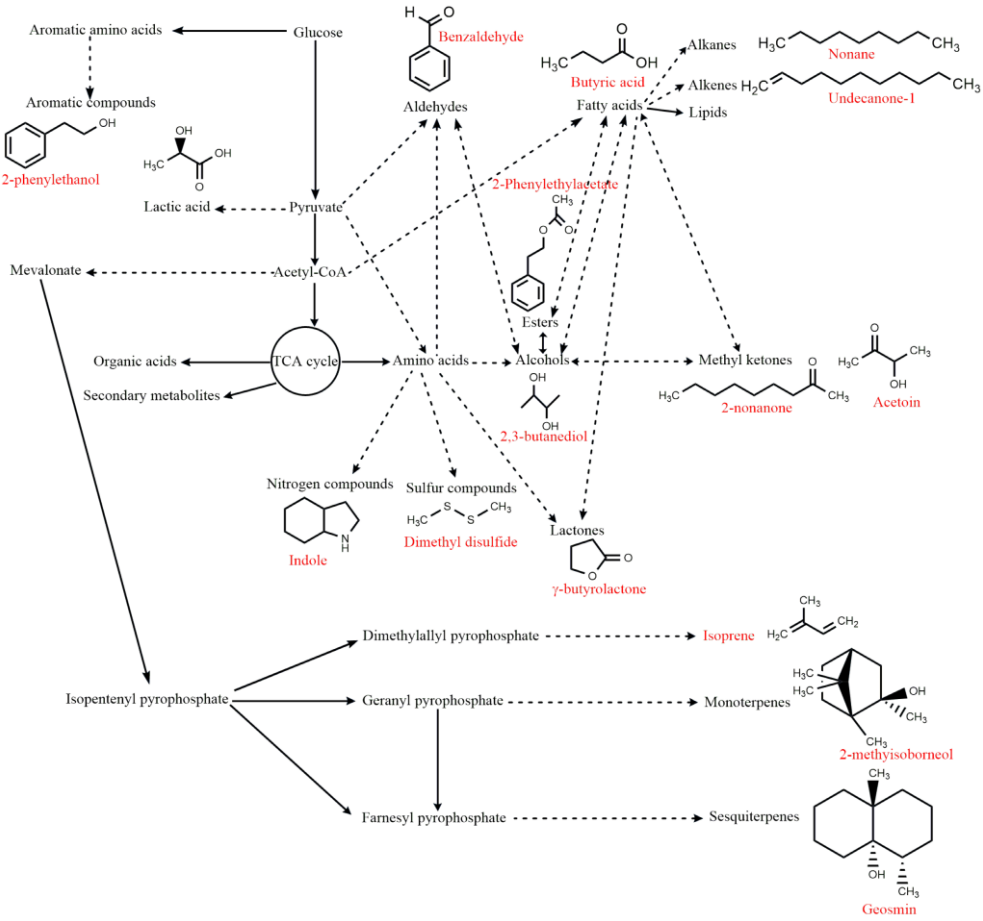


Figure 1-16: Main biosynthetic route of volatile organic compounds from bacterial origin. The volatile end-products are coloured in red. Plain arrows indicate intermediate reactions, while dashed arrows show the direct formation of volatile compounds.

4.3. Mechanisms underlying the biocontrol potential of *Bacillus*

Plant protection provided by strains of *B. subtilis* clade against various soil- and air-borne phytopathogens involves four different mechanisms including the competition for space and nutrients, direct antibiosis, induced systemic plant resistance (ISR) and signal interference (J. Lee et al., 2023; N. Zhang et al., 2023). These mechanisms are directly or indirectly mobilized for the successful antagonism of plant pathogens and involve the above-described panoply of BSMs produced by these bacilli (Figure 1-17).

4.3.1. Competition

Competition for space and nutrients between microbial phytopathogens and plant beneficial microorganisms such as *Bacillus* spp. play an important role in plant disease suppression in limiting disease incidence and severity (Karačić et al., 2024). Rhizosphere bacilli possess several chemoreceptors for specific nutrients exudated by plant roots such as sugars, amino acids and organic acids, etc. enabling their successful root colonisation and establishment at the expense of their competitors (Boubsi et al., 2023; H. Feng et al., 2018; Hashem et al., 2019; N. Zhang et al., 2014). For instance, it was shown that maize root exudates attract and favor *B. amyloliquefaciens* OR2-30 root colonization as a strategy to combat the maize pathogen *F. graminearum* (Xie et al., 2022). Plant root colonization by *Bacillus* spp. results in the formation of biofilm on root surfaces, acting as a shelter against invading soil-borne phytopathogens and/or other competitors (Y. Liu, Xu, et al., 2024; Nishisaka et al., 2024). It was reported, for example, that *B. subtilis* supresses tomato wilt caused by *Fusarium* sp. by forming robust biofilm on tomato roots (Altaf et al., 2017; Y. Chen et al., 2013).

Competition for iron is another well-studied mechanism in *Bacillus* ecology and involves the siderophore bacillibactin that chelates all available ferric ion and thus starve this key element from their prominent (phytopathogens) competitors and inhibit their proliferation (Arguelles-Arias et al., 2009; Mazumdar et al., 2020). Indeed, bacillibactin was found antagonistic in iron-limited conditions against plant pathogens *P. syringae*, *Verticillium dahlia*, *F. oxysporum*, *F. fujikuroi, Aspergillus flavus*, *R. solani*, *Magnaporthe oryzae* and *Phytophthora capsici* (Dimopoulou et al., 2021; Y. Liu, Dai, et al., 2024).

4.3.2. Antibiosis

Antibiosis is a biological process in which bioactive secondary metabolites secreted by microorganisms suppress or inhibit in direct way plant pathogens (Fira et al., 2018). Most of the above-presented BSMs produced by strains of the *B. subtilis* clade are tainted with antimicrobial potential and participates in this direct antagonism against phytopathogens. The various cyclic lipopeptides (CLPs) act solely or synergically against plant pathogens by disrupting target cell wall or membranes integrity in creating pores or enhancing membrane permeability which favour the ion and/or cytosol leakage (thus osmotic imbalance) and thus the cell death (Helmy & Parang, 2023; Salazar et al., 2023). The role of surfactin alone as a direct antagonist is relatively negligible compared to iturin and fengycin, but it is often reported to act synergically and enhance the antagonistic potential of iturin and/or fengycin. The antifungal potential of the latter has been extensively investigated and they are reported, for example, to be involved separately or

in synergy against *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Fusarium solani*, *Botryosphaeria berengriana f. sp. piricola*, *Botrytis cinerea*, *Podosphaera fusca, Fusarium graminearum*, *Bipolaris maydis*, *Colletotrichum orbiculare*, *Fusarium verticillioides*, *Candida albicans*, *Aspergillus fumigatus*, *Cryptococcus neoformans, Fusarium graminearum*, *Gaeumannomyces graminis* var. *tritici, Colletotrichum acutatum,* etc. (Helmy & Parang, 2023; T. Mahmood, 2022; Sreedharan et al., 2023; Yaraguppi et al., 2023).

The three families of polyketides i.e. bacillaene, difficidin and macrolactin are well known for their antibacterial activity against bacterial pathogens mostly by interfering with protein synthesis in blocking the action of some key enzymes like peptide deformylase (Puan et al., 2023; C. Tran et al., 2022). These BSMs were reported to mediate antibacterial activity against plant pathogens such as *Erwinia carotovora*, *E. amylovora*, *Ralstonia solanacearum*, *Pseudomonas chlororaphis*, *Xanthomonas oryzae*, *Dickeya chrysanthemi* (Y. Wang et al., 2024). Moreover, bacillaene and macrolactin are described to exhibit antifungal activity against *Penicillium digitatum* and *B. cinerea*, respectively (Miao et al., 2023; J. Ni et al., 2023). Bacillaene plays other ecological roles like in enabling coexistence of its producer strain with another beneficial bacterium *Streptomyces* sp. Mg1 and in protecting the producer *B. subtilis* strain from predation by another soil-borne bacteria *Myxococcus xanthus* (Barger et al., 2012; Miao et al., 2023; Müller et al., 2014, 2015).

The dipeptide bacilysin has multiple antagonistic activity against fungi and bacteria and its mode of action relies on blocking the activity of glucosamine 6-phosphate synthase mediating the synthesis of the glucosamine 6-phosphate (GlcN6P), an important constituent of bacterial murein or fungal mannoprotein (Islam et al., 2022). This oligopeptide has been reported to interplay in the inhibition of *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* causing the rice leaf blight disease, the fire blight disease-causing agent *E. amylovora* (X. H. Chen et al., 2009; L. Wu et al., 2015). It is the main inhibitor of the oomycete *Phytophthora sojae* (a soybean pathogen) (X. Han et al., 2021) and active against many human pathogens such as *Escherichia coli*, *Salmonella enterica*, *Candida albicans*, etc. (Kenig & Abraham, 1976; Nannan et al., 2021), as well as against harmful algae *Microcystis aeruginosa*, *Aphanizomenon flos-aquae*, *Anabaena* sp. and *Nostoc* sp. (L. Wu et al., 2014).

The huge diversity of RiPPs produced by *B. subtilis* strains are great antibacterials, mostly against Gram positive bacteria including *Bacillus* closely related strains, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Their action is either by forming the pores in the target cytoplasmic membrane or depolarizing the cell envelope through targeting the peptidoglycan precursor lipid II or the

undecaprenyl pyrophosphate that results into the blockage of cell wall synthesis and/or cell lysis (Olishevska et al., 2019; Ongpipattanakul, Desormeaux, van der Donk, et al., 2022). Indeed, subtilin of class I lantibiotic harbours great antibacterial activity at nanomolar concentrations against several Gram-positive bacteria encompassing *Micrococcus luteus*, *Lactococcus* spp., *Listeria monocytogenes*, *S. aureus* and *Bacillus* spp. (Chan et al., 1993; Qin et al., 2019; Wei et al., 2021; Q. Zhang et al., 2022). Plantazolicin, a LAP, produced by *B. velezensis* FZB42 and *B. pumilus* (Scholz et al., 2011) retains strong antibacterial effect against the obligate pathogen *B. anthracis* (Molohon et al., 2016) and a moderate nematicidal activity against *Caenorhabditis elegans* (Z. Liu et al., 2013; Mhatre et al., 2024).

The cell wall degrading enzymes secreted by *Bacillus* spp. such as chitinases, glucanases, cellulases, proteases are interplayers in the direct antifungal antagonism where they weaken or disrupt the fungal cell wall, leading to cytosol release or enabling the successful action of other antibiotics (Dimkić et al., 2022). For instance, protease and glucanase produced by *Bacillus* spp. were found to be overproduced in presence of fungi *Alternaria triticina* and *Bipolaris sorokiniana* and hence would mediate the antifungal activity against the latter pathogens (Saini et al., 2024). β-glucanase secreted by *B. subtilis* exerts an antifungal activity against the mycotoxin producer *Aspergillus ochraceus* and was found effective in protecting soybean from fungal spoilage with 97% (M. Zhao et al., 2022). In addition, chitosanase from *B. subtilis* SH21 exhibited also antagonistic effect against *F. solani*, a soil-borne fungal pathogen affecting many agricultural crops (Pang et al., 2021). Volatile organic compounds (VOCs) produced by strains of the *B. subtilis* group exert antifungal and antibacterial activities against the plant pathogens such as *Alternaria solani*, *Monilinia fructicola*, *Colletotrichum gloeosporioides*, *Botrytis cinerea*, *Curvularia lanata, Clavibacter michiganensis* spp. *sepedonicus, Xanthomonas oryzae* pv. *Oryzae*, etc. (Awan et al., 2023; H. Chen et al., 2008; Rajer et al., 2017; Xie et al., 2018, 2020; D. Zhang et al., 2020; P. Zhao et al., 2019).

4.3.3. Signal interference

Bacteria live in communities inside their ecological niches and have evolved to communicate for co-existence or exclusion or competition for nutrients, as well as for performing other metabolic functions and responding to their host and external biotic or abiotic factors (S. Sharma & Araujo, 2024; Tadrosova et al., 2024). Cell-to-cell communication within bacteria, known as quorum sensing (QS), through small and diffusible chemicals permits bacteria to engage in diverse physiological processes on a cell density-dependent rule (Kalamara et al., 2018). Strains of the *B. subtilis* group are

equipped with QS ComQXPA system, where ComX activated by ComQ plays a pheromone role as an autoinducer when its secretion accumulates above threshold concentrations. This will hence regulate many upstream metabolic processes including secretion of specialized secondary metabolites like surfactin (Danevčič et al., 2023). Another QS system in *B. subtilis* strains is the universal LuxS/AutoInducer-2 that is involved in biofilm formation (Duanis-Assaf et al., 2016). For pathogenic bacteria (*Pseudomonas* spp., *E. carotovora*, *Staphylococcus* spp., etc.), several QS-dependent molecules including N-acylhomoserine lactones (AHLs), alkylquinolones, oligopeptides and LuxS/autoinducers are important for bacterial phenotype and virulence, biofilm formation and other physiological processes (Raju et al., 2022; Sikdar & Elias, 2020; Striednig & Hilbi, 2022).

These pathogenic QS-mediated metabolites can be chemically or enzymatically degraded or its secretion inhibited by quorum quenching (QQ) molecules such as lactonase, acylase, peptides, oxidoreductase produced by *Bacillus* spp. and other PGPR (Noor et al., 2022; Prazdnova et al., 2022). For instance, the virulence-associated biofilms of *S. aureus* were successfully destroyed by a surfactin variant produced by *B. subtilis* 6D1 through quorum quenching (Leistikow et al., 2024). Another compound produced by *B. subtilis* BR4, stigmatellin Y, was reported to interfere with the QS systems autoinducer protein (AIP)/Arg and PQS-pqsR of *P. aeruginosa* (Boopathi et al., 2022). *B. subtilis* DZ17 is reported to have antibacterial activity by QQ against *P. aeruginosa*, *P. carotovorum* and *Streptococcus mutans* by either degrading AHLs via lactonase activity or disrupting the biofilm formation (El Aichar et al., 2022; Roca et al., 2024). This phenomenon of signal interference can tentatively be considered as part of the above described biocontrol mechanisms, antibiosis and/or competition, due to their common direct inhibition of the pathogens. However, their modes of action are different and are rather complementary mechanisms.

4.3.4. Induced systemic resistance

Induced systemic resistance (ISR) is the process by which plants treated with PGPR species, or their secreted molecules induce defence genes and develop immunity to subsequent plausible pathogen attack (Mahapatra et al., 2022). PGPR-induced ISR is an indirect mechanism of pathogens' inhibition and is phenotypically similar to the well-studied systemic acquired resistance (SAR), which is activated after a first infection by an incompatible or necrotizing pathogen (Pršić & Ongena, 2020). ISR process is regulated by the jasmonate and/or ethylene phytohormone pathways, whereas SAR is dependent on the salicylic acid regulation pathway (Salwan et al., 2023). The main elicitors of ISR produced by *B. subtilis* strains are cyclic lipopeptides (surfactin, iturin and fengycin), volatile compounds, flagellin, peptidoglycan

and other putative metabolites with antagonistic functions like siderophores (Orozco-Mosqueda, Fadiji, et al., 2023; L. Zhu et al., 2022). These elicitors act as microbe-associated molecular patterns (MAMPs) and may be perceived by specific transmembrane pattern recognition receptors (PRRs) or interact in a physico-chemical way with the cell membrane sphingolipids (Balleux et al., 2024; Riseh et al., 2025). This leads to the activation of the MAMPs-triggered immunity (MTI) marked by a rapid burst of reactive oxygen species (ROS), Ca^{2+} influx and phosphorylation cascades (M. M. Ansari et al., 2024; Balleux et al., 2024). However, for a successful symbiosis with plant root, PGPR have evolved to evade or suppress or modulate this MTI developed by the plant as the first line of defense (Riseh et al., 2025). This strategy implies either the secretion of modified MAMP or several MAMPs with reduced eliciting potential or producing compounds with binding affinity to the MAMP, creating a masking effect as it was shown with the subtilomycin produced by *B. subtilis* that binds to the bacilli flagellin (Riseh et al., 2025; L. Zhu et al., 2022). The above rapid and early immune events will result in the induction of plant defense hormones jasmonic acid and ethylene leading to the secretion of resistance proteins and molecules including lipoxygenase, glucanase, chitinase, phenylalanine ammonia-lyase, production of phenolic compounds, phytoalexins and deposition of lignin, callose (Abdelaziz et al., 2023).

For instance, *B. velezensis* MS20 was reported to protect maize against *R. solani* through surfactin mediated-ISR, evidenced by the accumulation of plant defense enzymes such as phenyl ammonia lyase (PAL), ascorbate peroxidase (APx), polyphenol peroxidase (POx), chitinase, catalase (CAT) and superoxide dismutase (SOD) (S. A. M. Ali et al., 2022). Mycosubtilin produced by *B. subtilis* BS-Z15 is reported to trigger the upregulation of ISR-related genes families (jasmonate-zim domain (JAZ), jasmonate response locus (JAR), lipoxygenases (LOXs), etc.) in *Arabidopsis* infested by *Verticillium dahlia*, resulting in disease reduction (Q. Yang et al., 2023). In addition, *B. subtilis* KB21 was found to control effectively the *Colletotrichum acutatum* in Pepper plants and the lipopeptide iturin A interplayed this antagonism by activating the ISR-related genes *PAL* and *LOX* (J. S. Park et al., 2022). The lipopeptide fengycin secreted *B. subtilis* XF-1 was recently shown as the main elicitor of defense systems in Chinese cabbage for counteracting the clubroot disease (caused by *Plasmodiophora brassicae*) (P. He et al., 2023). VOCs emitted by *B. subtilis* GB03 was found to be the main mediators of biocontrol of *B. cinerea* in *Arabidopsis* through priming and defense-related genes *PR1* and *PDF1.2* were found upregulated (Sharifi & Ryu, 2016). In addition, VOC 2,3-hexanedione emitted by *B. halotolerans* NYG5 was recently described to trigger overexpression of plant resistance genes PR1 and PR2 in *A. thaliana* (Rana et al., 2024).

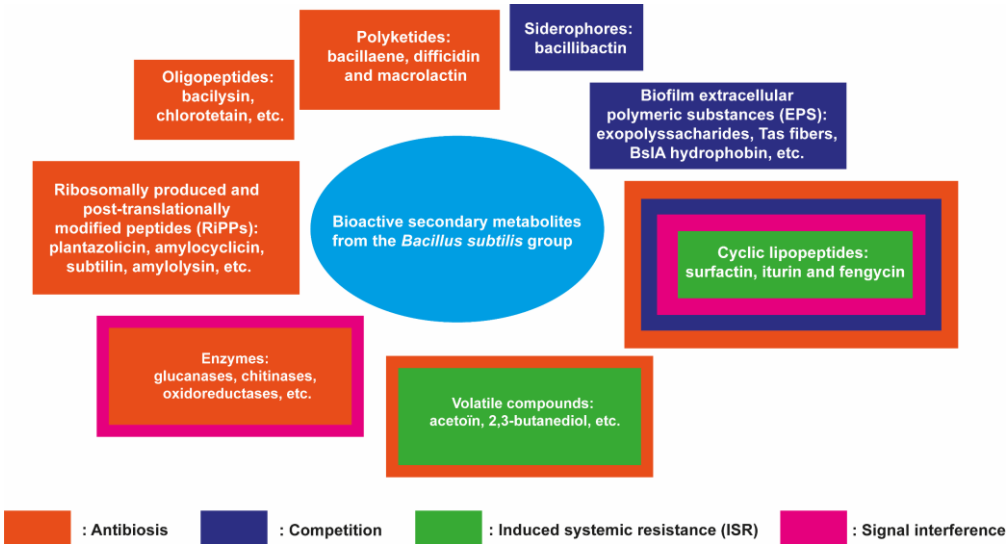


Figure 1-17: Different *Bacillus* biocontrol mechanisms and overview of generally involved bioactive secondary metabolites.

5. *Bacillus* spp. in the plant growth promotion and development

The plant growth promotion encompasses phytostimulation and biofertilization and constitutes another virtue of *Bacillus* spp. Phytostimulation by beneficial bacteria implies a secretion of phytohormones, small molecular weight compounds with abilities to modulate key plant physiological processes including growth, development and homeostasis (Asari et al., 2017; Soni & Keharia, 2021). Growth promoting phytohormones include auxins, cytokinins, gibberellins, and plant defense-related phytohormones salicylic acid, jasmonic acid and ethylene or 1-aminocyclopropane-1-carboxylate (ACC) (Asari et al., 2017; Poveda & González-Andrés, 2021).

Auxin, mostly indole acetic acid (IAA), is biosynthesized in a tryptophan-dependent way and involves different genes such as *dhaS*, *patB*, *yclB*, *yclC*, *yhcX* and *ysnE* with implication in plant root cell elongation and vascular tissue development (Poveda & González-Andrés, 2021; J. Shao et al., 2015). For instance, several *Bacillus* strains including *B. subtilis* ALC_02, *B. subtilis* 168 Gö, *B. licheniformis* FMCH001, *B. subtilis* FMCH002 and *B. megaterium* WW1211 are reported to induce the overgrowth of plant shoot and an increase of the root surface area through the mediation of the auxin IAA (de O. Nunes et al., 2023; Jensen et al., 2024; S. Wang et al., 2021).

Cytokinins play a key role in cell division and differentiation (Kieber & Schaller, 2018) and its synthesis by *Bacillus* spp. implicates *recA* and *recX* genes, and *ipt* genes encoding for isopentenyltransferase (Arkhipova et al., 2006; S. Iqbal et al., 2021). Cytokinins produced by *Bacillus* spp. are largely reported to improve growth and root system development of various plants (Barrera-Ortiz et al., 2023; Zerrouk et al., 2020). Gibberellins are co-produced by plants, fungi and bacteria and are known to have a positive impact in stem elongation and seed germination (Keswani et al., 2022; Salazar-Cerezo et al., 2018). Bacteria produces mostly four types of gibberellins GA1, GA3, GA4 and GA20, but GA3 is the main abundant and bioactive. Their biosynthesis involves key genes like *GA3ox* (GA3-oxidase gene) and *GA20ox* (GA20-oxidase gene) and other putative cytochrome genes P450 such as *P450-1*, *P450-4* (Hao et al., 2019; Q. Hu et al., 2024). Several *B. subtilis* group strains such as *B. velezensis* HNH9, *B. altitudinis* HNH7, *B. subtilis* QM3, *Bacillus* sp. PG-8, etc. were described to promote plant growth through gibberellins production (Abdelmoteleb et al., 2023; Gohil et al., 2022; N. Hasan et al., 2022; Q. Hu et al., 2024).

Biofertilization effect of PGPR (including *Bacillus* spp.) implies that microorganisms enhance nutrient uptake by plants and hence promote their growth and development (Chadhary et al., 2022; Jacob & Paranthaman, 2023). Amongst the pivotal mineral elements to plant growth, Nitrogen (N) and Phosphorus (P) are the two main elements that lack often in soil ecosystems and thus limit the plant growth (Aloo et al., 2022; Zeng et al., 2022) and this explains the overall reliance on chemical fertilizers (NPK-Nitrogen, Phosphorus and Potassium) in agriculture worldwide. However, only 5-25% of the P amended to soils as chemical fertilizer is utilized by plants and the remaining part is converted into insoluble complexes and bound to soils especially in calcareous soils (H. P. Li et al., 2023; Mehta et al., 2015). They can also be leached and brought by erosion to aquatic ecosystems where it constitutes the sole of eutrophication (Bindraban et al., 2020; Conijn et al., 2018). Rhizobacteria including *Rhizobium, Azobacter, Erwinia, Serratia, Bulkholderia, Pseudomonas* and *Bacillus* have the ability to solubilize the inorganic or organic phosphorus form into the soluble form absorbable by plants; they are hence termed Phosphate Solubilizing Bacteria (PSB) (Mehta et al., 2015; Ricci et al., 2019). These could serve as alternatives to the costly and environmentally damaging phosphate fertilizer.

This beneficial trait is mediated through the action of secreted organic acids (by-products of acetate kinase and citrate synthase), alkaline phosphatase and pyrophosphatase that solubilize the inorganic phosphorus, and the action of phytase that solubilize the indigestible organic phosphorus (phytate) (Backer et al., 2018; D. Zhao et al., 2022). Studies emphasizing on the effect of *Bacillus* spp. in mineral or organic phosphorus solubilization are steadily

increasing and examples include, not exhaustively, *B. amyloliquefaciens* H-2-5 and *B. velezensis* Ag75 that promote the growth of soybean plants (M.-J. Kim et al., 2017; Mosela et al., 2022).

Nitrogen (N_2) is involved in metabolic functions by synthesizing proteins, nucleic acids including DNA, RNA. Though freely available in the atmosphere, plants are unable to assimilate it unless the aid of specific naturally occurring microorganisms which convert it into ammonium (NH_4^+) and nitrate (NO_3^-) forms, directly utilisable by plants (Chadhary et al., 2022; Shahwar et al., 2023).This valuable task is accomplished by PGPR called diazotrophs. Diazotrophs encompass symbiotic and non-symbiotic/associative bacteria. Symbiotic bacteria (*Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Sinorhizobium*, *Mesorhizobium*) invade the leguminous plant root system and develop nodules in which atmospheric nitrogen (N_2) is fixed and enzymatically transformed into NH_4^+ by nitrogenases (Alzate Zuluaga et al., 2024; Berde et al., 2022). Non-symbiotic nitrogen fixing bacteria (also called free-living bacteria), including species of genera *Azoarcus, Azotobacter*, *Acetobacter*, *Azospirillum*, *Burkholderia*, *Diazotrophicus, Enterobacter*, *Gluconacetobacter*, *Pseudomonas, Bacillus* and cyanobacteria, play a crucial role in mobilizing nitrogen for non-leguminous plants (e.g. rice, vegetables, etc.) (Jalal et al., 2022; Patra & Mandal, 2022). The nitrogen metabolism in bacteria involve main enzymes and proteins such as carbon-nitrogen family hydrolase, nitrite reductase, nitrate reductase, P II family nitrogen regulator and NifU family protein and NifS family protein, and urease for urea digestion (Zaid et al., 2022). Some reports have shown that *B. altitudinis*, *B. aryabhattai*, *B. barbaricus*, *B. circulans*, *B. endophyticus* and *B. fusiformis* (Verma et al., 2016), *B. subtilis* B9 (Di et al., 2023), *B. velezensis* HNA3 (Zaid et al., 2022), etc. exhibited a good nitrogen fixation activity and thus contribute to plant growth promotion.

6. Impact of abiotic factors on the fitness of *Bacillus* spp.

Rhizobacteria in general and bacilli in particular, are seriously dependent on abiotic parameters such as nutrient availability (C, N, K, P, Ca, Fe, etc.), environment temperature and light intensity, soil pH and texture, soil pool of O_2 and CO_2, etc. (Lopes et al., 2021). These affect their growth and the secretion of their secondary metabolites and thus revert implications on their beneficial attributes to host plants (Lopes et al., 2021; Santoyo et al., 2017). These factors are interdependent from one another and their plausible effect (positive or negative) to soil microbiome or the whole holobiont is indiscutably an outcome of both factors (Pandey et al., 2024). For instance, the availability of some nutrient elements is directly linked with the soil pH,

temperature and moisture and this will thus impact the microbiome fitness and ecology (Etesami, 2020; Mahmood et al., 2019). Particularly, soil pH exhibits severe impacts on soil dwelling-microbiome and shape its diversity and abundance (C. Wang & Kuzyakov, 2024b). Indeed, it was found that acidic soils are mostly dominated by fungi, while neutral and alkaline soils were more colonized by bacteria (Lopes et al., 2021). In harsh conditions of soil such as acidic pH, drought, UV light exposure, heavy metals, scarcity of nutrients, most bacilli evolve in dormant state of endospores until favorable conditions re-established, whereas non-endospore forming bacteria won't survive (Akinsemolu et al., 2024; Mahapatra et al., 2022). Nervertheless, owing to geographic diversity of ecological niches in which *B. subtilis* strains were isolated, some have acquired specific adaptations to plausible local stressful conditions. These include, for example, chaperones against cold and heat stress, compatible solutes against osmotic stress, anti-oxidative stress enzymes, etc. (Jayakumar et al., 2021; L. Shen et al., 2021). However, the adaptation strategies were shown to be energy-consuming with possible negative implications to bacterial overall fitness including bacterial biomass, secreted bioactive metabolites and consequently related-plant beneficial attributes (Prasad et al., 2023; Sagar et al., 2022).

Chapter 2

Objectives

Soil microorganisms, especially the plant growth promoting rhizobacteria such as *Pseudomonas*, *Streptomyces*, *Paenibacillus*, *Bacillus* have already proven their potential to mitigate plant diseases and enhance plant growth and development through diverse mechanisms involving their secreted metabolites (Igiehon et al., 2024; Ryu et al., 2024). Their based-formulations are already marketed, dominated by the resistant endospore-forming *Bacillus*-based products (S. Kumar et al., 2024; Ramírez-Pool et al., 2024). Despite the relative worldwide transition to these safe and eco-friendly tools, Burundi and other developing countries such as the Great Lake countries of Central Africa are increasingly relying on chemicals for which negative effects on human health and environment are not be demonstrated. This situation is jeopardizing the attainment of the sustainable development goals. However, the deployment of non-native bacterial agents in a typical agroecosystem is without any concern since the introduction of foreign species can lead to ecological imbalance or possible inadaptation of this bacterial bioproduct due to unfavourable local abiotic conditions (Hossain et al., 2023; O'Callaghan et al., 2022).

Within this context, this thesis is aimed to bioprospect from Burundi local agro-ecosystems new and adapted plant beneficial *Bacillus* with biocontrol and growth promotion potential. In a more specifical way, this works is subdivided into four main chapters.

1. **Isolation, molecular identification, and functional-based genetic analysis of the Burundi isolated *Bacillus nakamurai* BDI-IS1.** From root samples collected from a farm lands, *Bacillus*-like colonies were isolated, and the best antibacterial isolate against an array of bacterial phytopathogens was selected. After 16S rRNA, DNA genome sequencing, the taxonomical identity of the isolate was established, genomic-based functional analysis and assessment of its potential to promote plant growth were performed using bioinformatic tools such as TYGS, NCBI-Blast, RAST Server, OrthoVenn.
2. **Characterisation the secondary metabolome and biocontrol potential of BDI-IS1.** In light of the antagonistic potential of BDI-IS1, genomics, metabolomics and reverse genetics were used first to mine (AntiSMASH and Bagel 4) all the biosynthetic gene clusters of known secondary metabolites at the strain and species level, and second to screen soluble secondary metabolites from the cell-free culture supernatants using the UPLC q-TOF MS technique, and third to establish the correlation between the secondary metabolome and the observed antagonistic activities. The biocontrol potential of the strain was then evaluated against tomato early blight and nothern corn leaf blight, caused by *A. solani* and *E. turcicum*, respectively, through greenhouse assays.
3. **Impact of abiotic factors on BDI-IS1 fitness and its antagonistic potential**. As it has been demonstrated that the *in-vitro* efficacy of biocontrol agents fails to be reproduced in field conditions due possibly to the harsh conditions prevailing in these natural ecosystems, the isolate BDI-IS1 was subjected *in*

vitro to the effect of variable temperature (15°C-30°C) and pH (pH 4.6 to pH 7.0) reproducing the natural soil conditions of tropical regions and we assessed their impact on its growth, biofilm forming ability, metabolome production and the antagonistic activities toward bacterial phytopathogens.

4. **Bioformulation of BDI-IS1 on local and affordable lignocellulosic substrates**. *Bacillus*-based biocontrol agents are majorly available in the form of endospores powders produced via the submerged fermentation (SmF) requiring sophisticated and expensive equipments, not easily implementable in the socio-economic context of Burundi and many developing countries. We proposed the solid state fermentation (SSF) that uses the cost-effective and locally available lignocellulosic substrates for BDI-IS1 spores' production and a dry formulated product made of the residual fermented substrates embedded with BDI-IS1 spores and the excreted metabolites was developed and assessed for biocontrol against nothern corn leaf blight.

Chapter 3

Isolation, molecular identification, and functional-based genetic analysis of the Burundi isolated *Bacillus nakamurai* BDI-IS1

This chapter uncovers a mixture of already published data and others not. The unpublished data are original and were generated by me. However, the two first sub-sections of the results (sections 3.1 & 3.2) about isolation and bio-guided selection of BDI-IS1, and the phenotypical and molecular identification of the strain are adapted from:

Nimbeshaho F., Nihorimbere G., Arias A. A., Liénard C., Steels S., Nibasumba A., Nihorimbere V., Legrève A. and Ongena M. (2024). Unravelling the secondary metabolome and biocontrol potential of the recently described species *Bacillus nakamurai*. *Microbiological Research* 288, 127841. https://doi.org/10.1016/j.micres.2024.127841.

In this paper, GN and I are co-first authors. My contribution consisted in the isolation and the selection the bacterium based on antagonistic activities, its identification, characterisation of its secondary metabolome and the understanding of the relationship between the bioactivity and secreted bioactive compounds. In a collaborative framework, I provided the bacterial material to GN, and he carried out all the plant experiments aimed to assess the biocontrol efficacy of the above characterised bacterium. We jointly wrote the manuscript from the first draft until to responding to reviewers' comments and submission of the final manuscript.

1. Introduction

The development of bacterial inoculants to control plant diseases and improve plant growth is one of the most promising eco-friendly and efficient alternatives to the use of chemical inputs in sustainable modern agriculture (Elnahal et al., 2022; Galli et al., 2024). A group of bacteria is particularly thriving in the close vicinity of plant roots where they establish a beneficial association with their hosts, by promoting plant growth and ensuring good health while feeding on plant secreted root exudates. This group bacteria was hence termed "plant growth promoting rhizobacteria" and include several bacterial genera including *Pseudomonas, Streptomyces, Acetobacter, Azospirillum, Paenibacillus, Serratia, Burkholderia, Rhizobium and Bacillus* (Backer et al., 2018; A. Hasan et al., 2024).

Bacillus spp. have been amply investigated for their potential to both promote the plant growth and development and preserve plants from diseases, especially species of the *Bacillus subtilis* clade. These bacterial species are renown for their ability to form resistant endospores, colonizing quite efficiently the rhizosphere through motility, biofilm formation and secreting effectors helping to cope with inherent soil unfavourable conditions, and establishing a strong mutualistic relationship with plant host (Bhadrecha et al., 2023; A. R. Khan et al., 2022). Indeed, rhizospheric bacilli have been shown to possess the innate potential of secreting an arsenal of phytohormones (auxins, cytokinins, etc.) and hormones-like substances (polyamines) stimulating plant growth and tolerance to stress (Jang et al., 2023; Luo et al., 2022). They have also the potential to produce stress resistance-related proteins and specific compounds, a vast array of lytic enzymes necessary for biodegradating the polymeric nutrient-sources and fungal cell wall lysis (Jang et al., 2023; Luo et al., 2022). In addition, they possess the ability to dissolve insoluble forms of phosphorus and fixing atmospheric nitrogen and make them available to plants into assimilable forms (Patani et al., 2024). Strains of the *B. subtilis* clade provide also their benefits to plants by secreting a wealth of BSMs which suppress directly prominent soil-borne phytopathogens or indirectly induce plant systemic resistance to subsequent biotic and abiotic stresses (S. Iqbal et al., 2023; Salazar et al., 2023; Y. Wang et al., 2024).

Furthermore, as for other microbial species, the success of these *Bacillus*-based products as soil bioinoculants suffers from variable efficacy and inconsistency in real-world agricultural conditions (Marian & Shimizu, 2019). This is due to the high complexity of the ecological context, and many factors may restrict proper invasion of the soil or rhizosphere niche by the introduced strain. This would therefore hamper bacterial establishment and persistence in this highly competitive zone or may not attain threshold

populations necessary to provide the beneficial effects (Hossain et al., 2023; O'Callaghan et al., 2022). Successful invasion does not only depend on the ability of bioinoculants to cope with specific abiotic soil factors including pH, temperature, water content, nutrient status but it is also driven by intricate interactions with the host plant and with the associated microbiome communities whose structure, functions and compositions are also dictated by the plant genotype and inherent soil physico-chemical conditions (Bonaterra et al., 2022; J. Hu et al., 2021; Santos & Olivares, 2021). This supports the importance of keeping mining soils to discover new bacterial isolates with plant growth promotion and biocontrol potential and well-adapted to the field-specific abiotic conditions in which they will be deployed (Novello et al., 2023; Thakur et al., 2022).

In this chapter, we describe a new strain BDI-IS1, isolated in the highlands of tropical Africa (Burundi), belonging to the *Bacillus nakamurai* species and displaying a broad-spectrum antagonistic activity against bacterial and fungal phytopathogens. We provide also genomic insights into its capacity to colonize plant root and adapt to subsequent abiotic stresses that would hamper its persistence in soils, and its potential to enhance plant growth and development.

2. Materials and Methods

2.1. Sample collection, isolation of beneficial bacteria from Burundi

Arable soil samples containing bean and potato roots were collected in Murwi (North-west of Burundi) and Isare (West of Burundi) communes, respectively, for isolation of beneficial bacteria.

Small roots were then selected from soil samples and mixed with peptone water + Tween 80 (2%) and glass beads in a falcon (15 mL). The mixture was vortexed (5 min), serially diluted and plated (100 µL) on LBA (tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L and agar 15 g/L). After 16 h of incubation (30˚C), *Bacillus*-like colonies were selected based on their morphological traits and subcultured (three times) on LBA for purification. Pure isolates were cultured in LB broth at 30°C overnight and stored at -80°C in peptone water + 20% glycerol until use.

2.2. Biological materials

Six plant pathogenic bacteria i.e. *Clavibacter michiganensis* subsp. *michiganensis*, *Rhodococcus fascians* D188, *Pectobacterium carotovorum* LMG6663, *Xanthomonas campestris* subsp. *campestris* LMG582,

Pseudomonas cichorii LMG2162, *Pseudomonas fuscovaginae* LMG2158 and six phytopathogenic fungi i.e. *Fusarium oxysporum* MUCL31742, *Botrytis cinerea* R16, *Rhizoctonia solani* MUCL49235, *Aspergillus niger* MUCL28689, *Pyricularia oryzae* MUCL9191 and *Colletotricum* sp. available in the collection of the Microbial Processes and Interactions (MiPI) Laboratory, Gembloux Agro-Bio Tech, University of Liège, were used in the antagonism assays with the bacterial isolates from Burundi. In addition, fungi i.e. *A. solani*, *E. turcicum* and *A. rabiei* isolated from Burundi-collected diseased leaves of tomato, maize and bean, respectively, were tested. *Bacillus velezensis* QST713 (Serenade Aso®), also from the MiPI collection, was used as a reference standard antagonist.

2.3. Culture preparation

Bacillus-like isolates and plant pathogenic bacteria initially stored at -80˚C were plated on LBA and incubated at 30˚C for 24 h. Individual colonies were then pre-cultured the day before the experiment. On the day of the experiment, all bacterial cultures were centrifuged, and the pellet was washed twice with LB broth, resuspended in the same medium and adjusted to OD_{600} 0.1 and OD_{600} 2 per mL for pathogenic and isolated bacteria, respectively. *B. velezensis* QST713 (hereafter referred to as QST713) was prepared under the same conditions. Five days' old fungal mycelia (cultured on PDA at 25°C) were used in dual confrontation assay with the most promising isolate based on antibacterial activity.

2.4. Antagonistic activity assessment

A dual culture assay was performed to evaluate the antibacterial and antifungal activity of the bacterial isolates. Following the historical background of the sampling site where potatoes were previously grown, solidified Solanaceae root exudates-mimicking medium (REM) (glucose 1 g/L, fructose 1.7 g/L, maltose 0.2 g/L, ribose 0.3 g/L, citrate 2 g/L, oxalate 2 g/L, succinate 1.5 g/L, malate 0.5 g/L, fumarate 0.5 g/L, casamino acids 0.5 g/L, $(NH_4)_2SO_4$ 1 g/L, KH_2PO_4 0.3425 g/L, MOPS 11.5 g/L, $MgSO_4 \times 7H_2O$ 0.25 g/L, KCl 0.25 g/L, yeast extract 0.5 g/L, 50 µL of $Fe_2(SO_4)_3$ 1.2%, 50 µL of $MnSO_4$ 0.4%, 50 µL of $CuSO_4$ 1.6%, 50 µL of Na_2MoO_4 4%, Agar 15 g/L) (V. Nihorimbere et al., 2012) was selected for antibacterial activity assessment. Suspension of pathogenic bacteria (OD_{600nm} 0.1) was spread homogeneously on REM agar medium in square plates (12 cm×12 cm) and were incubated at room temperature for approximately one hour to dry. Bacterial isolates and QST713 suspensions (OD_{600nm} 2) were spotted (5 µL) on the plate coated with the pathogenic bacteria. Incubation was performed at

30˚C for 48 h. Each bacterial suspension was tested in triplicate against each plant pathogen, with two independent experiments (n = 6). Activity was expressed as mean values (± δƐ) of the inhibition diameter (mm) around the bacterial spot.

For antifungal activity evaluation, a mycelial plug (5 mm of diameter) of each fungus grown on PDA was placed at the centre of a PDA plate and 5 µL of the best bacterial isolate (based on antibacterial potential) suspension (OD_{600} 2) was spotted 25 mm away from the centre. The inhibition radius was recorded when control growth reached a radius of 25 mm and the percentage of inhibition was calculated using the following formula: Inhibition (%) = ((A-B)/A) X 100), where A and B are the growth radius of the control and treated fungi, respectively. Each confrontation was tested in triplicate, and two independent experiments were performed (n = 6).

2.5. Molecular identification and comparative genomic functional analysis

2.5.1. Molecular identification

Genomic DNA of the promising bacterial isolates was extracted using the Thermo Fischer Scientific Gram-positive bacteria genomic DNA Extraction Kit according to the manufacturer's standard procedure and was quantified using a Nanodrop ND-1000 spectrophotometer. The 16S rRNA gene was amplified by PCR using the primers 8F (5' - AGAGTTTGATCHTGGCTCAG - 3') and 1492R (5' - GGHTACCTTGTTACGACTT - 3'), Q5® High-Fidelity DNA Polymerase (New England BioLabs). After electrophoresis (1% agarose gel), the amplicons were further purified using the Thermo Fischer Scientific GeneJET Genomic DNA Purification Kit according to the manufacturer's standard procedure. The 16S rRNA amplified fragments were sequenced by Eurofins Genomics (Anzinger, Germany) and were blasted for homology searching to the NCBI Genebank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Whole genome sequencing (WGS) was also performed for the most striking isolate. Libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina), and the sequencing was performed on the Illumina MiSeq v3 platform using 600 bp paired-end chemistries (2 X 300 bp read length). Illumina read adapters and low-quality bases were trimmed with Trimmomatic0.39. Draft genomes were assembled using SPAdes v3.13.0.

2.5.2. Genomic characterization, phylogenetic analysis and comparative genetic-based functional analysis

Quality of the assembled WGS was evaluated by NCBI-inserted CheckM tool and annotation was carried out by GenMarkS-2+ method of NCBI to provide the intrinsic features of the genome. Proteomic sequence was generated and annotated by Prokka webtool (Seemann, 2014) and a circular map of chromosomal DNA was generated using Proksee software (Grant et al., 2023). Antibiotic resistance genes and self-immune system genes were analysed by Proksee incorporated webtools CARD/RIB (comprehensive antibiotic resistance database/ Resistance Gene Identifier) (Alcock et al., 2023) and CRISPR/Cas Finder (Clustered Regulary Interspaced Short Palindromic Repeats) (Couvin et al., 2018).

Phylogenetic analysis was performed using the automated online tool Type Genome Server (TYGS) comparing the submitted genome against a set of selected related species by considering the digital DNA-DNA hybridization (dDDH) and G+C content (Meier-Kolthoff et al., 2014; Meier-Kolthoff & Göker, 2019) and the tree was generated by the TYGS-integrated method GBDP (Genome BLAST Distance Phylogeny) using the classical neighbour joining technique (Henz et al., 2005; Meier-Kolthoff, Auch, et al., 2013). Average Nucleotide Identity (ANI) analysis was performed with FastANI (Jain et al., 2018) to appreciate the taxonomic status of the sequenced strain among its pairs of the same species and other close related species. Comparative analysis of orthologous gene clusters of the sequenced strain with close related species was performed and visualized on Venn diagrams using the OrthoVenn 3 webtool (J. Sun et al., 2023). In parallel, this webtool also carried out expansions and contractions analysis which helps to decipher the genetic modifications in line with environmental adaptation and invasion all along species/strain evolution and to provide estimation of split time between the strain of study and closely related species/strain (J. Sun et al., 2023).

Comparative functional analysis between the strain genome and closely related species/strains was undertaken using the RAST (Rapid Annotation using Subsystems Technology) server that classifies protein-encoding genes into functional categories (herein termed subsystems) which provide insights into the potential of the sequenced strain to metabolize proteins and carbohydrates, mobilize and metabolize iron, cope with environment stress, etc. (Brettin et al., 2015; Overbeek et al., 2014). Plant growth promotion and development (PGPD) genes within the strain genome were mined by aligning the PGPD genes from the *B. subtilis* 168 (Pedreira et al., 2022) and *B. velezensis* FZB42 (Fan et al., 2019) databases against the studied strain genome using the NCBI Blast online platform (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

3. Results and discussion

3.1. The isolate BDI-IS1 as strong antagonist of plant pathogens

This work was initiated by screening bacteria in rhizosphere soil samples collected from crop fields at two different locations in Burundi. Our objective was to isolate endemic bacilli, and we selected a subset of sixteen isolates (Figure 3-1) characterized by a rapid growth (≤ 20h) and typical white *Bacillus*-like colonies on solid LB medium. These isolates were first tested for their antagonistic potential against a range of phytopathogenic bacteria including species known as important plant pathogens with high economic impact notably in tropical and subtropical regions (Ndayihanzamaso et al., 2016; Nyabyenda, 2005, 2006). Antibacterial activity was chosen as prime criterion for screening because this trait is not only critical to the overall efficacy of broad-spectrum biocontrol agents (S.-Y. Wang et al., 2022) but also important for the ecological fitness and persistence in the competitive rhizosphere niche (Afridi et al., 2022; Andrić et al., 2023; Weiland-Bräuer, 2021). Moreover, the outcome of bacterial interactions in general and of antagonistic competition in particular may vary according to various conditions including the medium (Garrido-Sanz et al., 2023; X. Sun et al., 2022). It is why, instead of common laboratory media, we performed these confrontation assays on an agar-solidified Root Exudates-Mimicking medium (REM) designed to reflect the nutritional context of the rhizosphere in Solanaceae plants (Hoff et al., 2021).

Based on the size of the inhibition zone developed around the colony, the isolate BDI-IS1 displayed the highest and most consistent antibacterial activity against all the tested bacterial pathogens, followed by the isolates BDI-M4, BDI-M12, BDI-M13 and BDI-IS3 with antagonistic effect against five (*X. campestris*, *C. michiganensis*, *R. fascians*, *P. cichorii* and *P. fuscovaginae*) out the six selected bacterial pathogens, except *P. carotovorum* (Figure 3-1A & B). The broad range antibacterial activity of the most striking isolate BDI-IS1 was comparable to the one observed for the commercial strain *B. velezensis* QST713, a well-known biocontrol agent in agriculture (Serenade Aso®, Bayer Crop Science, Germany) (Anastassiadou et al., 2021). A very high inhibition was observed against *C. michiganensis*, *R. fascians* and *X. campestris* for both BDI-IS1 and QST713, while *P. carotovorum*, *P. cichorii* and *P. fuscovaginae* were moderately inhibited (Figure 3-1A).

The most promising isolate BDI-IS1 (BDI: Burundi, IS1: the 1st isole of Isare site) was also evaluated for its antifungal activity against a range of phytopathogenic fungi reported as threats to agriculture in tropical regions

including species that we isolated from diseased plants in Burundi (Figure 3-1E). Data revealed the broad-spectrum activity of BDI-IS1, at levels comparable to QST713, with very high antifungal potential against *P. oryzae* and *Colletotrichum* sp., good inhibitory potential against *A. rabiei*, *A. solani* and *E. turcicum*, and moderate inhibition towards *A. niger*, *B. cinerea* and *F. oxysporum* (Fig.3-1C & D).

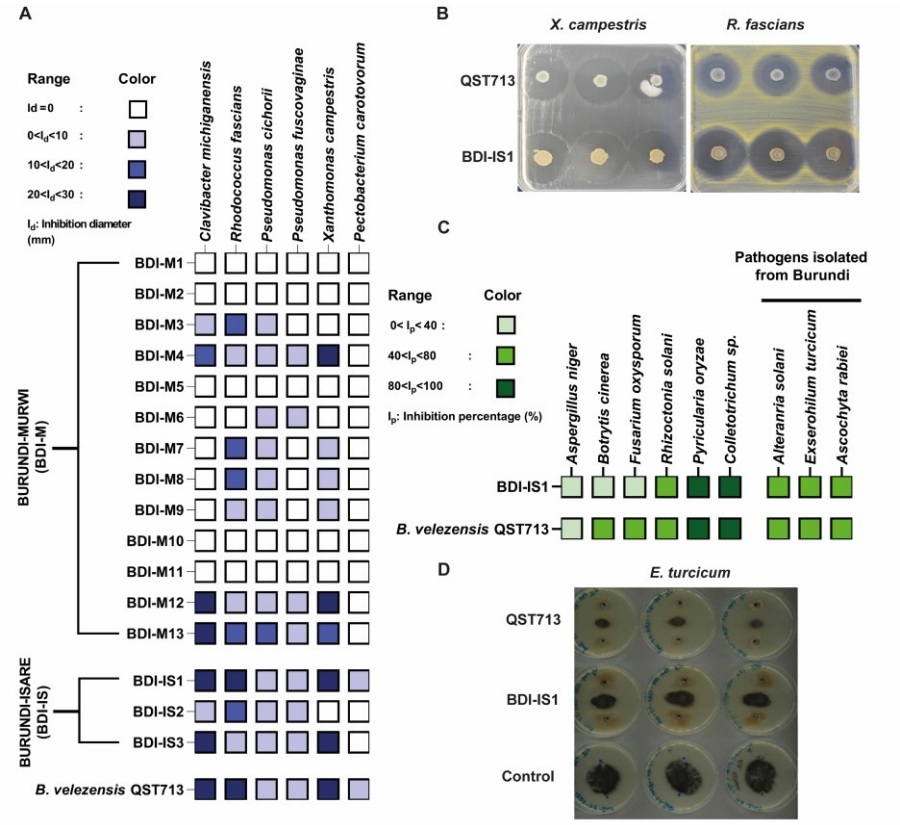


Figure 3-1: Antagonistic potential of the Burundi isolated strain BDI-IS1 against phytopathogens. A. Antibacterial activity assessment of bacterial isolates (BDI-M1 to BDI-M13 and BDI-IS1 to BDI-IS3) against a set of bacterial phytopathogens in comparison to *Bacillus velezensis* QST 713 (Serenade®), through dual culture assay on gelified REM (30ml, 30°C, 48h). The inhibition potential is illustrated in blue boxes with a gradual increase of color font in line with the increasing range of inhibition diameter (I_d); data are means of inhibition diameter (± SE, mm) and evolve from two independent experiments conducted each in triplicate (n = 6) (Table S2). B. Illustration of inhibition growth of *X. campestris* and *R. fascians* exhibited by the isolate BDI-IS1 and *B. velezensis* QST713. C. Antifungal activity evaluation of the isolate BDI-IS1 and *B. velezensis* QST713 against fungal

phytopathogens through dual confrontation assay. Inhibition percentages (partitioned into different ranges) are represented in green boxes with color font intensity increasing with inhibition percentage range, data are means (± SE) of triplicates repeated independently twice (n = 6) (Table S3). D. Illustration of antagonistic activity of BDI-IS1 and QST713 against *E. turcicum* isolated from diseased maize leaves from Burundi, in a dual culture assay set-up.

3.2. Molecular and phenotypical identification of the promising bacterial isolates

Genomic DNA of the six best antagonistic isolates (BDI-M4, BDI-M12, BDI-M13, BDI-IS1, and BDI-IS3) were further extracted and the 16S rRNA gene was sequenced for identification purpose. The respective 16S rRNA gene sequences were blasted on NCBI Genebank and were found closely related to *B. altitudinis*, *B. wiedmannii*, *B. australimaris* and *B. nakamurai*, respectively, for the duo BDI-M4 & BDI-M13, BDI-M12, BDI-IS3 and the best antagonistic isolate BDI-IS1 (Table 3-1). *B. altitudinis* and *B. australimaris* are close phylogenetically to *B. pumilus* species (Y. Liu et al., 2016), known for producing bioactive secondary metabolites with antibacterial activity (Rudakova et al., 2023). *B. wiedmannii* is however a strain that belongs to the *B. cereus* group, which comprises the bioinsecticide *B. thuringiensis* and other pathogenic or potentially dangerous species (Miller et al., 2016).

Table 3-1: 16S rRNA gene sequence-based molecular identification of the best bacterial isolates from Burundi. b implies that the NCBI acc. # stand for the closely related species and not the accession number of deposited sequence.

Bacterial isolate	Closely related species[b]	Query cover (%)	% of identity	NCBI acc. #[b]
BDI-M4	*Bacillus altitudinis* 11-1-1	98	99.66	CP054136.1
BDI-M12	*Bacillus wiedmannii* HBUM207173	100	99.14	MT239515.1
BDI-M13	*Bacillus altitudinis* H31	100	99.58	OQ552799.1
BDI-IS1	*Bacillus nakamurai* NRRL B-41091	100	97.7	NR_151897.1
BDI-IS3	*Bacillus australimaris* ROA051	99	99.09	MT525306.1

The species name *B. nakamurai* to which could belong the most promising antagonistic isolate BDI-IS1 was first described in 2016 and was isolated

from soil samples collected from Argentina (Dunlap et al., 2016). Phenotypically, the species *B. nakamurai* was described as black pigment producer on tryptic soy agar (TSA) medium (Dunlap et al., 2016) and our strain BDI-IS1 conserved this particular trait upon cultivation on TSA and on REM Agar after 48h of incubation (Figure 3-2), bringing more evidence to its close relatedness to *B. nakamurai* strains. This black pigment observed with *B. nakamurai* strains is also reported in other members of *Bacillus subtilis* group including among others *B. atrophaeus* (L. K. Nakamura, 1989), and *B. subtilis* 4NP-BL (Ghadge et al., 2020). This melanic pigment biosynthesized mainly via two pathways involving tyrosinases or laccases is associated to environmental stresses resistance such as UV rays and oxidative stress (Ghadge et al., 2020; D. Saxena et al., 2002) and was found to have antibacterial activity against some plant pathogens including *X. campestris* (Ghadge et al., 2020).

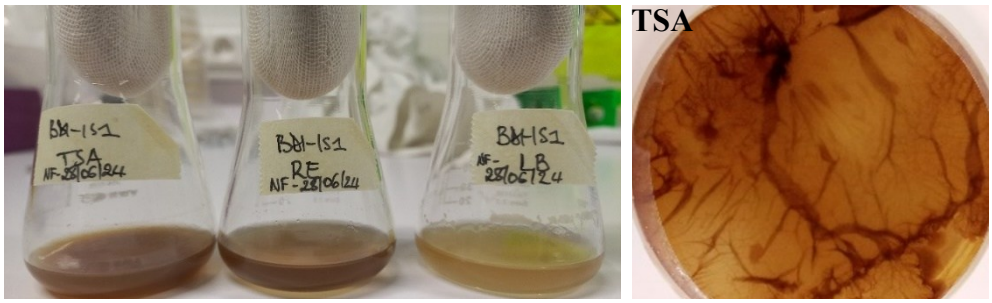


Figure 3-2: Black pigment production by BDI-IS1. Culture was set in different media including TSA, REM and LB (OD_{600nm} 0.1) and incubated for 72h (30°C, 150rpm).

Beyond the antagonistic activity of *B. nakamurai* occasionally reported against different strains of the fire blight causing agent *Erwinia amylovora* (Leathers et al., 2020), *Fusarium poae* causing the head blight disease in many cereals (Zanon et al., 2024) and the broad host range necrotrophic *B. cinerea* (Chaouachi et al., 2021), this is the first report of such a broad-antagonistic property against bacterial and fungal pathogens affecting different crops of worldwide economic importance. Our data harness that this broad antimicrobial potential is comparable to the well-known biocontrol agent *B. velezensis* QST713 (Serenade Aso®, Bayer Crop Science, Germany) and this provides an important basis to its consideration as potential biocontrol candidate. After the isolate identification by 16S rRNA gene sequence-based technique (NCBI # : OP546292) and phenotypic observation, this strain was fully sequenced for a better characterization and an in-depth appreciation of its plant beneficial potential at genomic level.

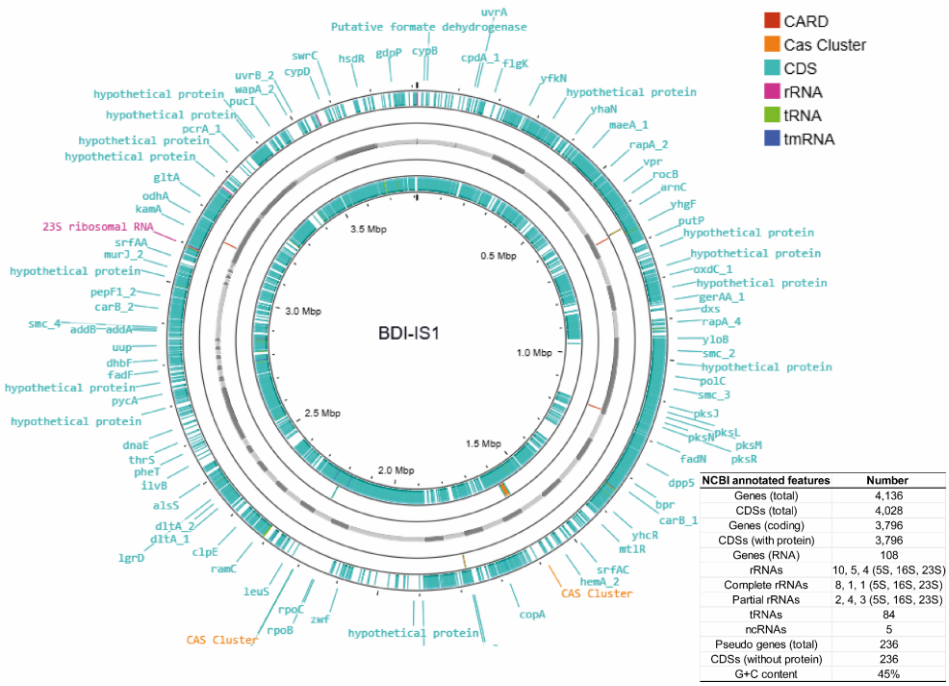
Since the description of this species in 2016, only five strains including BDI-IS1 were described and sequenced up to date including *B. nakamurai* NRRL B-41091 (GCF_001584325.1) and *B. nakamurai* NRRL B-41092 (GCF_001584345.1), *B. nakamurai* MZ03-67 (GCF_024241155.1) and *B. nakamurai* B-41093 (GCF_036210565.1). Other not fully sequenced isolates were also described as putative strains of *B. nakamurai* based on 16S rRNA gene sequencing (Chaouachi et al., 2021; Shaikh et al., 2023).

3.3. Genomic features of BDI-IS1

The genomic DNA of BDI-IS1 was *de novo* sequenced by Illumina MiSeq v3 (600 bp pair-ended) and the raw reads were quality controlled (2 X 300 bp) and assembled by SPAdes method with a genome coverage of 43.0X. The assembled draft genome sequence (111 contigs, N50: 89.5 kb, L50: 13) was deposited on NCBI genebank (GCA_020905065.1), reviewed by NCBI RefSeq (GCF_020905065.1). Further information can be found through https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_020905065.1/.

The GenMarkS-2+ v6.9 method-based NCBI annotation revealed that BDI-IS1 genome has a size of 3,811,863 bp, 45% of guanine-cytosine content (GC content) and 4,136 total genes with 4,028 coding sequences (CDSs) and 108 RNA genes (Figure 3-3). The sequence was complete at 99.41% (60th percentile) and no contamination was detected through NCBI-inserted CheckM analysis (v1.2.3) method.

By using Proksee mapping webtool (Grant et al., 2023), a circular map of BDI-IS1 genome sequence was generated along with Prokka-derived protein annotation (Seemann, 2014). The different genes such as rRNA, tRNA, tmRNA, antibiotic resistance genes generated from the CARD (comprehensive antibiotic resistance database) and RIB (resistance gene identifier) programmes (Alcock et al., 2023) were also localized on the map. In addition, the genes derived from CRISPR/Cas Finder encoding for bacterial self-immunity against virus, phages and other external dangerous elements (Couvin et al., 2018; Y. Xu & Li, 2020) were also mounted on the map. Indeed, three resistance antibiotic genes were detected by the software including *vanT*, *vanY* encoding for the glycopeptide vancomycin antibiotic cassette and *qacJ* encoding for the disinfecting agent benzalkonium chloride. Moreover, four Cas (**C**RISPR-**as**sociated) clusters were found, providing insights into the capacity of the strain to protect itself against external viral enemies.



NCBI annotated features	Number
Genes (total)	4,136
CDSs (total)	4,028
Genes (coding)	3,796
CDSs (with protein)	3,796
Genes (RNA)	108
rRNAs	10, 5, 4 (5S, 16S, 23S)
Complete rRNAs	8, 1, 1 (5S, 16S, 23S)
Partial rRNAs	2, 4, 3 (5S, 16S, 23S)
tRNAs	84
ncRNAs	5
Pseudo genes (total)	236
CDSs (without protein)	236
G+C content	45%

Figure 3-3: Circular genome sequence of BDI-IS1 with annotated key features. The map of BDI-IS1 was generated by Proksee software after uploading the FASTA file of BDI-IS1 whole genome sequence. Annotation of genes including ribosomal RNA (rRNA), transfer RNA (tRNA), transfer messenger RNA (tmRNA) and coding sequences (CDSs) were generated by PROKKA inserted within Proksee software and added by the latter on the map. The table on the bottom-right corner denotes the intrinsic features ad their magnitude as provided by NCBI Genebank.

3.4. Phylogenetic analysis of BDI-IS1 and its genomic comparison with related Bacillus spp.

3.4.1. Phylogenetic analysis

For better appreciation of the phylogenetic status of this newly isolated and described strain BDI-IS1, we used an automated online tool Type (strain) Genome Server (TYGS) (Meier-Kolthoff & Göker, 2019). The latter combines the latest techniques devoted for species delineation such as digital DNA-DNA hybridization (dDDH) and the classic, but still consistent, DNA G+C content assessment (Meier-Kolthoff et al., 2014). Instead of relying on the 16s rRNA marker with reduced dealination efficiency for closely related strains with more than 97% of similarity (Meier-Kolthoff, Göker, et al., 2013), the whole genome sequences were used in our phylogenetic

assessment. The optimized TYGS-integrated programme Genome BLAST Distance Phylogeny (GBDP) calculates the distances between the high-scoring segment pairs (HSPs) from a BLAST-based pairwise comparison and generate a phylogenetic tree from these genome-to genome distances by the neighbour joining technique (Henz et al., 2005; Meier-Kolthoff, Auch, et al., 2013). BDI-IS1 was phylogenetically compared to other 17 related *Bacillus* strains based on their whole genome sequences, and a tree was generated (Figure 3-4). The most closely related strain was found to be *B. nakamurai* NRRL B-41091 and this confirms the prior assignment of this strain to *B. nakamurai* species based on 16S rRNA gene sequence. This species shares the direct common ancestor of the strains such as *B. amyloliquefaciens* DSM7, *B. siamensis* KCTC 13613 and *B. velezensis* strains (Figure 3-4), confirming its membership into the *B. amyloliquefaciens* operational group (Ngalimat et al., 2021).

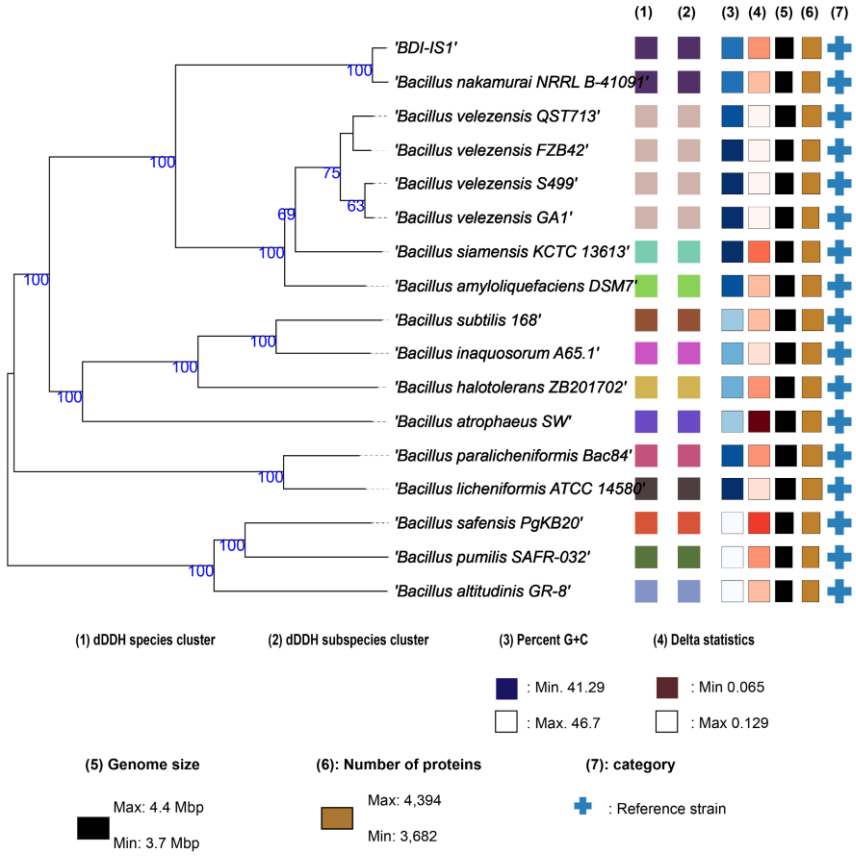


Figure 3-4: TYGS-derived phylogenetic tree of BDI-IS1 compared to other strains of the *B. subtilis* group. The tree was inferred with FastME 2.1.4 from GBDP distances calculated from genome sequences. Branch lengths are scaled in terms of GBDP distance formula d_5; numbers above branches are GBDP pseudo-bootstrap support values from 100 replications. Leaf labels are annotated by affiliation to species cluster (1) and subspecies cluster (2), genomic G+C content (3), δ values (4), overall genome size (5), number of proteins (6), and the category of strain (7).

3.4.2. Orthologous gene clusters-based comparative analysis

To better understand the level of relatedness between BDI-IS1 and other *B. nakamurai* strains, on one hand, and some other common *Bacillus* strains of agricultural importance, comparative analysis using average nucleotide identity (ANI) and orthologous gene clusters was carried out. FastANI analysis conducted within Proksee software (Jain et al., 2018) leverages the close relatedness between the five *B. nakamurai* strains with ANI values more than 98%, while the ANI value was 86% when compared to *B. velezensis*

strains and *B. amyloliquefaciens*, and around 80% against *B. subtilis* 168 (Table 3-2). These ANI values cope with golden rule for species delineation where the threshold of ANI value is set at 95-96% (M. Kim et al., 2014) and, thereby, evidence the taxonomic identity and position of the strain BDI-IS1.

Table 3-2: ANI-based comparison of BDI-IS1 and closely related strains.

Strains	ANI (%)	Protein count	Singletons count (B)	Singletons count (C)
B. nakamurai BDI-IS1		3,787	231	284
B. nakamurai NRRL B-41091	98.8014	3,526	120	-
B. nakamurai NRRL B-41092	98.575	3,743	46	-
B. nakamurai B-41093	98.6721	3,834	59	-
B. nakamurai MZ03-67	98.9532	3,643	169	-
B. velezensis FZB42	86.7807	3,710	-	81
B. velezensis QST713	86.9031	4,056	-	192
B. amyloliquefaciens DSM7	86.9645	3,922	-	266
B. subtilis 168	80.8068	4,243	-	709

Furthermore, comparative analysis of orthologous gene clusters (J. Sun et al., 2023) among *B. nakamurai* strains outlined onto the Ven diagram (Figure 3-5) shows that the strains have a share of 3,020 core orthologous cluster genes (75.5%) out of the total of 3,996 detected gene clusters at species level (Figure 3-5A). Correlatively, the overall orthologous gene families for each strain represent 88.4% (3,534), 84.7% (3,384), 92.1% (3,682), 93.9% (3,754) and 86.4% (3,455) for BDI-IS1, NRRL B-41091, NRRL B-41092, B-41093 and MZ03-67, respectively. On the other hand, comparison of *B. nakamurai* BDI-IS1 with other strains of the *B. subtilis* group revealed a total of 4,119 gene families, with 2,844 (69%) highly conserved orthologous gene clusters (Figure 3-5B) and 3,457 (83,9%), 3,602 (87.4%), 3,591 (85.5%), 3,807 (92.4%), 3,482 (84.5%) genes groups respectively found in *B. nakamurai* BDI-IS1, *B. amyloliquefaciens* DSM7, *B. velezensis* FZB42, *B. velezensis* QST713 and *B. subtilis* 168. This gene family distribution between species, especially the conserved orthologous genes group shows that BDI-IS1 is phylogenetically closer to other *B. nakamurai* strains than the other related *Bacillus* spp. considered in our analysis, which corroborates with the already established phylogeny and derived-evolutionary relationship between species *B. nakamurai*, *B. amyloliquefaciens*, *B. velezensis* and *B. subtilis*.

This same analysis revealed that BDI-IS1 genome bear more singletons genes (Table 32-2), also called taxonomically restricted genes (TGRs) (Karlowski et al., 2023), than its close related *B. nakamurai* strains and falls behind *B. subtilis* 168 when compared to the other *Bacillus* spp. This richness

in orphan genes (TGRs) with no corresponding sequence into the considered strains for alignment implies the existence of additional proteins with putative new biological functions (Karlowski et al., 2023) or environment adaptive roles (Cubry et al., 2017). Furthermore, expansions and contractions analysis (Figure 3-5C) for environmental adaptation and invasion (J. Sun et al., 2023) revealed that BDI-IS1 has undergone three expansions and 144 contractions compared to its close ancestor. The estimated split time is about 2 million years between BDI-IS1 and its closely related species *B. amyloliquefaciens* DSM7 and *B. velezensis* strains, and approximately 4.15 million years when compared to *B. subtilis* 168 (Figure 3-5C). These insights into expansion and contraction features are very useful in evolutionary studies since they denote specific strategies developed by different strains to adapt to various and fluctuating niches where abiotic conditions exert a positive or negative pressure on living organisms (M. Y. Chen et al., 2021), mounting thereby genetic differentiation and strain diversity.

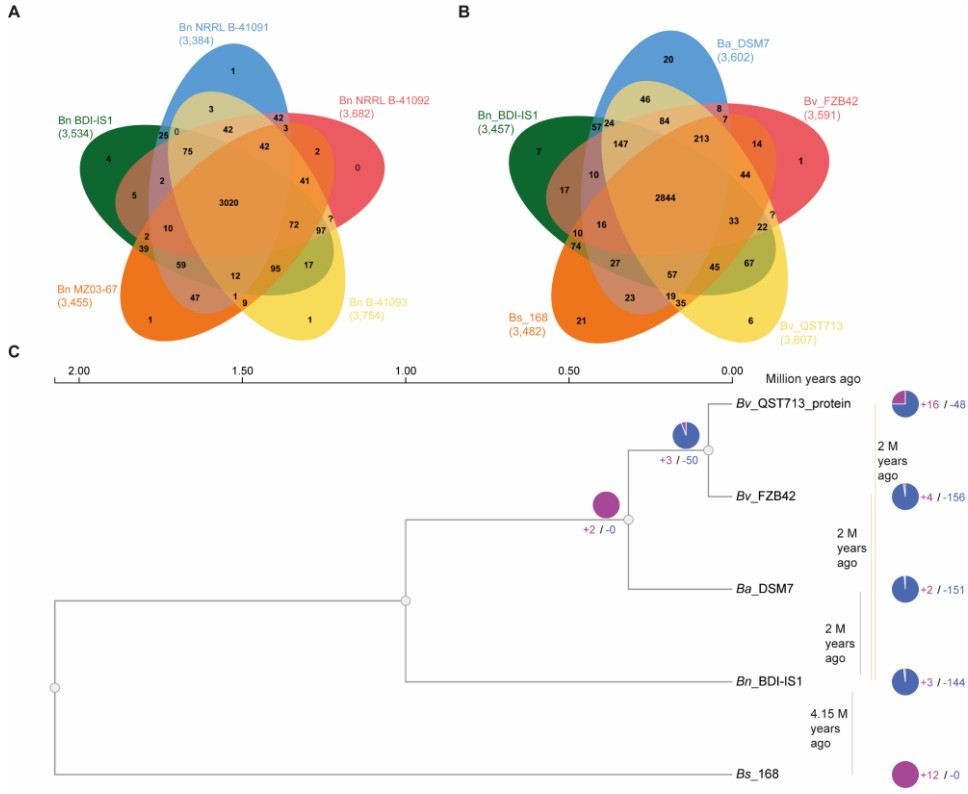


Figure 3-5: Evolutionary relationship analysis of *B. nakamurai* BDI-IS1. A & B Venn diagrams constructed based on the comparative study of orthologous gene

clusters between BDI-IS1 and its closely related *B. nakamurai* strains and between BDI-IS1 and other related *Bacillus* spp. including *B. subtilis* 168 (Bs 168), *B. amyloliquefaciens* DSM7 (Ba DSM7), *B. velezensis* FZB42 (Bv FZB42) and *B. velezensis* QST713 (Bv QST713). C. Phylogenetic tree shows the evolutionary timeline of the species, while the pie chart indicates the number of gene families that have expanded (purple) and/or contracted (blue) during species evolution. The split time data were calculated inside the OrthoVenn3 software (J. Sun et al., 2023) by adding the values corresponding to the different branches.

3.5. Functional annotation of coding sequences and comparison with related *Bacillus* species

3.5.1. Functional analysis of BDI-IS1

In a bid to profile the functional diversity associated with BDI-IS1 proteome, the multiple CDSs of the BDI-IS1 genome were assigned to different functional groups by the aid of RAST (Rapid Annotation using Subsystem Technology) server. The latter identifies and annotates protein-encoding genes (CDSs), and distributes them into functional categories, here named subsystems (Aziz et al., 2008; Brettin et al., 2015; Overbeek et al., 2014). The RAST functional annotation of BDI-IS1 covers 36% (1,542) of the total number of CDSs described in the genome by RAST pipeline (4,268). This coverage is relatively low due to reduced number of *in-house* completely defined metabolic pathways, but this can be improved by merging RAST with other tools such as KEGG for a more complete functional annotation. Nevertheless, this RAST-based functional annotation provides great insights into the ability of BDI-IS1 to colonize plant, especially into the highly competitive rhizosphere niche. Indeed, about 11% of the clustered CDSs belong to subsystems involved in efficient bacterial root colonization and secretion of bioactive secondary metabolites with antagonistic effects against phytopathogens i.e. “motility and chemotaxis”, “secondary metabolism”, “membrane transport”, “virulence, disease and defense”, and “stress response” (Figure 3-6). Genes involved in nutrients sequestration were also found in this classification and encompass subsystems like “iron acquisition and metabolism”, “potassium metabolism”, “phosphorus metabolism” and “carbohydrates” with recognized implication in plant growth promotion and development.

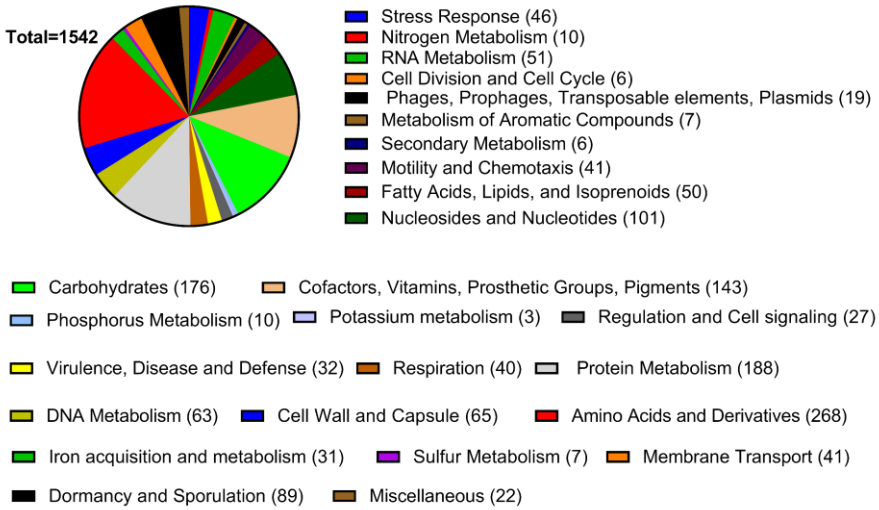


Figure 3-6: RAST-Seed functional annotation of *B. nakamurai* BDI-IS1 coding DNA sequences. The pie chart shows the distribution of different annotated subsystems. The count of each subsystem is indicated in brackets in front of each subsystem name and is illustrated by the width of corresponding coloured slice on the pie chart.

3.5.2. Comparative analysis of the functional diversity

Comparative analysis of the RAST functional annotated subsystems of BDI-IS1 and its closely related *B. nakamurai* strains do not reveal substantial differences. However, BDI-IS1 has one more gene family involved in oxidative stress response, in protein metabolism (except for B-41093) and in amino acids and derivatives uptake (Table 3-3). Indeed, function-based comparison of BDI-IS1 and NRRL B-41091 highlights a unique presence in BDI-IS1 of gene clusters relative to D-serine/D-alanine/glycine transporter, two set of genes encoding enzymes involved in the catabolism of the sugar alcohol inositol and gene clusters encoding the phenolic acid decarboxylase. In contrast, BDI-IS1 is the least equipped in proteins sustaining the metabolism of carbohydrates (Table 3-3). For instance, the function-based comparison revealed that it lacks some enzymes like fructokinase, and lactate 2-monooxygenase implicated in acetogenesis from pyruvate.

Furthermore, comparison of the RAST functional annotated subsystems of *B. nakamurai* strains, represented by BDI-IS1, with other related *Bacillus* strains of the *B. subtilis* clade represented by *B. velezensis* QST713, *B. velezensis* FZB42, *B. amyloliquefaciens* DSM7 and *B. subtilis* 168, denotes important differences related to stress response, iron acquisition and metabolism, and aromatic compounds metabolism. For instance, in

comparison with *B. velezensis* strains, BDI-IS1 harbours more stress response-related genes clusters including two oxidative stress enzymes lactoylglutathione lyase and alkyl hydroperoxide reductase subunit C-like protein and has evolved with additional iron acquisition and metabolism systems namely the ferrous iron transporter proteins EfeUOB (elemental Fe, efe). EfeB is a bifunctional periplasmic transporter protein which binds with specific high affinity to ferrous iron (Fe II) while inducing its oxidative conversion to ferric iron (Fe III), and it reverts a peroxidase activity protecting cells from oxidative stress. This ferric iron is then taken up by another transport periplasmic protein EfeO, which henceforth transfers it to the transmembrane transport permease EfeU responsible of its delivery to the cytosol. This operon *efeUOB* has the ability to mobilize the two forms of iron Fe (II) and Fe (III), and this suggests either a tripartite combined efforts of all the genes *efeB-efeO-efeU* in the case of ferrous iron or the concert of only *efeO* and *efeU* in the case of uptake of the extracytosolic ferric iron (Miethke et al., 2013). This Fur-independent iron uptake system is conserved also in *B. amyloliquefaciens* DSM7 and *B. subtilis* 168 and represent an additional tool for environmental ferric iron sequestration or an interesting alternative enabling the uptake of ferrous iron in micro/anaerobic and/or acidic environments where this reduced form (Fe^{2+}) is predominant (Miethke et al., 2013; Okumura et al., 2024; E. M. Roy & Griffith, 2017).

In addition, this comparative study shows that BDI-IS1 houses a set of heme/hemin efflux genes that are missing in *B. velezensis* strains, *B. amyloliquefaciens* DSM7 and *B. subtilis* 168, and which are responsible for bacterial cells' protection against heme/hemin toxicity (H. Nakamura et al., 2022; Stauff et al., 2008). Indeed, both considered *Bacillus* spp. are equipped with genes involved in the uptake and utilization of heme and hemin, two congeneric iron-complexed porphyrin (a tetrapyrrole) molecules, respectively containing ferric and ferrous iron and which are found in both metazoans and plants (Dutt et al., 2022; Kaya et al., 2023). Heme/hemin does not constitute only an interesting alternative source of iron for animal- or plant-associated bacteria (either beneficial or pathogenic bacteria) (Choby & Skaar, 2016; S. Park et al., 2014), but also it is an important detoxifying agent by limiting oxidative stress caused by heavy metals at the benefit of producing organism (Anita et al., 2024; Cui et al., 2024; N. Singh & Bhatla, 2022). However, this iron-containing complex can be very harmful for host-associated bacteria when available in excessive concentrations as it has been described in pathogenic bacteria, hence the rationale of efflux system into the latter (Anzaldi & Skaar, 2010; M. Wang et al., 2023). Noteworthy, this efflux system is, for the first time, described in beneficial bacilli and underscores the adaptive ability of *B. nakamurai* strains in diverse environments.

Moreover, *B. nakamurai* species, represented by BDI-IS1, was found to conserve, together with *B. subtilis* 168, a specific enzyme biphenyl-2,3-diol 1,2-dioxygenase that is described to have a strong degrading activity of the highly toxic polychlorinated biphenyls (PCBs) (Behan-Bush et al., 2023; Ngoubeyou et al., 2022; Sakai et al., 2002; C. Yu et al., 2022a). Although other genes involved in biphenyls biodegradation were detected in *Bacillus* spp. (Z. Han et al., 2023; Semerikova et al., 2022), this enzyme previously described in strains of *Pseudomonas*, *Rodococcus*, *Stenotrophomonas* (Garrido-Sanz et al., 2018; Waigi et al., 2015; C. Yu et al., 2022b) is an additional trait of these soil-dwelling *Bacillus* spp. enabling them to survive even in PCBs-polluted environments and can, therefore, be exploited in soil remediation strategies.

Table 3-3: Comparative RAST server functional annotation of *B. nakamurai* BDI-IS1 and other related *Bacillus* strains.

Subsystems	Bn BDI-IS1	Bn B-41091	Bn B-41092	Bn B-41093	Bn MZ03-67	Bv QST713	Bv FZB42	Ba DSM7	Bs 168
Stress Response	46	45	44	44	44	40	41	40	44
Nitrogen Metabolism	10	9	9	9	10	20	20	19	19
RNA Metabolism	51	50	51	50	49	52	50	50	53
Photosynthesis	0	0	0	0	0	0	0	0	0
Cell Division and Cell Cycle	6	6	6	6	6	6	6	6	4
Phages, Prophages, Transposable elements, Plasmids	19	19	24	24	10	21	0	14	12
Metabolism of Aromatic Compounds	7	8	7	7	7	9	10	9	10
Secondary Metabolism	6	6	6	6	6	6	6	6	6
Motility and Chemotaxis	41	41	41	41	41	42	42	42	45
Fatty Acids, Lipids, and Isoprenoids	50	50	50	50	50	52	51	50	49
Nucleosides and Nucleotides	101	100	104	104	101	101	93	99	110
Cofactors, Vitamins, Prosthetic Groups, Pigments	143	141	145	145	141	145	142	142	145
Carbohydrates	176	184	183	180	182	213	211	193	243
Phosphorus Metabolism	10	10	10	10	10	10	10	10	11
Potassium metabolism	3	4	3	3	3	3	3	3	3

Regulation and Cell signaling	27	25	26	26	25	26	26	26	27
Virulence, Disease and Defense	32	32	33	33	32	35	36	36	35
Respiration	40	40	40	40	39	38	38	37	36
Protein Metabolism	188	180	159	190	177	202	207	210	168
DNA Metabolism	63	62	62	62	63	64	62	63	69
Cell Wall and Capsule	65	68	67	67	64	77	67	76	79
Amino Acids and Derivatives	268	265	258	258	267	271	273	279	279
Iron acquisition and metabolism	31	31	31	31	30	22	22	26	31
Sulfur Metabolism	7	7	7	7	7	6	6	6	7
Membrane Transport	41	42	41	41	41	41	41	42	42
Dormancy and Sporulation	89	90	92	91	90	88	88	93	90
Miscellaneous	22	23	22	22	22	22	22	22	22

3.6. Genetic basis of plant growth promotion and development properties of BDI-IS1

Plant growth promotion and development is an interesting feature for plant root-associated bacteria, and this multifaceted trait is supported by dedicated genes that can be mined within the bacterial genome by BLAST alignment with known public and interconnected database i.e. *subti*wiki and Amylowiki (Fan et al., 2019; Pedreira et al., 2022), in case of the strains of the *B. subtilis* clade. Indeed, as shown above (Figure 2-4), BDI-IS1 is closely related to the model strain of root-associated bacilli, *Bacillus velezensis* FZB42 (previously *B. amyloliquefaciens* subsp. *plantarum* FZB42) known for its broad plant growth promotion virtues (Fan et al., 2018).

3.6.1. Polymeric-substrates degradation and niche establishment

Genome mining revealed the presence of several genes involved in primary nutrients acquisition such as exoenzymes interplaying the breakdown of polysaccharides, proteins and lipids, and in the successful establishment of *Bacillus* spp. in rhizosphere such as the proteins and enzymes implicated in motility and chemotaxis, biofilm formation and root colonization (Table 3-4). An array of genes required for carbohydrates bio-digestion into mono/dimers of sugars directly assimilable by bacteria were detected in the BDI-IS1 genome (Table 3-4). These include for example α-amylases (*amyE*) degrading starch (Rajesh & Gummadi, 2022), cellulases (*licH, bglA, licABC, yckE* genes) involved in degradation of cellulose and derived products (Ejaz et al., 2021; G. Liu et al., 2023), chitosanase (csn) for chitin-derived chitosan hydrolysis into chitobiose and chitotriose (Guo et al., 2024), pectate lyases (*pelBC*) responsible for the plant pectin biodegradation (Boubsi et al., 2023; Pavlović et al., 2024), and laccase, a heme-containing peroxidase and manganese catalase described to have a biodegradation potential of the agricultural lignocelluosic residues (Dhankhar et al., 2020; Pfanzagl et al., 2018; Rashid & Bugg, 2021).

In addition of the above-described carbohydrates degradation-associated genes, dbCAN3-based automated CAZyme annotation coupled to CGC-Finder detecting CAZyme gene clusters (J. Zheng et al., 2023) revealed that the carbohydrates metabolism-associated enzymes encoded in BDI-IS1 are classified into six CAZymes sub-families including 38 glycosyltransferases (GTs), 15 carbohydrate esterases (CEs), 30 glycoside hydrolases (GHs), 2 polysaccharide lyases (PLs), 5 carbohydrate-binding module proteins (CBMs) and 6 auxiliary activities-related enzymes.

Furthermore, BDI-IS1 is equipped with neutral (*nprE*) and alkaline (*aprE*) proteases required not only for the degradation of nitrogen-containing polymeric substrates but also for the release of carbon and amino acids essential for the anabolism of new proteins (Harwood & Kikuchi, 2022; Rosazza et al., 2023). BDI-IS1 possesses lipid-degradating enzymes (*lip* genes) which enable it to strive even oil-polluted environments and can be used for food waste recycling or wastewater treatments (Cai et al., 2022; Roets-Dlamini et al., 2022; W. Wang et al., 2024).

Noteworthy, these lytic enzymes, above their fundamental role of degrading biopolymers, interplay in other ecological functions accomplished by the producer strain. For instance, chitosanase enzyme secreted by *B. cereus* B25 was found to enhance the antifungal activity of this *Bacillus* against the soil-borne fungae *F. verticillioides* (Báez-Astorga et al., 2022). Homogalacturonan, pectin backbone, was shown to induce an improved biofilm formation and root colonization, sporulation and enhanced production of some secondary metabolites (surfactin and iturin) of the plant-associated *B. velezensis* GA1 through the interplay of the pectinolytic enzymes (encoded by *pel* genes) (Boubsi et al., 2023).

Table 3-4: BLAST-based genome mining in BDI-IS1 of genes involved into the catalysis of complex nutrient sources and niche colonization.

Genes	Function	Related protein ID #
	Genes involved in carbohydrates degradation	
amyE	alpha-amylase involved in starch degradation	MCC9023321.1
bglH	6-phospho-beta-glucosidase	MCC9021925.1
bglP	beta-glucoside-specific PTS transporter subunit IIABC	MCC9021926.1
bglS	family 1 glycosylhydrolase	MCC9024245.1
licA	Phosphotransferase system (PTS)-lichenan transporter subunit IIA	MCC9021865.1
licB	PTS-lichenan transporter subunit IIB	MCC9021867.1
licC	PTS-cellobiose transporter subunit IIC	MCC9021866.1
licH	6-phospho-beta-glucosidase	MCC9021864.1
yckE	aryl--glucosidase/glycosylhydrolase for utilisation of aryl--glucosides	MCC9024245.1
csn	chitosanase implicated in chitin and chitosan degradation	MCC9023639.1
pel	pectate lyase required in the pectin backbone (polygalacturonic acid) degradation	MCC9022428.1
cotA	Laccase/multicopper oxidase family protein, involved in lignin breakdown and dye decomposition	MCC9022060.1
hemQ	heme-specific managnese peroxidase/coproheme decarboxylase in *B. subtilis* 168, also involved in lignin breakdown	MCC9022792.1
cotJC	putative manganese catalase with implication in lignin biocatalysis	MCC9022002.1
	Genes required for protein degradation	
aprE	extracellular alkaline serine protease (subtilisin E), the major extracellular protease	MCC9022538.1

vpr	minor extracellular serine protease	MCC9022750.1
aprX	intracellular alkaline serine protease	MCC9020873.1
bpr	bacillopeptidase F	MCC9023288.1
epr	minor extracellular serine protease, implicated the control of swarming motility	MCC9021854.1
nprE	extracellular neutral protease, involved in degardation of many proteins	MCC9024250.1
	Genes required for lipid degradation	
lip	triacylglycerol lipase involved in lipid degradation	MCC9022936.1 MCC9022935.1
	Genes implicated in biofilm formation	
sinR/flaD	Transcriptional regulator of post-exponential phase responses genes, involved in the control of biofilm formation	MCC9023119.1
RicF/ylbF	Antagonist of biofilm repressor *sinR*	MCC9023261.1
ricFsigD/flaB	RNA polymerase sigma factor for flagellar operon and repressor of biofilm formation	MCC9020797.1
luxS	S-ribosylhomocysteine lyase, involved in quorum sensing, sliding and biofim formation	MCC9024016.1
sigL	RNA polymerase sigma-54 factor involved in biofilm formation, utilization of arginin, acetoin and fructose, and required for cold adaptation	MCC9023471.1
csrA	carbon storage regulator for biofilm formation and motility	MCC9022374.1
flgM	Repressor of *sigD*, negatively controls the flagellin synthesis and favors biofilm formation	MCC9022380.1
mnaA	UDP-N-acetylglucosamine 2-epimerase required for cell wall teichoic acids, biofim formation and root colonization	MCC9022403.1
epsA	Putative transmembrane modulator of EpsB activity implicated in extracellular polysaccharide synthesis, a component of biofilm	MCC9023487.1
epsB	protein tyrosine kinase involved in extracellular polysaccharide synthesis	MCC9023486.1
epsC	UDP-N-acetylglucosamine 4,6-dehydratase required for extracellular polysaccharide synthesis	MCC9023485.1
epsD	UDP-acetylglucosamine-dependent N-acetyl glucosamine transferase involved in extracellular polysaccharide synthesis	MCC9023484.1
epsN	UDP-2,6-dideoxy 2-acetamido 4-keto glucose aminotransferase, required for extracellular polysaccharide synthesis	MCC9023474.1
tasA	major component of biofilm matrix by forming bundles of fibres	MCC9023120.1

tapA	primer for TasA oligomerization implicated in biofilm formation	MCC9023122.1
sipW	signal peptidase I controls surface-adhered biofilm formation and processes TasA and TapA	MCC9023121.1
bslA	bacterial hydrophobin, a water-repellent surface layer of the biofilm required for sliding and inhibitor of KinA autophosphoryaltion	MCC9024200.1
pgsB *pgsC*	poly-γ-glutamic acid synthase and poly-γ-glutamate biosynthesis protein, required for capsule synthesis involved in biofilm formation	MCC9024232.1 MCC9024233.1
	Genes involved into motility and chemotaxis	
flgB	flagellar basal-body rod protein	MCC9020767.1
flgD	flagellar hook cap necessary	MCC9020777.1
flgK	flagellar hook-assiocated protein, involved in filaments junction	MCC9022378.1
flhA	flagellar biosynthesis protein, flagellar type III secretion system	MCC9020789.1
flhF	flagellar biosynthesis protein, signal recognition particle-like GTPase involved in placement and assembly of polar flagella	MCC9020790.1
flhG	MinD/ParA family proteins activing as ATPase/GTPase involved in the activation of FlhF	MCC9020791.1
swrAA	swarming motility protein involved in solid surfaces colonization, controls DegU activity and upregulates *sigD*	MCC9022346.1
swrB	control of SigD activity and induces the flagellar type III secretion export system	MCC9020798.1
cheA	two-component sensor kinase acting as a chemotactic signal modulator	MCC9020793.1
cheB	Chemotaxis response regulator protein, MCPs-glutamate methylesterase	MCC9020792.1
cheD	protein deaminase, regulating the activity of *cheC*	MCC9020796.1
cheC	control of chemotaxis by interacting with *cheD*, CheY-P phosphatase and inhibition of CheR-mediated methylation of MCPs	MCC9020795.1
cheR	MCP-methytransferase involved in motility and chemotaxis	MCC9021030.1
cheY	chemotaxis protein, modulation of flagellar switch bias, two-component response regulator	MCC9020783.1
cheV	chemotaxis protein involved in modulation of CheA activity in response to attractants	MCC9023801.1
hag	flagellin	MCC9022373.1

fliD	flagellar hook-associated protein, periplasmic chaperone involved in flagellin polymerization into helical filament	MCC9022372.1
fliS	chaperone for the flagellin export	MCC9022371.1
motAB	flagellar stator subunit coupled to H^+	MCC9024115.1 MCC9024114.1
motPS	flagellar stator subunit coupled to Na^+	MCC9021304.1 MCC9021303.1
mcpA	methyl-accepting chemotaxis protein, acting also as an attractant signal to decreasing pH	MCC9024189.1
mcpB	methyl-accepting chemotaxis protein, receptor of aspargine, ethanol and other short-chain alcohols, attractant signal to increasing pH	MCC9024187.1
mcpC	methyl-accepting chemotaxis protein that controls chemotaxis to proline, threonine, glycine, serine, lysine, valine and arginine	MCC9023794.1
	Genes encoding heat and cold shock proteins	
dnaJ *dnaK*	heat shock protein (*dnaJ*) and its activator (*dnaK),* also termed as class I heat-shock protein	MCC9023565.1 MCC9023566.1
groES	Chaperonin, necessary in protein folding and re-folding after a thermic shock	MCC9022078.1
cspB *cspC* *cspD*	major cold shock protein, an RNA chaperone	MCC9023355.1 MCC9023355.1 MCC9021510.1
	Genes involved into osmotic stress tolerance	
gbsA	glycine betaine- aldehyde dehydrogenase involved in glycine betaine synthesis	MCC9024202.1
gbsB	choline dehydrogenase (FAD-dependent), involved in glycine betaine synthesis	MCC9024203.1
gbsR	transcription repressor, regulating the osmoprotection	MCC9024201.1

Moreover, our *in-silico* investigation revealed the presence of all the panoply of genes involved in motility and chemotaxis, biofilm formation and genes encoding stress-resistance proteins such as heat and shock proteins and osmoprotectant proteins (Table 3-4). Indeed, these genes are very important in bacterial niche invasion such as in the root colonization for example. Motility and chemotaxis proteins help in the movement of bacterial cells on solid surfaces (swarming, twitching and gliding) or in liquid media (swimming), responding to a chemoattractant such as root exudates or a chemorepellent like toxin secreted by competitors or diverse hazardous compounds found in the environments (e.g. pollutants) (H. Feng et al., 2019; R. Fu & Feng, 2024; L. Yang et al., 2023). Biofilm is a state of life of bacterial cells where they are organized in complex communities encased and protected by self-produced exopolysaccharides matrix (products of *eps* genes), and where other biofilm structures

such as the amyloid fibres (Tas proteins) and hydrophobin (surface layer) strengthen its protective role against antibiotics and abiotic stresses (Sauer et al., 2022; Shree et al., 2023; Xia et al., 2024). In addition, these biofilm structures are key determinants to the successful colonization of roots by rhizobacteria. They adhere firmly to the root surfaces, provide a shelter for bacterial cells against competitors, but also enable the bacteria to tightly interact with the plant host and exert its plant growth promotion and protective benefits (Gastélum et al., 2024; Y. Liu, Xu, et al., 2024a; Nishisaka et al., 2024).

Environmental physico-chemical parameters such as temperature and osmolality exert a pressure on inhabiting micro/organisms which can impede bacterial protein homeostasis and thus bacterial fitness (Matavacas & von Wachenfeldt, 2022). In response, bacteria have evolved to conserve the so called "quality control proteins" (cold and heat shock proteins) to counteract protein misfolding and aggregation, and/or secrete specialized metabolites called chemical chaperones like the osmolyte glycine betaine which re-equilibrates the intra-extracellular osmotic imbalance upon osmotic stress (Denaxa et al., 2023; Valencia-Marin et al., 2024). Following a cold shock, the nucleic acid chaperones (cold shock proteins, Csp) block the synthesis of secondary mRNA at low temperature, and thus hinder the formation of misfolded proteins (Z. Xue et al., 2024), while the cytosolic chaperones (heat shock proteins, DnaJK and GroES) work either by decreasing the rate of folding of compromised protein species or by tightly binding to the unfolded proteins and prevent their aggregation (Matavacas & von Wachenfeldt, 2022). In harsh saline environments leading to hyperosmotic stress, bacteria such as BDI-IS1 are able to secrete in higher amounts the compatible solute glycine betaine (encoded by *gbs* genes) in order to stop the cell water efflux and thus maintain turgor pressure and envelope plasticity, necessary for fundamental cellular processes (deformation, division and motility), as well as for preserving the proteins and other organelles integrity (Rath et al., 2020; Xia et al., 2024).

3.6.2. Plant growth stimulation and biofertilization

Plant growth and development sustained by plant-associated bacteria passes either through the secretion of phytohormones and hormones-like substances or by aiding in the mobilization of specific nutrients (N, P and Fe). Phytohormones and hormones-like substances stimulate in an indirect way the growth of host-plants by modulating the plant developmental genetic traits upon different exogenous cues, while the biofertilization implies the secretion of organic acids and enzymes that enhance the uptake of critical nutrient elements that often lack or present in limited concentrations in soils (Jha et al., 2024; Orozco-Mosqueda, Santoyo, et al., 2023; Patel et al., 2015).

Genome mining depicted that BDI-IS1 harbours the gene clusters governing the biosynthesis of volatile organic compounds (acetoin and 1,2-butanediol), the phytohormones indole acetic acid (IAA) and cytokinins, and polyamines (spermidine and polyamine) (Table 3-5). These biological molecules were described to mount genetic, physiological and morphological changes favouring the overall plant growth

and development, and stress tolerance. For instance, volatile compounds 2,3-butanediol and benzyl alcohol produced by *B. subtilis* strain isolated in the rhizosphere of *Haloxylon ammodendron* were found to induce overgrowth of root hair and enhanced lateral root elongation in *Arapidopsis* and *H. ammodendron* (A. L. He et al., 2023). In addition, a set of bacterial volatiles secreted by rhizobacteria were reported in other studies to boost the rice shoot growth compared to the control and enhanced maize growth and stress tolerance to heavy metals (Almeida et al., 2023; Rojas-Solis et al., 2023).

Indole acetic acid molecules (auxin) produced by root-associated bacteria such as *Pseudomonas* spp. and *Bacillus* spp. are reported, for example, to improve tomato seed germination rate (Pappalettere et al., 2024), to boost maize and canola growth by increasing total root length, aerial part dry matter and germination index (Figueredo et al., 2023; M. Iqbal et al., 2023). Moreover, cytokinin production by the rhizobacteria *B. velezensis* 83 and *Bacillus* sp. LZR216 was associated with improved mitosis in root meristems and overall root and shoot biomass increment in *A. thaliana* (Barrera-Ortiz et al., 2023; J. Wang et al., 2018).

Polyamines consisting in spermine, spermidine and putrescine are excellent plant growth promoters and stress alleviators and were described to accelerate plant seed germination, tissue lignification, flowering, pollination and fruit development and ripening (Dunn & Becerra-Rivera, 2023; Tyagi et al., 2023). They help also plants in coping with acidic, oxidative, drought, salt stresses by either alkalizing the environment via its biosynthesis (protonation in aqueous solution) that consumes protons, or upregulating the genes involved in anti-oxidant enzymes production (Amiri et al., 2024; Nandy et al., 2023). They can also regulate ion exchange for ionic homeostasis or elicit the general plant defense hormones (salicylic acid, jasmonic acid, ethylene, nitric oxide, abscisic acid, gibberelins) that enhance the plant resistance to abiotic stresses (Amiri et al., 2024; Nandy et al., 2023).

Table 3-5: BLAST-based genome mining in BDI-IS1 of genes involved into plant growth promotion and biofertilization.

Gene name	Protein encoded by the gene	NCBI protein ID #
Genes involved into volatile compounds production		
alsS	acetolactate synthase	MCC9024215.1
alsD	acetolactate decarboxylase	MCC9024216.1
alsR	regulation of acetoin synthesis	MCC9024214.1
bdhA	acetoin/butanediol dehydrogenase	MCC9022064.1
acuA	Acetoin utilization proteins	MCC9021300.1
acuB		MCC9021301.1
acuC		MCC9021302.1
acoA	Acetoin dehydrogenase	MCC9022476.1
acoB		MCC9022477.1
acoC		MCC9022478.1

acoL		MCC9022479.1
acoR	Transcription activator of *acoABCL*	MCC9022480.1
Genes involved into indole acetic acid (IAA) synthesis		
trpA *trpB*	tryptophan synthase alpha unit (*trpA*) and beta unit (*trpB*)	MCC9021021.1 MCC9021022.1
trpC	indole-3-glycerol-phosphate synthase	MCC9021024.1
trpD	anthranilate phosphoribosyltransferase	MCC9021025.1
trpE	anthranilate synthase (subunit I)	MCC9021026.1
trpF	phosphoribosylanthranilate isomerase	MCC9021023.1
trpP	tryptophan transporter	MCC9022564.1
trpS	tryptophanyl-tRNA ligase	MCC9021410.1
dhaS	aldehyde dehydrogenase	MCC9021598.1
ysnE	N-acetyltransferase	MCC9022745.1
patB	cystathione-β-synthase/methionine synthesis	MCC9023768.1
ywkB	auxin efflux carrier	MCC9022629.1
yhcX	putative amidohydrolase (amidase)	MCC9023367.1
Genes involved into cytokinin biosynthesis		
miaA	tRNA (adenosine(37)-N6)-dimethylallyltransferase	MCC9020880.1
miaB	tRNA (N6- isopentenyl adenosine (37)-C2)-methylthiotransferase	MCC9020851.1
dapF	diaminopimelate epimerase	MCC9023241.1
pdeH	c-di-GMP degrading phosphodiesterase putatively involved in the dephosphorylation of isopentenyladenosine monophosphate (N6-iAMP) to yield the cytokinin active form, isopentenyladenosine (N6-iPR)	MCC9023739.1
Genes involved in polyamines (spermine, spermidine et putrescine) biosynthesis		
bioI	cytochrome P450 enzyme for biotin synthesis	MCC9021665.1
speA	polyamine biosynthesis	MCC9024445.1
speB	N^1-aminopropylagmatinase	MCC9024338.1
speE	spermidine synthase/N^1-aminopropylagmatinase synthase	MCC9024339.1
speD	S-adenosylmethionine decarboxylase	MCC9021237.1
metE	methionine synthase	MCC9023664.1
bltD	spermidine/spermine acetyltransferase	MCC9024372.1
Genes involved in nitrogen metabolism		
ureA *ureB* *ureC*	urease for utilisation of urease as alternative source of nitrogen	MCC9022667.1 MCC9022668.1 MCC9022669.1
nrgB	P (II)- family nitrogen regulator	MCC9022682.1

nfrA	oxygen-insensitive NADPH-nitroreductase specific for nitroaromatic compounds	MCC9023424.1
glnA	type I glutamate-ammonia ligase	MCC9021680.1
	Genes involved into phosphorus mineralisation	
phy	3-phytase for utilisation of the organic phosphate source, phytate	MCC9021542.1
citZ	citrate synthase	MCC9021250.1
mmgD	2-methylcitrate synthase	MCC9023068.1
mdh	malate dehydrogenase	MCC9021248.1
ackA	acetate kinase	MCC9021271.1
pstA	phosphate ABC transporter, binding protein	MCC9023152.1
pstBA		MCC9023151.1
pstBB		MCC9023150.1
pstC		MCC9023153.1
pstS		MCC9023154.1
pit	low-affinity inorganic phosphate transporter, proton symporter	MCC9023701.1
phoAB	Alkaline phosphatases for phosphate acquisition upon its starvation	MCC9023381.1
phoC	HAD family sugar phosphate phosphatase, involved in dephosphorylation of N-acetyl-glucosamine 6-phosphate	MCC9022699.1
yqeG	HAD IIIA-type phosphatase	MCC9023588.1

Furthermore, our investigation revealed the presence of genes involved in the acquisition of some nutrients such as N and P (Table 3-5). Their availability in the soil is not always guaranteed due to their intrinsic physico-chemical characteristics or to inadequate agricultural practices or erosion/drought, especially in the current context of the global climate change (Marschner & Rengel, 2023). Albeit the conventional soil amendment by chemical fertilizers for which the effectiveness is limited and tainted with undesirable effects, some plant-associated bacteria have the ability to enhance the soil-nutrient pool by fixing atmospheric nitrogen, metabolize alternative nitrogen-source such as urea or by solubilizing organic and inorganic sources of phosphorus (Zeng et al., 2022). Indeed, soil urease activity was improved after application of soil-beneficial bacteria *B. subtilis* and *P. fluorescens* and thus regenerates and enhances the availability of nitrogen in the bulk soils that can directly be assimilated by plant roots (NG et al., 2022). Enhanced soil availability of soluble form of phosphorus and subsequent plant growth promotion following an amendment of *Bacillus* spp. were thoroughly reported. For instance, a set of *Bacillus* strains were shown *in vitro* to trigger an increased P solubilization from an insoluble source $Ca_3(PO_4)_2$ by secreting several low-molecular weight organic acids and phosphatase (Z. Iqbal et al., 2024). *B. velezensis* Ag75, above its antifungal potential, enhanced the growth and yield of maize and soybean crops by its ability to solubilize soil-inorganic

phosphorus and it was shown that it can partly compensate the chemical amendment of P_2O_5 (Mosela et al., 2022).

4. Conclusion

Isolated from residual roots collected from cultivated land in Isare- west of Burundi, BDI-IS1 was selected among a panoply of *Bacillus*-like isolates following its *in vitro* strong antibacterial activity against a set of phytopathogenic bacteria, and at comparable level as the commercial strain *B. velezensis* QST713 (Serenade Aso®). In addition, this isolate BDI-IS1 demonstrated a consisting and promising antifungal potential against an array of fungal pathogens of great agricultural importance in a similar way as QST713. Phenotypical coupled with molecular techniques established the identity of this promising isolate BDI-IS1 as a new strain of the recently described *B. nakamurai* species, *B. nakamurai* BDI-IS1. The chromosomal genome sequence of this strain has a size of 3.8M bp, 45% GC content and 4,123 genes and ANI and orthologous gene clusters-based comparison showed that BDI-IS1 shares the same close ancestor as *B. siamensis*, *B. velezensis* and *B. amyloliquefaciens*, confirming its membership in the *B. amyloliquefaciens* operational group. Comparative functional analysis by RAST revealed that BDI-IS1 harbours particularly additional genes implicated in oxidative stress responses, ferrous and ferric iron uptake and biodegradation of hazardous biphenyl containing compounds, constituting a marked adaptation strategy to unfavourable soil conditions.

Furthermore, BLAST-based genome mining revealed that BDI-IS1 is a prominent plant growth promoting rhizobacteria, equipped with the set of genes dedicated to the degradation of the root pectin, colonize root surface, form robust biofilm, as well as the genes devoted to the synthesis of quality control chaperones and to the secretion of osmoprotectants. We also found the gene clusters supporting the synthesis of the phytohormones, hormone-like substances polyamines and VOCs, the secretion of organic acids and phosphatases interplaying the solubilization of insoluble forms of phosphorus.

Altogether, these findings provide useful insights into the ability of *B. nakamurai* BDI-IS1 to thrive in harsh environments, to successfully colonize the plant roots and provide growth promotion benefits to its plant host. Beyond this plant growth promotion potential, BDI-IS1 is a prominent biocontrol candidate owing to its strong antagonistic potential against plant bacterial and fungal pathogens at better or comparable level as QST713. However, the mechanisms underlying that observed antagonistic potential of this strain are missing and a thorough investigation of the secondary metabolome of the strain coupled with reverse genetics would provide a clear overview of the cues behind these antagonistic activities.

Chapter 4

Characterization of the secondary metabolome and evaluation of the biocontrol potential of BDI-IS1

This chapter is adapter from:

Nimbeshaho F., Nihorimbere G., Arias A. A., Liénard C., Steels S., Nibasumba A., Nihorimbere V., Legrève A. and Ongena M. (2024). Unravelling the secondary metabolome and biocontrol potential of the recently described species *Bacillus nakamurai*. *Microbiological Research* 288, 127841. https://doi.org/10.1016/j.micres.2024.127841.

In this paper, GN and I are co-first authors. My contribution consisted in the isolation and the selection the bacterium based on antagonistic activities, its identification (2nd chapter), characterisation of its secondary metabolome and the understanding of the relationship between the bioactivity and secreted bioactive compounds. In a collaborative framework, I provided the bacterial material to GN (UCLouvain), and he carried out all the plant experiments aimed to assess the biocontrol efficacy of the above characterised bacterium. We jointly wrote the manuscript from the first draft until to responding to reviewers' comments and submission of the final manuscript. With the approval of our respective supervisors, GN and I included all the data published in this paper in our thesis manuscripts.

1. Introduction

The biocontrol potential of the *Bacillus*-based formulations against plant diseases depends greatly on their ability to produce a vast array of bioactive secondary metabolites (BSMs) acting as antimicrobials and/or elicitors of host immunity (Abd-Elsalam & Mohamed, 2023; Lourenzi et al., 2022). However, other BSMs retain key ecological functions because they are involved in competition for nutrients (iron-chelating siderophores) or in root colonization (cyclic lipopeptides) (Deb & Tatung, 2024; Y. Liu, Xu, et al., 2024). Bacilli dedicate up to 10-13% of their genome to the biosynthesis of BSMs (Borriss, 2020; Fazle Rabbee & Baek, 2020) including volatile organic compounds (VOCs) (Kai, 2020) and a wide range of soluble metabolites of different types such as non-ribosomal (NR) cyclic lipopeptides, polyketides, oligopeptides, ribosomally produced and post-translationally modified peptides (RiPPs) encompassing bacteriocins and lantibiotics, as well as various lytic enzymes (S. Iqbal et al., 2023).

Bacillus-derived cyclic lipopeptides surfactin, iturin and fengycin are the most interesting bioactive compounds that have attracted attention of many researchers, and that are already commercialized as biocontrol agents owing to their dual ecological functions as antimicrobials and ISR inducers (Balleux et al., 2024; Théatre et al., 2022; Z. Wang et al., 2024). In fact, ISR is an interesting mechanism where beneficial bacteria inoculated to the roots enhances the defensive capacity of the entire plant against various pathogens including those infecting the aerial parts (Salwan et al., 2023). It is mediated mainly by surfactin, as well as fengycin and iturin in some pathosystems by eliciting the upregulation of defense hormones jasmonic acid and ethylene (Salwan et al., 2023; L. Zhu et al., 2022). On the other hand, the direct antibiosis is necessary for bacterial self-fitness in soil and rhizosphere niches by inhibiting or suppressing the proliferation of its competitors and plausible plant pathogens (Saiyam et al., 2024). It implicates surfactin by enhancing biofilm formation and lowering surrounding surface tension that exclude prominent enemies for the producer strain and/or plant host, and fengycin and iturin for their direct and strong antibiotic effect against pathogenic fungi and oomycetes (Lam et al., 2021; Saiyam et al., 2024). Moreover, the remaining of the secondary metabolome including polyketides (bacillaene, macrolactin and difficidin), bacilysin, a wide variety of RiPPs (amylocyclicin, amylolysin, etc.) are reputed antimicrobials against an array of pathogenic bacteria, some fungal pathogens and occasionally against close related strains (Dimkić et al., 2022; Mercado & Olmos, 2022; C. Tran et al., 2022).

From an ecological perspective, evolving such diversity of BSMs can also be considered as an adaptive trait to improve rhizosphere fitness (Anckaert et al., 2021; Etesami et al., 2023a). Additionally, the importance of low molecular weight organic compounds in communication among microorganisms and between microorganisms and plants is increasingly appreciated, extending their ecological roles beyond microbial warfare, plant defence and development (Mithöfer & Boland, 2016; Vaishnavi & Osborne, 2021; Z. Xu et al., 2023). Furthermore, strains of the *B. subtilis* clade have evolved to distinguish among themselves by secreting a different and

specialized panel of BSMs that can serve for taxonomical identification, as shown for *B. velezensis* for which its secreted non-ribosomal compounds are typical to all strains (Steinke et al., 2021; Q. J. Yin et al., 2023). These include the tree families of cyclic lipopeptides (surfactin, iturin and fengycin), three family of polyketides (bacillaene, difficidin and macrolactin), bacillibactin and bacilysin (Steinke et al., 2021; Q. J. Yin et al., 2023).

Bacillus nakamurai species, a member of the *B. amyloliquefaciens* operational group (Balleux et al., 2024; Ngalimat et al., 2021), was first described in 2016 (Dunlap, Saunders, et al., 2016) and actually comprises five sequenced strains including the Burundi-isolated strain *B. nakamurai* BDI-IS1 described in the previous chapter. Although some rare studies have reported the ability of this species to secrete the bioactive cyclic lipopeptides surfactin and iturin as well as volatile organic compounds (Chaouachi et al., 2021; Dunlap et al., 2019; Leathers et al., 2020), the entire bioactive secondary metabolome of this species remains to be characterized. Correlatively, the uncertainty about the nature of the bioactive metabolite (s) supporting the inhibition of *Erwinia amylovora* by *B. nakamurai* NRRL B-41091 still prevails (Leathers et al., 2020). Furthermore, the previously described broad-spectrum antagonistic activity of *B. nakamurai* BDI-IS1 against phytopathogenic fungi and bacteria speculates for the great potential of the species to secrete multi-target antimicrobial molecules. Thus, this underscores the necessity for in-depth characterization of the chemistry of this species and decipher the chemical mediators of this antagonism.

Hereafter, we henceforth provide a first characterization of the whole range of specialized metabolites formed by this understudied species by combining genomics and metabolomics. We identified some compounds that play a key role in the antimicrobial activity observed *in vitro*, but our data also suggest that this secondary metabolome may also contribute to the strong biocontrol potential of BDI-IS1 against tomato early blight and northern corn leaf blight caused by *Alternaria solani* and *Exserohilum turcicum*, respectively.

2. Material and methods

2.1. Biological materials

Six plant pathogenic bacteria, including *C. michiganensis* subsp. *michiganensis*, *R. fascians* D188, *P. carotovorum* LMG6663, *X. campestris* pv. *campestris* LMG582, *P. cichorii* LMG2162, *P. fuscovaginae* LMG2158 and five phytopathogenic fungi, including *B. cinerea* R16, *R. solani* MUCL49235, *A. niger* MUCL 28698, *E. turcicum and A. solani*, were used in the antagonism assays with the *B. nakamurai* BDI-IS1 mutants. All the biological materials were available in the collection of the Microbial Processes and Interactions (MiPI) Laboratory, Gembloux Agro-Bio Tech, University of Liège, except *E. turcicum and A. solani* which were isolated from diseased leaves from Burundi.

2.2. Culture preparation

B. nakamurai BDI-IS1 and the plant pathogenic bacteria initially stored at -80˚C were plated on LBA and incubated at 30˚C for 24 h. Individual colonies were then pre-cultured the day before the experiment. For dual culture assay, bacterial cells were centrifuged, and the pellet was washed twice with LB broth, resuspended in the same medium, and adjusted to OD_{600} 0.1 and OD_{600} 2 per mL for pathogenic and BDI-IS1 (and its mutant strains), respectively. Five days' old fungal mycelia (cultured on PDA at 25°C) were used in dual confrontation assay with BDI-IS1.

2.3. Antagonistic activity evaluation of BDI-IS1 mutants

The antibacterial potential of the cell-free REM culture supernatant (22µm pore size filters) was tested in a 96-well microtitre plate (200 µL per well including a mixture of LB culture of phytopathogenic bacteria adjusted at $OD_{600\,nm}$ 0.1 and 15 % of cell-free REM culture supernatants) and incubated at 30°C, 250 rpm in microplate incubator. Optical density readings (at 600 nm) were performed on a microplate reader (TECAN, SPARK) and the experiment was performed two times independently with three replicates per experiment (n = 6). For monitoring the kinetic growth of pathogens treated with BDI-IS1 cell-free extracts, incubation of microplates was performed however inside the TECAN machine with continuing shaking (105 rpm, 30°C) and readings (at 600 nm) recorded automatically each 30 min. For the antifungal activity of cell-free supernatants, increasing concentrations (10-40%) were added to the PDA before pouring the medium. The inhibition of fungi was appreciated after 5 days of incubation at 25°C (n = 3). The antibacterial and antifungal activities of various constructed BDI-IS1 mutant strains were assessed using the dual culture assay protocol (see chapter 3) on gelified (1.5% agar) REM and potato dextrose agar (PDA), respectively for antibacterial and antifungal confrontation assays. The assays were conducted in triplicates and repeated twice independently (n = 6).

2.4. Bioactive secondary metabolites identification of the isolate BDI-IS1

Metabolome characterization of the BDI-IS1 cell-free supernatant (48h, 160 rpm, 30°C and REM/PDB/LB) was performed by LC-ESI-qTOF-MS (Agilent 1290 Infinity II coupled with mass detector (Jet Stream ESI-Q-TOF 6530)) in positive mode with the following source parameters: capillary voltage of 3.5 kV, nebuliser pressure of 35 psi , drying gas of 8 $L.min^{-1}$, gas temperature of 300°C, sheath gas flow rate of 11 $L.min^{-1}$, sheath gas temperature of 350°C, fragmentor voltage of 175 V, skimmer voltage of 65 V, and octopole radiofrequency of 750 V. Accurate mass spectra were recorded in the m/z range of 100 to 1,700 (acquisition rate 2 spectra/s). For an optimal separation, a C18 Acquity UPLC BEH column (2.1 mm; 50 mm; 1.7 µm; Waters) was used at a flow rate of 0.6 $mL.min^{-1}$ and a temperature of 40°C (injection volume: 10 µL). A gradient of acidified water (0.1 % formic acid) (solvent A) and acidified acetonitrile (0.1% formic acid) (solvent B) was chosen as the mobile phase, starting

at 10 % B, and rising to 100 % B in 20 min. Solvent B was kept at 100 % for 4 min before returning to the initial ratio. Where possible, metabolites were identified based on retention time, by comparison with *in-house* database constructed with *B. velezensis* mutants impaired in the production of the different metabolites (Andrić et al., 2023), and accurate mass. In addition, all structures were confirmed by MS/MS fragmentation.

2.5. BDI-IS1 mutants' construction

Knock-out mutant strains of the promising isolate BDI-IS1 depleted in the biosynthesis of each of the bioactive secondary metabolites and in all non-ribosomally produced peptides (*sfp* mutant) were constructed by gene replacement and homologous recombination. The primers used for this purpose are listed in the supplementary material (Table S4). A cassette containing a chloramphenicol resistance gene flanked by about 1 kb of the upstream and downstream regions of each target gene was constructed by three fragments joining PCR. BDI-IS1 transformation was carried out according to the protocol of Hoff et al. (2021) with minor modifications. Shortly, one colony of BDI-IS1 was grown in LB medium (37°C, 150 rpm) for 4 h, and washed twice with MMG liquid medium (Anhydrous K_2HPO_4 19 $g.L^{-1}$, KH_2PO_4 6 $g.L^{-1}$, anhydrous $Na_3C_6H_5O_7$ 1 $g.L^{-1}$, $MgSO_4$ *7 H_2O 0.2 $g.L^{-1}$, Na_2SO_4 2 $g.L^{-1}$, $FeCl_3$ 50 mM, $MnSO_4$ 2 mM, glucose 8 $g.L^{-1}$, and L-glutamic acid 2 $g.L^{-1}$; pH 7.0) and the OD_{600nm} was adjusted to 0.01. The recombinant cassette (0.5 – 1 µg) was added to 1 mL of the latter prepared suspension and incubated at 37°C for 24 h. Transformed colonies that have integrated the recombinant cassette, were further selected on LBA medium supplemented with chloramphenicol (5 µg/mL).

2.6. Biocontrol assays against early blight and northern leaf blight

The biocontrol potential of the *B. nakamurai* BDI-IS1 against two important plant diseases prevalent in Burundi, tomato early blight and northern corn leaf blight, was assessed under greenhouse conditions. The bacterium *B. velezensis* QST713 (ingredient of Serenade Aso®) was tested under the same conditions for comparison purpose.

2.6.1. Plant material and culture conditions

The varieties Ruganda for tomato and ZM605 for maize were selected for their relative susceptibility to early blight of tomato and northern leaf blight of maize, respectively. Maize and tomato seeds were disinfected in sodium hypochlorite (0.05 %), washed 3 times in distilled water, pregerminated and then sown in pots (2 L) containing potting soil, then placed in greenhouse at a temperature of 22°C ± 2°C and 18°C ± 2°C day and night respectively, a relative humidity of 80 % ± 5 % and a photoperiod of 16 h.

2.6.2. Treatments and experimental design

The activity of the two tested bacteria (BDI-IS1 and QST713), after leaf or root application, against tomato early blight and northern corn leaf blight was evaluated in separate bioassays on tomato or maize, including twelve treatments : T_1 = plant + BDI-IS1 leaf + fungus, T_2 = plant + BDI-IS1 root + fungus, T_3= plant + QST713 leaf + fungus, T_4 = plant + QST713 root + fungus, T_5 = plant + BDI-IS1 leaf + water tween , T_6 = plant + BDI-IS1 root +water tween , T_7 = plant + QST713 leaf + water tween, T_8 = plant + QST713 root + water tween , T_9 = plant + $MgCl_2$ buffer on leaf + water tween , T_{10} = plant + $MgCl_2$ buffer on root + water tween, T_{11} = plant + $MgCl_2$ buffer on leaf + fungus ,T_{12} = plant + $MgCl_2$ buffer on leaf. The treatments were tested in a completely randomised block design (CRBD) in two independent experiments, with 16 (4 replicates of 4 plants: N=16) and 12 (3 replicates of 4 plants: N =12) plants per treatment in trial one and two, respectively.

2.6.3. Culture preparation, biocontrol agents' application and pathogen inoculation

A. solani was cultured on V8 media (V8 20 % (v/v), $CaCO_3$ 1 g/L, Agar 15 g/L) under neon lamp light, alternating light and darkness (12 h/12 h) for 10 days. *E. turcicum* was cultured on PDA (potato dextrose agar 39 g/L, agar 2 g/L) and incubated at 27°C for 21 days. To collect the conidia, 3 ml of sterilised water and Tween 20 (0.05%) were added to Petri dishes and the mycelia and conidia were repeatedly scraped with a sterilised blade and collected in Falcon tube. Conidia were obtained by filtration through a double layer of cheesecloth, and the concentration of the conidial suspension was counted using Fuchs Rosenthal cell and light microscopy. Adjusted concentrations of 2 x 10^4 conidia/mL for *A. solani* and 4.5 x 10^4 conidia/mL for *E. turcicum* were used for plant inoculation. The bacterial isolate BDI-IS1 and QST713 were revived from cryotubes (-80°C) on LBA, 16 h at 30°C. An individual bacterial colony was used to carry out a pre-culture incubated for 24 h at 30°C, 120 rpm, then used to perform a culture (initial OD_{600} = 0.001, 100 mL in 250 mL flasks) for 24 h, at 30°C, 120 rpm. The cultures were centrifuged; the pellets washed twice with $MgCl_2$ (2.5 g/L) and resuspended in 10 L of $MgCl_2$ (equivalent to10^6 CFU/mL) before application on plants.

Two application methods were tested, i.e. on roots (root treatment) or on leaves (leaf treatment). For the root treatment, 100 mL of the bacterial suspension was poured onto the plant substrate (about 2 L potting soil/plant), twice, at seven days and one day before pathogen (or control) inoculation, i.e. on 24 and 28 days-old tomato plantlets and 11 and 16 days-old maize plantlets, respectively, for trial one and two. For foliar treatment, the bacterial suspension was sprayed on all leaves of treated plants until run-off, the day before inoculation. The control plants were treated with $MgCl_2$ solution only.

The fungal inocula of *A. solani* or *E. turcicum*, as prepared above, were sprayed until run off on the leaf surface of plants of 25 and 29 days-old tomato plantlets and 12 and 17 days-old maize plantlets, respectively for trial one and two.

2.6.4. Data collection and analysis

Leaf necrotic area (LNA) was used to assess the severity of early blight and northern leaf blight on three (low, middle and top) and two leaves (middle and top) of each plant for tomato and maize, respectively. The LNA of tomato early blight was estimated daily using a scale from 1 to 9, where 1 = 0-5 % of leaf area affected by the disease, 3 = 5-10 %, 5 = 10-20 %, 7 = 20-50 % and 9 = 50-100 %. Northern leaf blight severity was scored at two-day intervals on a scale from 1 to 9 (Figure S4), where 1 = 0 % of leaf area affected by the disease, 2 = 10 %, 3 =20 %, 4 = 30 %, 5 = 40 %, 6 = 50 %, 7 = 60 %, 8 = 70 %, 9 = 80-100 %. The disease severity (DS) for nothern corn leaf blight (NLB) was obtained from the mean of the disease severity of the related scores recorded on two leaves, while DS for tomato early blight (TEB) represent the mean of disease scores recorded on three leaves obtained using the following formula as described by Willocquet et al. (2023):

$DS = \sum_{i=1}^{k}(SR_i \times NP_i)/TNP \times HS) \times 100$, where i is the disease score, k the number of classes in disease scale (k = 5), while SR_i and NP_i represent the score rating and number of plants with i score, respectively. TNP and HS stand for the total number of assessed plants and high score (HS = 9), respectively.

The area under disease progress curve (AUDPC) for TEB and NLB was calculated using the following formula:

$AUDPC = \sum_{i=1}^{n-1}\left(\frac{y_i+y_{i+1)}}{2}\right)X(t_{i+1} - t_i)$, Where y_i: disease severity of i^{th} day, y_{i+1}: disease severity of i^{th+1} day, t_i: i^{th} day, t_{i+1}: i^{th+1} day and n = total number of observations.

The Protection Index (PI) conferred by a treatment was calculated as described by Caulier et al., (2018) :

$$PI = \left[\left(1 - \frac{\text{AUDPC treatment}}{\text{AUDPC Control}}\right) * 100\right]$$

The data on disease severity collected in the greenhouse for TEB and NLB were analysed using Generalized Linear Mixed Model (GLMM) with R 4.0.5 software, where the bacteria, treatment method and trial were fixed factors, and block was a random factor. Degrees of freedom were calculated using the Kenward-Roger method, while the Estimated Marginal Means (emmeans) package was used to compare the different means. The differences between PI were assessed using t-test within R 4.0.5. For the *in vitro* antagonist assays, data analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test (p value = 0.05). All the graphs were constructed using Prism GraphPad 9.4.1 software.

3. Results

3.1. Involvement of soluble secondary metabolites in the antagonistic activity of BDI-IS1

The antagonistic potential of BDI-IS1 against bacterial and fungal phytopathogens was previously clearly demonstrated from dual confrontation assays on solid media, however the nature of the inhibitory compounds secreted by BDI-IS1 remained elusive. By microplate-based assays, we wanted to verify if the observed inhibition could be due to soluble secreted bioactive metabolites or not and cell-free extracts obtained from BDI-IS1 liquid cultures in REM were tested for this purpose. Growth of *C. michiganensis* and *R. fascians* cells were fully repressed upon addition of BDI-IS1 cell-free extract (15 % v/v); while *X. campestris*, *P. carotovorum* and *P. cichorii* growth inhibitions were partial, ranging from 90% to 40% depending on the pathogen (Figure 4-1A). PDB cell-free supernatants supplemented to the PDA plates (up to 40%) also exhibited growth inhibition of the two tested fungi *B. cinerea* and *A. solani*. The *B. cinerea* mycelium expansion was partially inhibited at 30%, but it was completely abolished at 40% (Figure 4-1C). *A. solani* revealed a moderate susceptibility to the cell-free supernatants with a residual growth even at the highest tested concentration of CFS (40%). The possible antagonistic potential of volatile organic compounds putatively produced by BDI-IS1 was tested *in vitro* by dual culture on superimposed plates, but there was no antagonism (Data not shown). Notably, these results correlate well with the ones obtained in plate confrontation assays (see Chapter III, section 3.1) and evidence the crucial role of secreted soluble BSMs in mediating antibacterial and antifungal activities.

Correlatively, the antibacterial effects of cell-free supernatants of two existing strains of *B. nakamurai* (NRRL B-41091 and NRRL B-41092) have also been reported against the *P. carotovorum*-closely related *Erwinia amylovora* species, but their effect was bacteriostatic rather bactericidal (Leathers et al., 2020). In the meantime, the reported VOCs-mediated antifungal activity of an Egyptian *B. nakamurai* strain against *B. cinerea* do not corroborate with our results (Chaouachi et al., 2021), but this can be explained by the already demonstrated variable susceptibility of *B. cinerea* isolates to antibiotics (Ajouz et al., 2011).

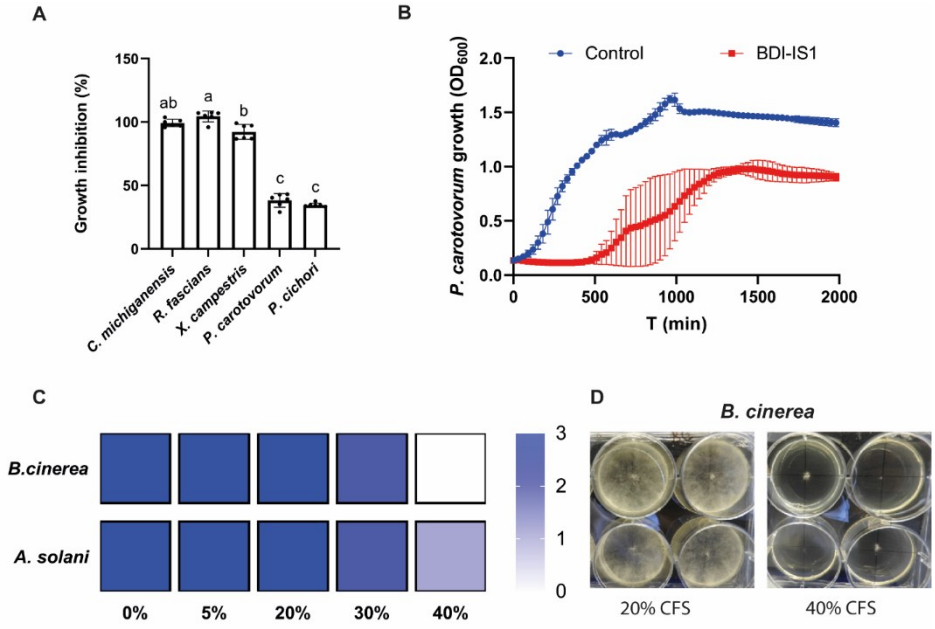


Figure 4-1: Antagonistic activity of cell-free culture supernatants (CFS) against selected phytopathogenic bacteria and fungi. A. Growth reduction of bacterial phytopathogens by cell-free REM culture supernatants (CFS, 15% v/v) of BDI-IS1. Bar graphs (with error bars) represent mean (± SD) values calculated from three repetitions with two independent assays (n = 6). Different letters a to c indicate significant statistical differences of means or no difference if same letters, following the one-way analysis of variance (ANOVA) coupled to Tukey's HSD test (Honestly significant different, $\alpha = 0.05$). B. Growth curve of *P. carotovorum* cultured with or without 15% (v/v) BDI-IS1 CFS (red and blue curves, respectively); data points are means (± SD) values calculated from three repetitions with two independent assays (n=6). C. Fungal growth inhibition of *B. cinerea* and *A. solani* (5 days) by increasing concentrations (0-40%) of BDI-IS1 CFS supplemented to PDA. The heatmap was generated in Prism GraphPad 9.5.0 by assigning discrete values 3, 2, 1 and 0 to scenarios of high, moderate, low, and no growth, respectively. D. Illustration of *B. cinerea* growth inhibition by 20% and 40% of BDI-IS1 CFS (5 days, 23°C).

3.2. Characterization of the BDI-IS1 secondary metabolome

The strong antagonistic potential of BDI-IS1 reflects the ability of the strain to efficiently secrete antimicrobial metabolites. Therefore, we first wanted to inspect its genomic content in biosynthetic gene clusters (BGCs) responsible for the synthesis of BSMs. Genome mining was performed using the software AntiSMASH 7.0.1 dedicated for non-ribosomal peptides and some lantibiotics (Blin et al., 2023) and completed by the specific RiPPs miner software BAGEL 4 (Van Heel et al., 2018). It revealed the presence of BGCs responsible for the biosynthesis of a range of non-ribosomal products such as the cyclic lipopeptides surfactin and iturin A (Z. Wang et al., 2024), the polyketide bacillaene (and its variant dihydrobacillaene) (Miao et al.,

2023), the siderophore bacillibactin (Dertz et al., 2006), and the dipeptide bacilysin (T. Islam et al., 2022). Genes encoding several ribosomally produced and post-translationally modified peptides (RiPPs) were also identified and include amylocyclicin (Scholz et al., 2014), plantazolicin (Kalyon et al., 2011; Molohon et al., 2016), bacinapeptin (D. Xue et al., 2022) and LCI (Gong et al., 2011) (Figure 4-2). Plantazolicin is a thiazole and oxazole heterocycle-containing molecule with a dimethylated bioactive form (plantazolicin A) and its inactive non-methylated precursor peptide (plantazolicin B) (Kalyon et al., 2011; Molohon et al., 2016). Bacinapeptin is a poorly described class III lanthipeptide characterized by a labionin ring that was recently reported in *B. nakamurai* NRRL B-41092 with two variants bacinapeptin A ($C_{106}H_{141}N_{27}O_{25}S$) and bacinapeptin B ($C_{104}H_{142}N_{26}O_{26}S$) (D. Xue et al., 2022). Bacinapeptins are structurally related to andalusicin A and its variants recently discovered into *B. thuringiensis* sv. *andalousiensis* (Grigoreva et al., 2021).

In addition, some orphan genes were also found in the AntiSMASH-based prediction (Figure S1) and they include one type III PKS BGC encoding putatively chalcone/stilbene synthase-like protein (Pandith et al., 2020), terpenes BGC putatively encoding for phytoene/squalene synthase family protein (M. Kim et al., 2020) and squalene-hopene cyclase (Nair & Kochupurackal, 2023), and one RRE (RiPP recognition element)-containing protein supporting putatively the synthesis of pyrroloquinoline quinone D (PqqD) family protein (Ren et al., 2023).

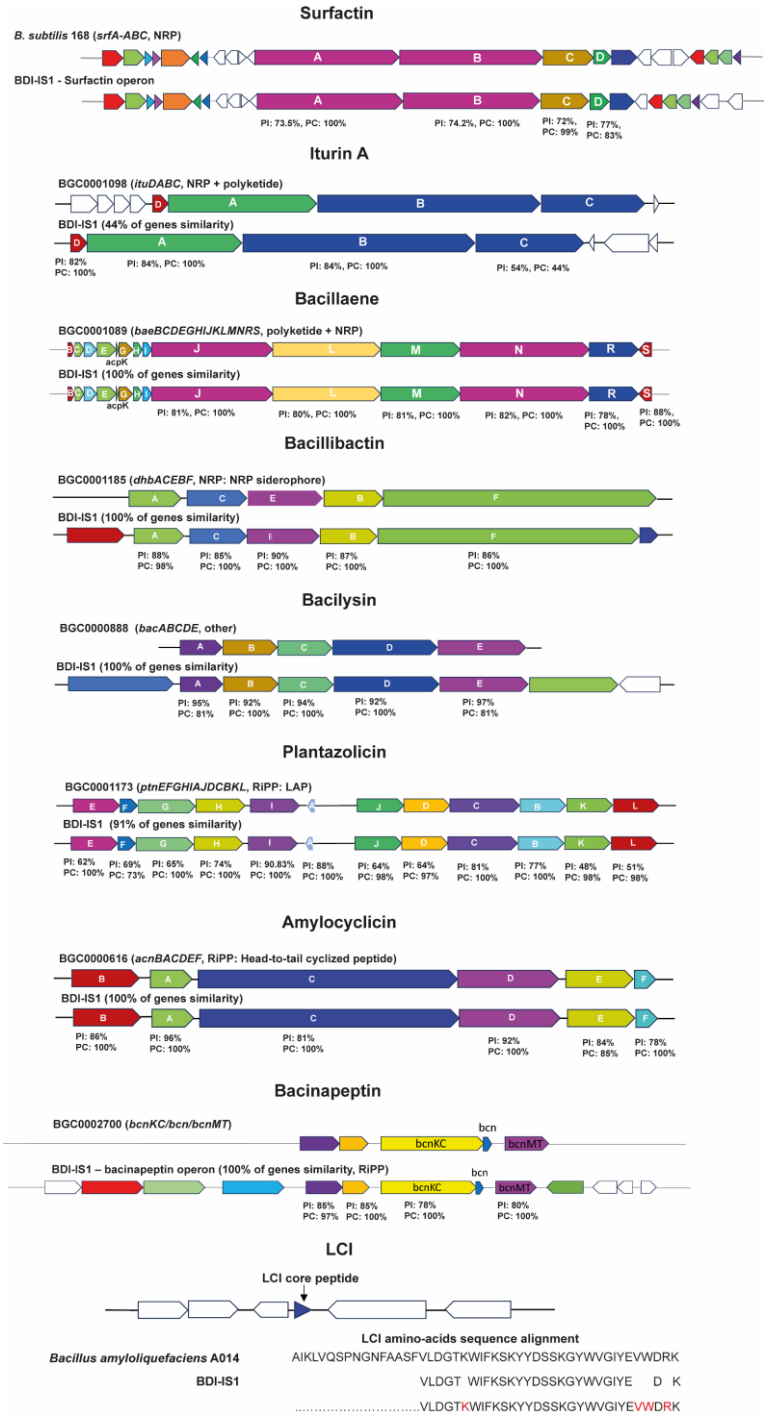


Figure 4-2: AntiSMASH and BAGEL prediction patterns for BGCs of important bioactive secondary metabolites. Each gene of the different BGCs of BDI-IS1 is presented with the

same colour as its corresponding gene in the reference BGC (top) and the percentage of identity is indicated below each gene. Colour assigned to each gene is depending on function prediction. Exception is for LCI, which was predicted only with BAGEL4.

Further chemical characterization of BSMs produced by BDI-IS1 in REM was performed via UPLC-q-TOF-MS analysis of cell-free extracts. We identified surfactin and iturin A along with their fatty acid variants C_{12} to C_{16} and C_{14} to C_{17} respectively, bacillaene (and its variant dihydrobacillaene), bacillibactin, bacilysin and the RiPP plantazolicin A (and its non-methylated form plantazolicin B) (Figure 4-3A). Thanks to MS^2, we could elucidate some controversial compounds with same or close LC-MS patterns (retention time and m/z ratio) like surfactin/pumilacidin or iturin A/ mycosubtilin. The MS^2 spectra patterns confirmed that the produced lipopeptides are surfactin and iturin A since valine (Val) and leucine (Leu) are in position 4 and 7, and serine (Ser) in position 7 in the respective amino-acids sequence of surfactin and iturin A (Figure 4-3B). The structure of plantazolicin was also confirmed by MS^2 based on specific fragment ions at m/z = 1145.4275, 630.2340, 584.2274, 455.0629; from the precursor ion at m/z = 1336.4900 (Figure 4-3B & S2).

In addition, BSM production by BDI-IS1 was tested in different laboratory media and obviously, the root exudates-mimicking medium supports better metabolite secretion compared to the classical laboratory rich media Luria Bertani (LB) and potato dextrose broth (PDB), except bacilysin for which high production was observed in PDB (Figure 4-3C). Surfactin production is very efficient (almost six times higher that iturin A) regardless the medium effect, which is a general trend for *Bacillus* spp. (D. Sun et al., 2019). However, the remaining metabolites are produced in reduced quantities compared to cyclic lipopeptides (ten-fold less) and in a medium-dependent manner. Bacillibactin is produced in the three media, but its secretion in LB is very insignificant compared to other media. In addition, bacillaene and plantazolicin were not detected in PDB and the dipeptide bacilysin was not produced in detectable amounts in LB cell-free supernatants. These results reveal the bacterium nutritional preferencies, which are in line with the natural conditions for rhizobacteria that feed mainly on plant root exudates (Lin et al., 2024) and constitute another evidence that BDI-IS1 could be a prominent root-associated bacterium.

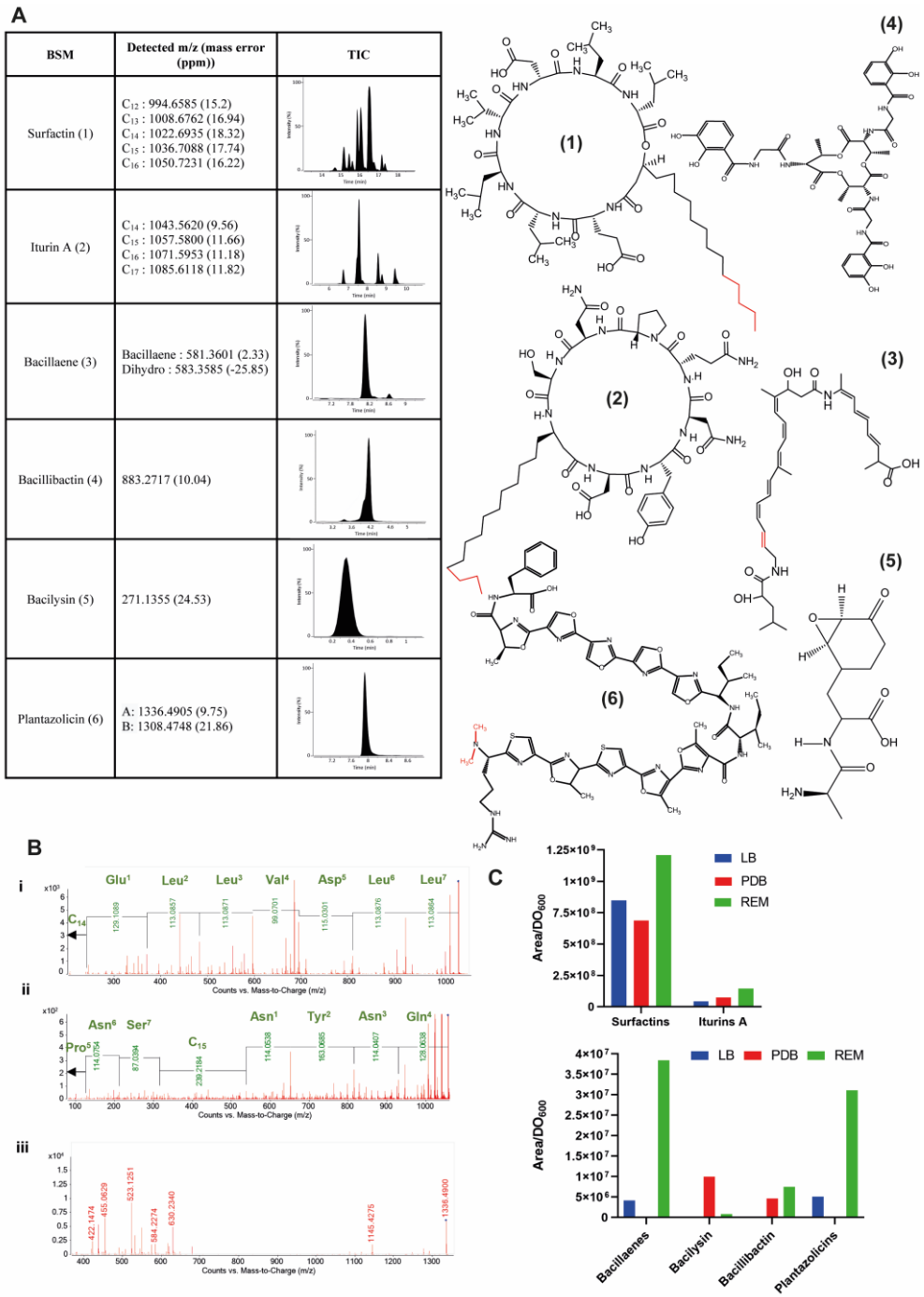


Figure 4-3: Chemical analysis of BDI-IS1 cell-free supernatants by UPLC-q-TOF-MS. Panel A illustrates the extract ion chromatogram (EIC) of each of the detected compound by the UPLC-q-TOF-MS technique along with the detected m/z value (error in ppm) (left) and

the ChemDraw software-derived chemical structures of the detected compounds (right). Panel B illustrates the MS^2 spectra with m/z corresponding to the precursor ion and the different fragmented ions of surfactin (i), iturin A (ii) and plantazolicin (iii). Panel C shows the plotted graphs of the relative production of BSMs by BDI-IS1 in LB, PDB and REM (30°C, 160 rpm).

3.3. Comparison of BSM potential in BDI-IS1 and related *Bacillus* strains

We next inspected the genomic content in BGCs responsible for BSMs biosynthesis in the other strains of *B. nakamurai* sequenced so far and in other related species of the *B. subtilis* clade to evaluate to what extent the BDI-IS1 secondary metabolome is conserved at the species and clade levels. This comparative genome mining analysis revealed that all non-ribosomal compounds mentioned above, at least one compound of the class III lanthipeptide and one metabolite of the circular lanthipeptide (amylocyclicin and/or skf) are conserved across the strains of the *B. nakamurai* species. Together with plantazolicin predicted in four out of the five strains, these molecules thus constitute the core metabolome typical of the *B. nakamurai* species (Figure 4-4). Conversely, the content in BGCs predicted to encode for other RiPPs (LCI, subtilin (ericin_S), pumilarin, mersacidin, and staphylococcin C55 α/β) and one putative non-ribosomal-like compound thermoactinoamide is more variable and strain-specific (Figure 4-4). On the other hand, surfactin (and their variants pumilacidin and lichenysin), bacilysin and bacillibactin are present in all the considered *Bacillus* strains and could hence be considered as characteristic of the whole *B. subtilis* clade, except *B. atrophaeus* which lack bacilysin BGC.

This comparative analysis also reveals that the species belonging to the *B. amyloliquefaciens* operational group (*B. velezensis*, *B. siamensis*, *B. nakamurai* and *B. amyloliquefaciens*) are characterized by at least two cyclic lipopeptides (surfactin and iturin/fengycin), at least one polyketide (bacillaene at least), bacillibactin, bacilysin and at least one circular lanthipeptide (amylocyclicin in general or skf). However, *B. velezensis* strains have the particularity to potentially secrete the whole three families of cyclic lipopeptides, three families of polyketides and additional lanthipeptide LCI compared to what described for the whole *B. amyloliquefaciens* group. The secondary metabolome characteristic to the *B. pumilus* and *B. licheniformis* groups are typically identical to what defining the *B. subtilis* clade as described above, except the additional RiPPs or RiPP-like compounds (plantazolicin, sonorensin, mersacidin, lichenicidin, bottromycin) that are more strain-specific. Peculiar to RiPPs, head-to-tail cyclized (circular) lanthipeptides appear to be more conserved in many of the considered strains of the *B. subtilis* clade, excluding the *B. pumilus* and *B. licheniformis* groups (Figure 4-4).

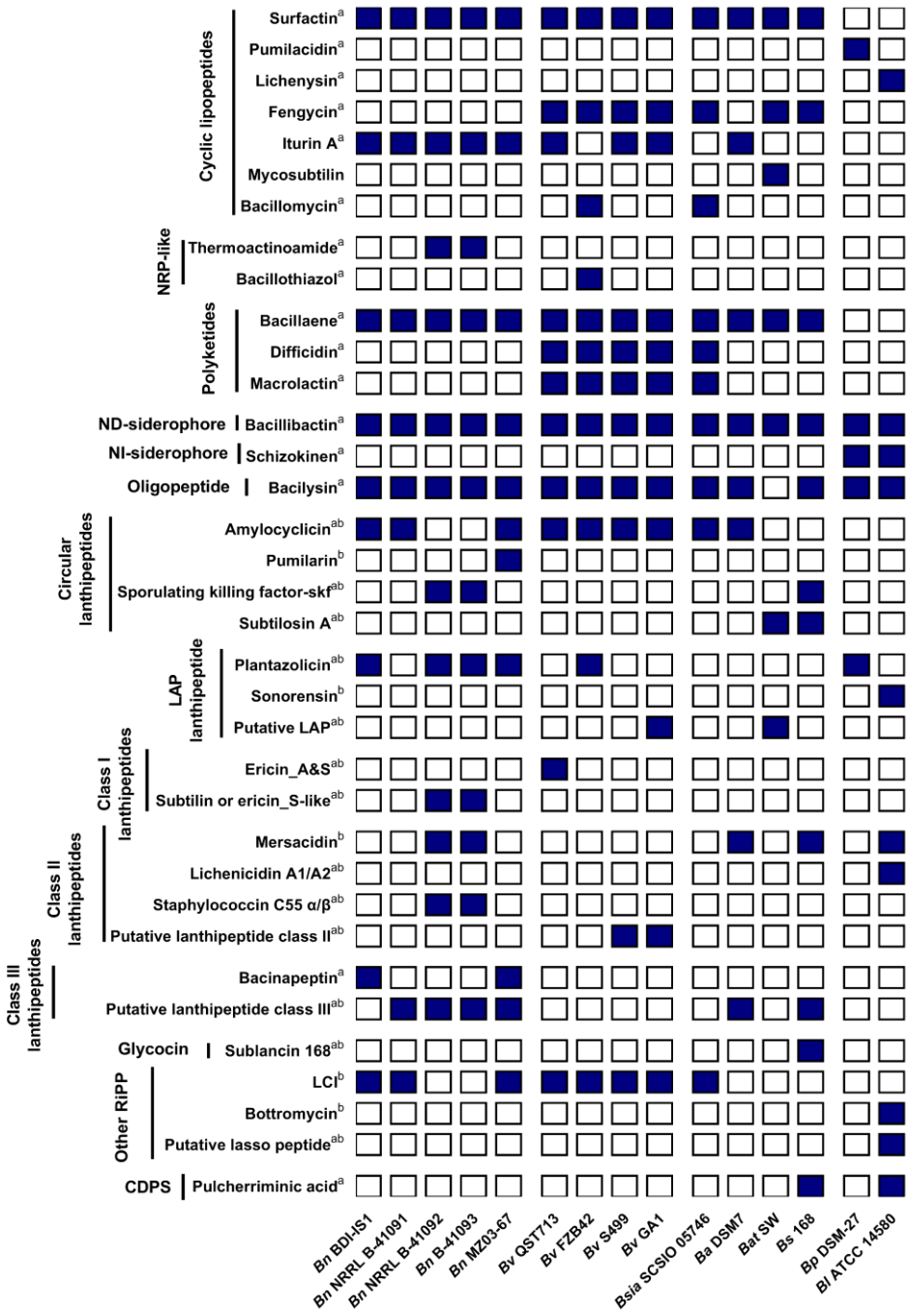


Figure 4-4: Comparative genome mining of secondary metabolites in *B. nakamurai* strains and related strains of the *B. subtilis* clade. BSMs prediction was carried out by AntiSMASH

7.0.1 (Relax mode) and BAGEL 4 and squares coloured in blue or not implies the presence or absence of corresponding metabolite BGC. BGCs were either detected by AntiSMASH ([a]) or BAGEL 4 ([b]) or the two of them ([ab]). ND and NI-siderophores stand for NRPS-dependent and NRPS-independent siderophores, LAP for Linear-azol(in)e-containing peptide and CDPS for tRNA-dependent cyclodipeptide synthases; while Bn, Bv, Ba, Bsia, Bat, Bs, Bp and Bl stand for the species name of *B. nakamurai*, *B. velezensis*, *B. amyloliquefaciens*, *B. siamensis*, B. *atrophaeus*, *B. subtilis*, *B. pumilus* and *B. licheniformis* respectively.

In addition, some of these predicted RiPPs either conserved or not at species level have undergone mutations within their amino acid sequences, a fact that is not clearly shown in the patterns generated from genome mining softwares. This is the case for bacinapeptin predicted in BDI-IS1 where some substitutions (highlighted in red) at position 14, 16 and 18 (Figure 4-5) can be noticed in its core amino acid sequence compared to bacinapeptin A *de novo* described in *B. nakamurai* NRRL B-41092 (D. Xue et al., 2022), which thus leads most probably to a bacinapeptin variant with raw formula $C_{105}H_{139}N_{27}O_{24}S$ (2,194.020705 Da). This observation is consistent also with other RiPPs such as the head-to-tail cyclized lanthipeptide amylocyclicin and the RiPP-like LCI (Figure S3).

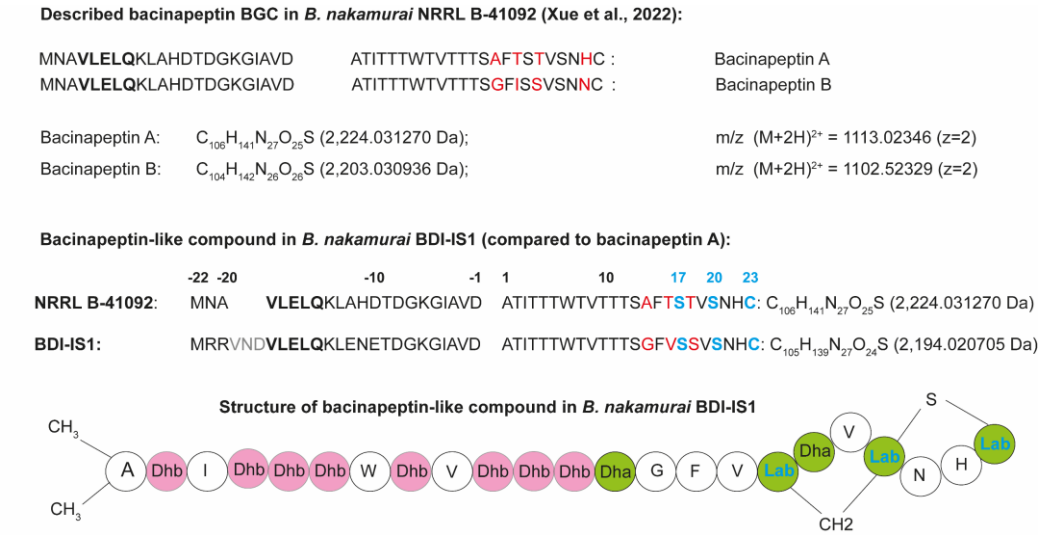


Figure 4-5: Amino acid sequence-based structural organisation of bacinapeptin-like compound from BDI-IS1. This amino acid sequence of bacinapeptin variant from BDI-IS1 was aligned against one of bacinapeptin A from *B. nakamurai* NRRL B-41092 (D. Xue et al., 2022). Amino acid symbols colored in red show where the substitutions occur, those in blue are constitutive of the labionin ring (S^{17}-S^{20}-C^{23}) which is represented on the structure with the same color. Pink and green colors on the structure represent Dhb and Dha, the dehydrated form of threonine and serine respectively.

3.4. Specific involvement of BSMs in the antimicrobial activity of BDI-IS1

We next wanted to determine the relative contribution of each BSM in the broad-spectrum antimicrobial activity displayed by *B. nakamurai* BDI-IS1. For that purpose, we generated a library of mutants specifically repressed in the synthesis of each compound together with a *Δsfp* mutant lacking the 4'-phosphopantetheinyl transferase (sfp) gene essential for the proper NRPS functioning (Wu et al., 2023) and thus impaired in the synthesis of all non-ribosomal metabolites (except bacilysin). Mutants were verified in two ways. First, DNA were extracted, amplified by PCR using the appropriate primers and verified by gel electrophoresis for proper insertion at desired position of the antibiotic cassette. Second and where possible (not for amylocyclicin, bacinapeptin and LCI), mutants were cultured in REM and their CFS were assessed by UPLC-qTOF-MS technique for absence of production of the depleted BSM.

All those mutants were tested for their antagonistic activity and data revealed different situations (Figure 4-6). In some cases, the antimicrobial activity clearly relies on one single compound such as bacillaene (and/or its -2H form) in the case of the antagonism against *P. carotovorum* and *R. fascians* or iturin A in the case of inhibition of the fungal pathogens *A. niger* and *R. solani*. This was also evidenced by the complete loss of inhibitory potential of the *Δsfp* mutant. In other cases, the antagonistic activity of *Δsfp* is drastically reduced and we could identify iturin A (against *E. turcicum*) as the main active ingredients, but other unidentified compound(s) also contribute to this inhibition. As a third situation, the polyketide bacillaene contributes at moderate level to the inhibition of *C. michiganensis*, *X. campestris* and *P. cichorii*, while iturin A is partly involved in the antagonism of *B. cinerea* and *A. solani.* The activity against *P. fuscovaginae* is mediated mainy by bacilysin, but the loss of activity for *Δsfp* suggests the interplay at some extent, and probably in synergical manner, of sfp-dependent NRPs. Ribosomally produced metabolites are also involved in pathogen inhibition like plantazolicin which plays some role in the antagonistic effect against *X. campestris* (partial loss of activity of *ΔpznA*). However, the main inhibitory molecule(s) toward *C. michiganensis*, *X. campestris*, *P. cichorii*, *B. cinerea* and *A. solani* still remain to be identified (Figure 4-6).

Nevertheless, some of these pathogens such as *B. cinerea* have been reported to be highly susceptible to purified or semi-purified iturin A (Ambrico & Trupo, 2017). Therefore, this suggests that the quantity of iturin A produced upon confrontation may not be sufficient to inhibit completely the growth of this pathogen and that the bacterium secretes other antifungal compounds or utilizes other strategies to reduce pathogen's proliferation.

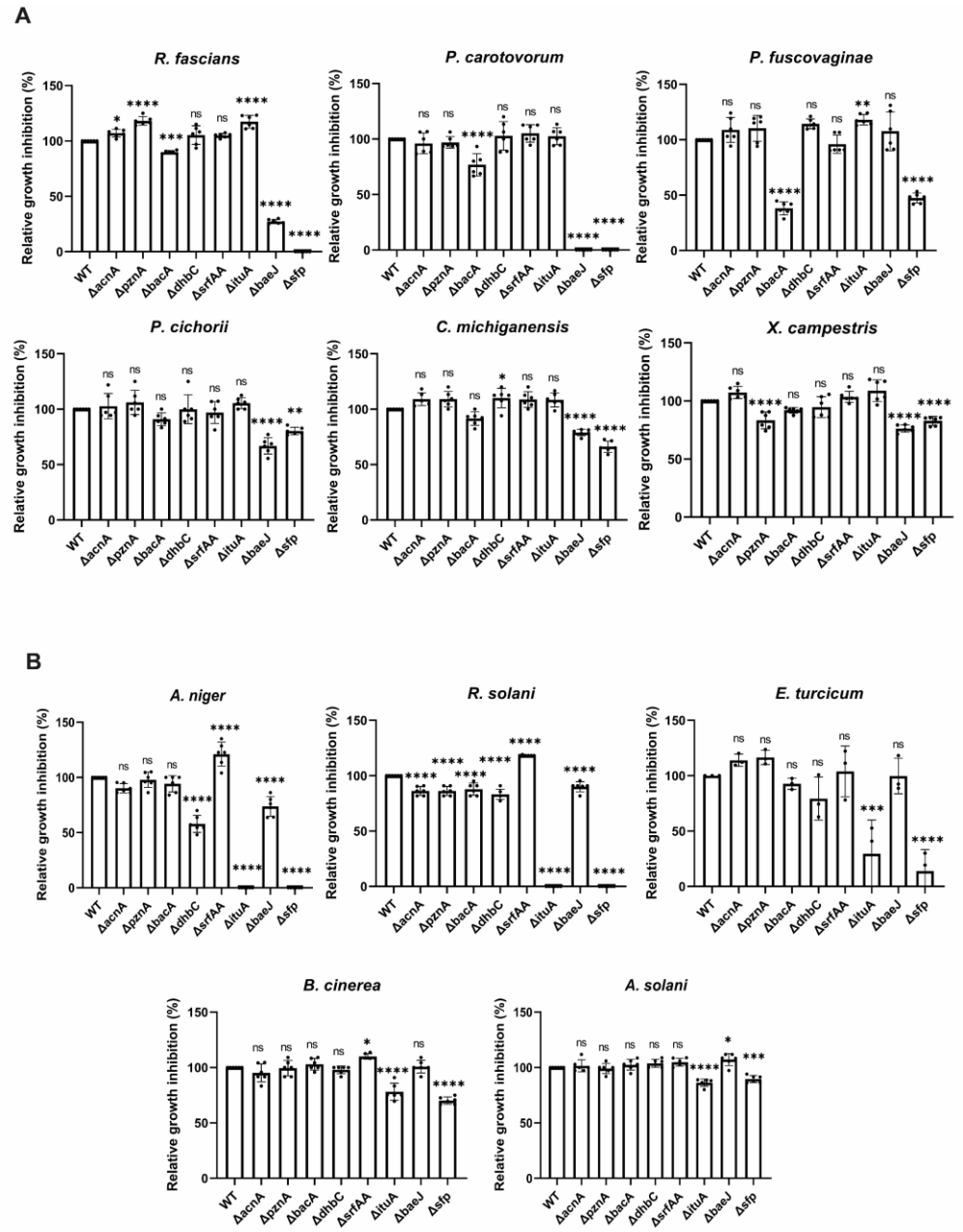


Figure 4-6: Antagonistic activities of BDI-IS1 mutants against bacterial and fungal phytopathogens. A. Antibacterial activity (relative growth inhibition) of different BDI-IS1 mutants against some bacterial phytopathogens, in comparison with the wild type. The confrontation assay was set on gelified REM (30ml, 30°C, 48h) and inhibition diameter (mm) recorded and expressed in percentage (compared to the wild type). B. Antifungal activity of BDI-IS1 mutants against some important fungal phytopathogens. Relative growth

inhibition of fungal pathogens by the different mutants was observed after seven days (20ml of PDA, 23-25°C), except for *A. solani* where incubation was for 21 days and grown on REM Agar. The plotted data (with error bars) for A&B are means (± SD) of three technical replicates, conducted twice in independent experiments (n = 6). Statistical comparison of means was performed with one-way ANOVA coupled with Tukey's test ($\alpha = 0.05$) and ns represent non-significant difference, while asterisks *, **, ***, **** imply significant differences at $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.0001$ respectively. WT represent the *B. nakamurai* BDI-IS1 wild type, while *ΔacnA*, *ΔpznA*, *ΔbacA*, *ΔdhbC*, *ΔsrfAA*, *ΔituA*, *ΔbaeJ* and *Δsfp* stand for BDI-IS1 mutant strains depleted in the biosynthesis of amylocyclicin, plantazolicin, bacilysin, bacillibactin, surfactin, iturin A, bacillaene and all sfp-dependent NRPs, respectively.

3.5. Biocontrol potential of BDI-IS1

Greenhouse experiments were next conducted to evaluate the potential of BDI-IS1 to control two major diseases prevalent in Burundi and caused by the foliar pathogens *A. solani* on tomato and *E. turcicum* on maize. Prior to infection, plants were treated with BDI-IS1 either by aerial spraying (as a foliar treatment) or by soil drenching (as a root treatment) to evaluate the biocontrol activity mediated via direct antagonism in the first case or via the induction of systemic resistance in the second case. The leaves of tomato or maize plants were then inoculated with *A. solani* or *E. turcicum*, respectively. Tomato early blight and northern leaf blight diseases were assessed by determining the necrotic leaf area according to a specific scale of symptoms (Figure S4). Comparison of the dynamics of disease severity revealed that symptoms occurrence was delayed by 1 to 3 days in plants pretreated with BDI-IS1, depending on the pathosystem and application method (Figure 4-7). We also observed a slower progression of disease severity compared to untreated plants, particularly on tomato plants upon leaf treatment with the bacteria, and on maize plants upon root and leaf treatments (Figure 4-7).

The reduction of disease severity was significant from the 13th day post inoculation until the time when symptoms no longer evolved on control plants (Table S5 & S6). BDI-IS1 delayed disease development by acting efficiently at early stage, as shown by the disease progression curves (Figure 4-7A&D). Root-treated plants did not show symptoms until 4 days post inoculation with *A. solani*, while a 10% disease severity (DS) was observed on control plants and for those treated on leaves. NLB symptoms appeared on control and treated plants from the 7th and 10th day post inoculation, respectively. Overall protection indexes of approximately 25% and 35% for TEB and 65% and 50% for NLB (Figure 4-7B&E) were obtained after root and leaf treatments in two independent greenhouse experiments under severe infection pressure, and no significant differences were observed between leaf and root treatments.

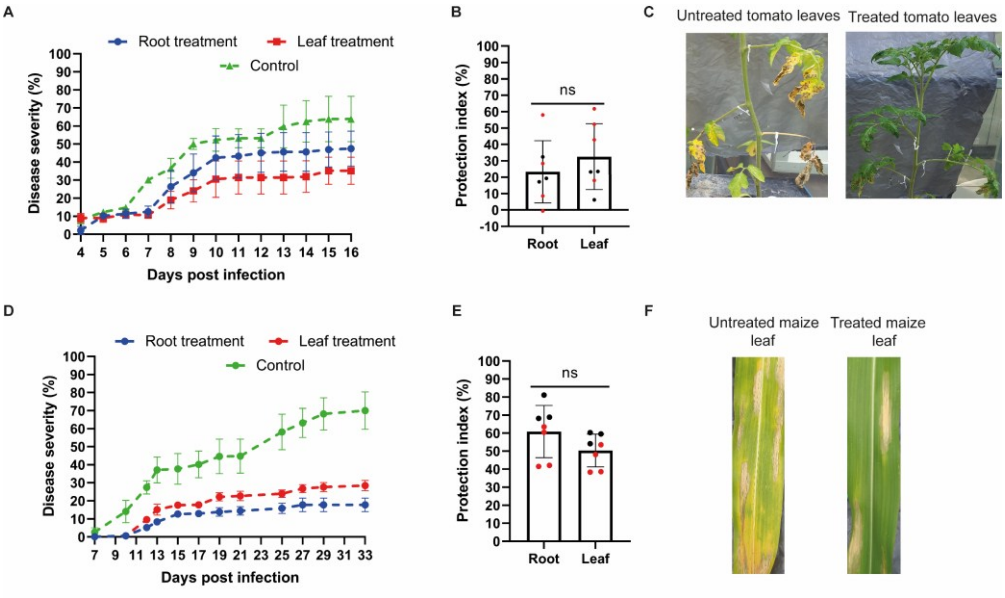


Figure 4-7: Biocontrol efficacy of BDI-IS1 against tomato early blight (TEB) and nothern corn leaf blight (NLB). A&D represent the reduction in disease severity of TEB and NLB on plants treated in the roots (blue) or leaves (red) with BDI-IS1 followed by inoculation with *A. solani* or *E. turcicum*, respectively, and control plants (in green) inoculated with the respective fungus, under greenhouse conditions. Each point in graphs A and D represents the mean (± SE) of disease severity from four replicates (n = 4) and three replicates (n = 3) for experiments one and two, respectively. B&E represent the protection index (PI) of BDI-IS1 when applied to roots or leaves of tomato and maize plants inoculated with *A. solani* and *E. turcicum*, respectively. PIs represent means (± SE) of two independent experiments (n = 7) with four (red) and three (black) replicates per treatment for experiment one (n = 4) and two (n = 3), respectively. Means of PI are calculated from area under progress curve (AUDPC) data (see formula in section 2.6.4 in this chapter). The differences between PI of BDI-IS1 obtained from root and leaf treatment were analyzed using t-test method and there was no significant difference (ns) at $p \leq 0.05$. C&F show the TEB and NLB necrotic lesions on tomato and maize plants treated on leaves with BDI-IS1 compared to untreated but inoculated plants.

These results highlight the biocontrol potential of BDI-IS1 acting locally as antagonist (leaf treatment) or systemically by eliciting plant resistance (root treatment). Furthermore, the levels of protection provided by BDI-IS1 against TEB or NLB are in the same range as the ones observed with *B. velezensis* QST713 tested under the same conditions, except for leaf treatment on tomato where no disease reduction was observed with that strain (Figure S5&S6 and Table S7). Despite the same global trend in protection levels for each treatment method adopted, the data collected from the two independent trials show a huge variation due to the different weather conditions in which the trials were conducted (Table S8).

4. Discussion

In this work, we provide a first comprehensive genomic and chemical characterization of the secondary metabolome produced by *B. nakamurai* BDI-IS1, described in the 2nd chapter with a great antagonistic activity against an array of important bacterial and fungal phytopatopathogens.

Comparative genome mining for cognate BGCs revealed that a subset of this metabolome with compounds produced via the non-ribosomal pathway is well conserved in all *B. nakamurai* strains sequenced so far. Due to the absence of the lipopeptide fengycin and the polyketides difficidin and macrolactin, this core metabolome of *B. nakamurai* appears less diversified compared to other closely related species such as *B. velezensis* in the *B. subtilis* clade but still can putatively be used as a chemical fingerprint typical for the species and could be exploited in taxonomic identification (Q. J. Yin et al., 2023). Correlatively, the core metabolome of the whole *B. subtilis* clade was found defined by three non-ribosomal peptides including a siderophore, bacilysin and surfactin (or its variant pumilacidin or lichenicidin) (Steinke et al., 2021). By contrast with the well-conserved non-ribosomal products at species level or species group/clade level with putative taxonomical significance, the type and number of RiPPs are more strain-specific, except the circular lanthipeptides which were found represented in the whole *B. amyloliquefaciens* group. This diversification of RiPPs at the strain level may be explained by the acquisition of BGCs from close relatives or other bacterial genera sharing the same agro-ecological niche via horizontal gene transfer (Grubbs et al., 2017; Malit et al., 2021). This may be facilitated by the *ComS* gene involved in natural competence and quorum-sensing (Rahman et al., 2021), and present in all the considered *Bacillus* strains. This chemodiversity of RiPPs among bacilli is not only reflected by their chemical classes, but also in the heterogeneity of substitutions occurring within their leader and core amino acid sequences leading to a vast array of putative structural variants of the same compound, either within the same strain or across the different *Bacillus* strains. Structural variants of the same compound imply modifications of the compound's intrinsic physico-chemical properties with plausible implications on their putative biological functions. These modifications are widespread in nature and impact most often the biological activities of these compounds, as it has been shown for iturin for example (Guez et al., 2022; Maget-Dana & Peypoux, 1994).

Furthermore, we could for the first time generate multiple mutants in this undervalued species, allowing us to further exemplify the key role played by some of these specialized metabolites in the broad-spectrum antimicrobial activity displayed by BDI-IS1 (Ajuna et al., 2024; Saiyam et al., 2024). Some bacterial phytopathogens were antagonized by BDI-IS1 via the production of bacillaene and/or its 2H-form or bacilysin or a synergy between the two compounds. The polyketide bacillaene (and its 2H-form) is an antibacterial compound against various pathogenic bacteria including *Campylobacter*, *Salmonella*, *Serratia*, *Streptomyces*, etc. by interfering with protein synthesis (Erega et al., 2021; Miao et al., 2023), and it is reported to sustain

other ecological roles like protecting the producing strain from predation by soil competitors such as *Mycoplasma xanthus* (Andrić et al., 2023). Although not evidenced in our study, this compound was also shown to interplay in the inhibition of some fungi like *Penicillium digitatum* and *F. oxysporum* (K. Chen et al., 2018; Miao et al., 2023; Z. Xu et al., 2014). Bacilysin retains strong antibacterial activity against a range of plant and food-borne pathogenic bacteria such as *Xanthomonas*, *cyanobacteria*, *Staphylococcus*, *Erwinia*, etc. presumably by inhibiting the synthesis of glucosamine 6-phosphate and hence the formation of peptidoglycan (X. H. Chen, Scholz, et al., 2009; Islam et al., 2022). This dipeptide was also shown to antagonize pathogenic yeasts and oomycetes by putatively blocking the synthesis of their cell wall mannoproteins (X. Han et al., 2021; Kenig & Abraham, 1976). Plantazolicin, a thiazole/oxazole containing-molecule was found to be involved in the inhibition of the brassica's black rot-causing pathogen *X. campestris*. Beyond exhibiting nematicidal activity against *Caenorhabditis elegans* (Z. Liu et al., 2013), this metabolite was already reported to have antibacterial activity, but only against Gram positive bacteria including the obligate pathogen *B. anthracis* (Molohon et al., 2016; Scholz et al., 2011).

In some cases, we could not identify the metabolites involved in the antimicrobial activity suggesting that other products may interplay solely or in synergy with known BSMs in these antagonisms. Among the possible products, the predicted antimicrobial peptide LCI was shown to have a strong inhibitory potential against *X. campestris* pv *oryzae* and *Pseudomonas solanacearum* PE1 (Gong et al., 2011) and *Aeromonas hydrophila*, the causal agent of red-sore disease in largemouth bass fish (Chang et al., 2024). Also, LCI-like APC_2 is reported to antagonize *Fusarium solani*, the causal agent of ginseng root rot (R. Wang et al., 2021) and LCI-like protein MD is related with antibacterial activity against the opportunistic human and animal pathogen *S. aureus* (Y. Wu et al., 2018). The predicted lanthipeptide bacinapeptin-like compound is structurally related to bacinapeptin and andalusicin which were described to exhibit antibiotic activity against various Gram-positive bacteria (Chang et al., 2024; Gong et al., 2011).

We suspect that other unknown metabolites derived from the T3PKS (type III polyketide synthase), terpene and RRE-containing protein BGCs (Figure S1) could be among the mediators of some antagonistic activities of BDI-IS1. Indeed, although not yet identified in *Bacillus*, some compounds synthesized via the T3PKS like diacetylphloroglucinol in *Pseudomonas* sp. are involved in biocontrol activity via inhibition of fungal phytopathogens (Katsuyama & Ohnishi, 2012). Most terpenes produced by strains of the *B. subtilis* group are volatile compounds (Caulier et al., 2019; S. Iqbal et al., 2023; Shafi et al., 2017), except the C-35 terpenes of *B. subtilis* KSM 6-10 (Takigawa et al., 2010) and the oxidative stress alleviator sporulenes, tetracyclic terpenoids found in *B. subtilis* spores (Bosak et al., 2008). The roles of these terpenes in the antimicrobial potential of bacilli against phytopathogens is not well described, but it was shown that isoprene and monoterpene α-terpineol from *B. subtilis* exhibit antagonistic activity against cyanobacteria and nematodes (Caulier et al., 2019). The RiPP Recognition Element (RRE) domain is represented in almost half

of RiPPs-producing prokaryotes (Ren et al., 2023) and this RRE-containing protein BGC in BDI-IS1 would code for a novel RiPP with putative antagonistic properties (Hudson & Mitchell, 2018). Other BSMs like surfactin and bacillibactin are not involved in direct antagonistic activity but still these compounds can play key functions for the ecological fitness of the bacterium considering their well-recognized role in space colonization through cell motility and biofilm formation for surfactin (Hoff et al., 2021; Stannius et al., 2025) and iron acquisition for bacillibactin (Gu et al., 2025; R. Kumar et al., 2024).

Globally, our data illustrate the huge chemical diversity of BSMs in *B. nakamurai*, which is likely to explain the broad-spectrum antimicrobial activity of strains such BDI-IS1. This extends much beyond the antagonistic activity of *B. nakamurai* occasionally reported against *Erwinia amylovora* (Leathers et al., 2020), *Fusarium poae* (Zanon et al., 2024) and *B. cinerea* (Chaouachi et al., 2021). As *B. nakamurai* targets bacterial and fungal pathogens affecting different crops of worldwide economic importance, this antagonistic potential is an important trait for any biocontrol candidate.

Basing our prospect on the new strain *B. nakamurai* BDI-IS1 isolated from arable soils collected from highlands of tropical region (1880m of altitude, Burundi), we also unravel the great biocontrol potential of this endemic *Bacillus* strain against two important tropical plant diseases, tomato early blight and northern corn leaf blight (de Sousa Ramos et al., 2024; Gupta et al., 2024). The biocontrol efficacy of BDI-IS1 against important fungal diseases such as northern corn leaf blight and tomato early blight is interesting as it reaches a level similar or higher than the commercial strain QST713 in greenhouse experiments. When applied preventively, BDI-IS1 delayed the severity of TEB and NLB by one to three days and thus reduced disease pressure on young plants, allowing faster growth and higher resistance to pathogen invasion or to abiotic stresses. This observation was also reported by Galiano-Carneiro and Miedaner (2017), where high yield losses were observed after early infection by *E. turcicum*, before silking.

Our results showed an average protection of 25% and 35% for TEB after root or leaf treatment with BDI-IS1. Previously, other *Bacillus* species such as *B. amyloliquefaciens* strain bact-03 have been reported to control TEB caused by *A. solani* strain As-9003, with a significant disease reduction (50%) after leaf treatment under greenhouse conditions (Imran et al., 2022). Although BDI-IS1 protects tomato plants by either root or leaf treatment, the fact that the leaf treatment guaranteed relatively high protection for BDI-IS1 strongly suggest that the strain utilizes mostly the direct route in controlling TEB. This may involve to some extent the antifungal iturin A active against several pathogens such as *Phytophthora infestans* (Y. Wang et al., 2020), *B. cinerea* (Ambrico & Trupo, 2017) and *Fusarium* spp. (Y. Liu et al., 2020). Noteworthy, these pathogens are also prevalent in the tropics, threatening various economic crops and could be controlled by BDI-IS1 alone or in combination with other plant disease control strategies. The efficacy of *Bacillus* spp. in controlling NLB was previously reported, with a reduction in disease severity ranging from 40% to 56% following leaf treatment in a greenhouse experiment (Sartori et al., 2017).

Here, we demonstrate for the first time the high potential of *B. nakamurai* to protect maize against NLB upon the two modes of application, suggesting a dual mode of action relying on direct antagonism and/or induction of plant systemic resistance. Based on *in vitro* assays, production of iturin A may be tightly involved in local restriction of *E. turcicum* development on leaves. Regarding ISR, the cyclic lipopeptide surfactin, produced quietly by all species of the *B. subtilis* clade including *B. nakamurai*, has been widely reported as elicitor of plant defense in several pathosystems (Hassanisaadi, 2024; Pršić & Ongena, 2020). However, iturin A has also been reported as trigger of ISR (Q. Han et al., 2015; Lam et al., 2021; Yamamoto et al., 2015) and further experiments are needed to identify the molecule(s) from *B. nakamurai* that is (are) active on tomato and maize in our study.

Although not equipped like *B. velezensis* QST713 in terms of secondary metabolites reported to be involved in the biocontrol of fungal diseases (i.e. absence of fengycin), BDI-IS1 demonstrated significant protection against TEB and NLB in a tropical mimicking-greenhouse conditions either upon leaf or root treatment at levels similar to QST713. However, QST713 did not reduce tomato early blight severity upon leaf treatment (Figure S5&S6). This suggests a specific adaptation of BDI-IS1 to tropical conditions to provide consistent protection against local plant diseases, as it has been shown that abiotic factors affect the *in planta* efficacy of biocontrol agents (Ayaz et al., 2023).

5. Conclusion

The newly isolated and prominent antimicrobial agent *B. nakamurai* BDI-IS1 relies on its soluble secreted metabolites to antagonize bacterial and fungal phytopathogens. We further characterized the core metabolome of the species and identified some non-ribosomal specialized metabolites for their involvement in pathogen inhibition. However, the weaponry of *B. nakamurai* is quite diverse and several RiPPs may also contribute to the global antimicrobial activity. A lot remains to be discovered in the diversity of bioactive natural products potentially formed by this understudied species. The biocontrol potential assessment against tomato early blight and northern maize leaf blight revealed that BDI-IS1 provides its protective effect upon leaf or root treatment and to a level similar or higher than *B. velezensis* QST713 (Serenade Aso®) under greenhouse conditions, suggesting two main modes of action including direct and indirect antagonism. Further studies should explore the mechanisms underlying these biocontrol properties and investigate other potential benefits in terms of plant growth promotion or resistance to abiotic stresses, as well as exploring to which extent this bacterium can cope with the harsh conditions prevailing in fields (Enebe & Babalola, 2018; Etesami et al., 2023b). The high potential of this locally isolated strain to control major plant diseases prevalent in the tropical region highlights the importance of seeking adapted solutions to local problems by exploiting indigenous microbial resources (Y. Liu et al., 2023; Novello et al., 2023).

Chapter 5

Modulation of the antagonistic potential of *Bacillus nakamurai* BDI-IS1 under acidic and mild-cold conditions

François Nimbeshaho, Augustin Rigolet, Guillaume Balleux, Anthony Arguëlles Arias, Farah Boubsi, Venant Nihorimbere and Marc Ongena. Manuscript in preparation. I carried out all the experiments, processed the data and wrote this manuscript. The co-authors AR, GB, AAA and FB contributed to the improvement of experimental protocols and AR, GB and AAA participated in the samples' analysis.

1. Introduction

The number of *Bacillus*-based biological control agents for plant diseases available on the market has increased steadily over the last few decades, with promising and reliable results in various pathosystems (DunhamTrimmer, 2023; J. Lee et al., 2023). However, many criteria have been underestimated in the assessment of the efficacy of these biopesticides such as the intrinsic soil properties (pH, soil texture, composition and soil moisture content) and environmental conditions (temperature and UV radiation). This has led, in some cases, to a lack of reproducibility in biocontrol results, from laboratory to field (Ayaz et al., 2023; Basu et al., 2021). Indeed, soil pH and its associated geochemical composition are very critical for nutrient cycling, soil microbial composition and processes, and dictate the type of vegetation that sprouts on such soils (X. Zhao et al., 2022). Unfortunetely, it is estimated that more than 30% of arable soils worldwide are acidic, and this trend is expected to increase due to antropogenic activities such as intensive soil fertilisation and greenhouse gases' emissions, as well as global climate change (Mosley et al., 2024; W. Sun et al., 2023; X. Zhao et al., 2022). Soil pH modification is a relatively slow process that is less prone to the immediate effects of natural climate perturbations than other abiotic factors. This relative stability of such hostile condition (acidic pH) may result in the growth failure of root-associated bioinoculants and thus their inability to provide beneficial attributes to host plants. On the contrary, other environmental parameters, such as moisture, UV radiation and temperature, are subject to seasonal variations but can still have a significant impact on the overall efficacy of these bioproducts (Lopes et al., 2021; Santoyo et al., 2017; Wei et al., 2017).

Nevertheless, bacteria have evolved mechanisms to cope with harsh abiotic factors. These mechanisms include upregulating the expression of stress-resistance genes and synthesizing specific chaperone proteins in response to cold or heat stress, as well as producing various antioxidants and polyamines in response to oxidative stress. They also produce specific solutes and metabolites against osmotic stress and pH stress. These mechanisms guarantee the bacteria's own protection, as well as that of their associated plants (El-Saadony et al., 2022; Etesami et al., 2023b; Jayakumar et al., 2021). However, these adaptive mechanisms in response to stress differ in expression level across different bacteria or strains, resulting in varying stress tolerance abilities (Chowdhury, 2020; Goswami et al., 2018; Reva et al., 2006; Schumacher et al., 2023). In this regard, many reports highlight the specific adaptation to local ecological conditions by strains isolated from that agroecosystem, whereas introduced foreign strains are unable to thrive and exert the expected level of biocontrol (Karthika et al., 2020; Magan, 2020). On the other hand, the limitations imposed on biocontrol agents by stressful abiotic factors affect not only their population dynamics, but also their capacity to produce BSMs or to form biofilms, which can strongly impact biocontrol efficacy (Moreno-Velandia et al., 2021; Pertot et al., 2013). More broadly, this environmental pressure also affects the fitness of the other microbes in the same ecological niche and therefore determines the outcome of interspecies or interkingdom interactions (Burpee, 1990; Magan, 2020; Youssef, 2024). The

biocontrol candidate *B. nakamurai* BDI-IS1, as characterised in the previous chapters, was isolated from soil samples collected in a specific area of Burundi and is intended to be used locally and/or in similar tropical regions. The ecological zone in which BDI-IS1 was isolated is characterized by acidic soil (pH < 5), an average ambient temperature varying ranging from 12 to 28.5°C and an average maximum precipitation of 170 mm (Figure 5-1 & S8).

In this chapter, we evaluated the ability of *B. nakamurai* BDI-IS1 to adapt to stressful temperature and pH conditions, corresponding to the real abiotic conditions prevailing in many farmlands of Burundi and other tropical regions. Through *in vitro* studies, we found out that BDI-IS1 withstands these abiotic stresses better than the commercial strain *B. velezensis* QST713, in terms of growth and biofilm formation. We also showed that abiotic factors can modulate BSMs' secretion and thereby reshape the antagonistic interactions between *Bacillus* spp. and plant-pathogenic bacteria.

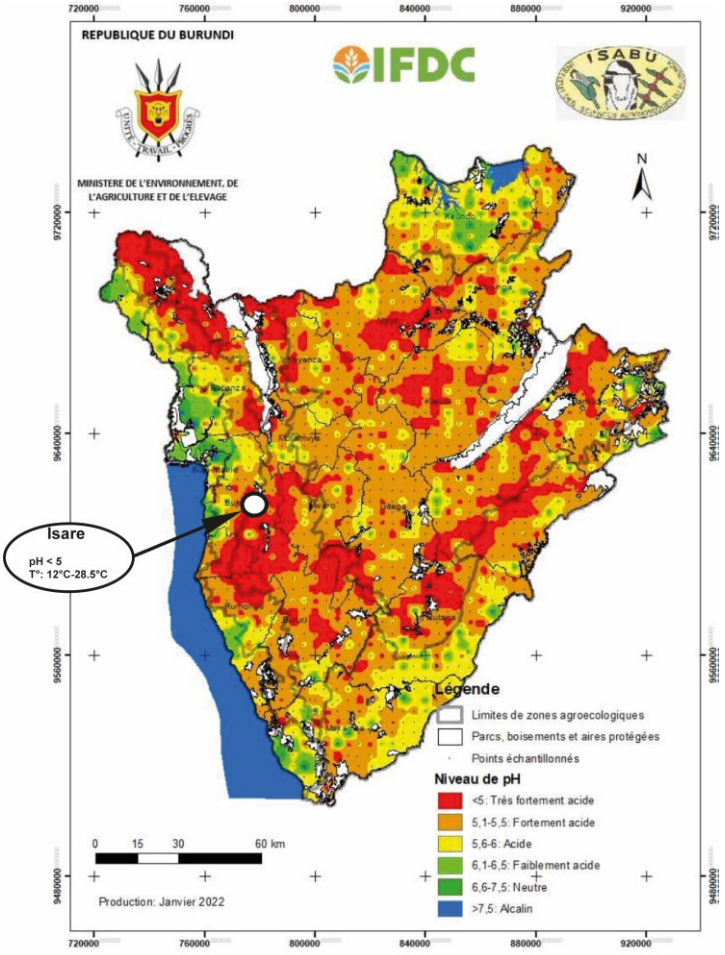


Figure 5-1: Soil pH map of Burundi (provided by Alain Kagisye, not published).

2. Materials and methods

2.1. Biological materials

Plant beneficial bacteria including *B. nakamurai* BDI-IS1, *B. velezensis* QST713, *B. velezensis* FZB42, *B. velezensis* QST713, *B. velezensis* GA1, *B. velezensis* S499, *B. amyloliquefaciens* DSM7, *B. pumilus* philippo, *B. subtilis* 168, *Paenibacillus polymyxa* 58 and *Pseudomonas fluorescens* pt-03 were used in the different assays of pH and temperature effect on growth, biofilm formation and BSMs production. Six phytopathogenic bacteria i.e. *C. michiganensis* subsp. *michiganensis*, *R. fascians* D188, *P. carotovorum* LMG6663, *X. campestris* subsp. *campestris* LMG582, *P. cichorii* LMG2162, *P. fuscovaginae* LMG2158 were used in the dual confrontation experiments between *Bacillus* spp. and phytopathogenic bacteria. All these bacteria were available in the collection of the Microbial Processes and Interactions (MiPI) Laboratory, Gembloux Agro-Bio Tech, University of Liège.

2.2. *Bacillus* spp. adaptation to variable culture conditions of pH and temperature

B. nakamurai BDI-IS1 and other plant beneficial bacteria initially stored at −80˚C were plated on LBA and incubated overnight at 30˚C. Liquid preculture was prepared by seeding one single colony in 20ml LB (150rpm, 30°C, 8h). Double washed cells were suspended into REM adjusted to different pHs (pH 4.6-5.0-6.0-7.0) to make a preparation of OD_{600nm} 0.1 for planktonic cell growth and biofilm formation assays. The bacterial suspension was added to flat-bottomed 48-well microplates (400µl) or round-bottomed 96-well microplates (150µl) for growth and biofilm assessment assays, respectively. For sessile cell growth assay, double washed cells were suspended into LB medium for a preparation of OD_{600nm} 2 and a spot (5µl) of bacterial suspension was deposited on agarose REM plates (agarose was chosen for low pH resistance properties compared to agar) with defined pH (pH 4.6 or pH 7.0). The planktonic cell growth assay was carried out on a shaker (150 rpm) at a fixed temperature (15°C, 20°C, 25°C and 30°C) and readings were performed into TECAN machine every 3h, while the sessile cell growth plates were incubated at 30°C for seven days with a daily photography for colony expansion observation.

For the biofilm formation assay, incubation was performed for 72h at different temperatures (15°C-20°C-25°C-30°C) without shaking. Medium and planktonic cells were gently removed by pipetting and wells were washed twice with phosphate-buffered saline (PBS). Biofilm pellicles were stained with crystal violet (0.1% v/v) for 10 min and wells were washed twice with PBS after a gentle removal of the dye. The stained biofilm pellicles were then dissolved in glacial acetic acid (30% v/v) for 30 min and absorbance readings were taken in TECAN machine at 595 nm. The assay was performed in two independent experiments, with eight technical replicates per each pH tested (n = 16). pH and growth dynamics were assessed in the BIOLECTOR machine (m2p labs) in special black (for light protection) round-bottomed 48-well flowerplates and the growth conditions were: initial OD_{600nm} 0.1, 1500 rpm, 30°C and

24h of incubation. The assay was conducted in triplicates with three independent experiments (n = 9).

2.3. Impact of temperature and pH on the antagonistic activity of BDI-IS1

Dual confrontation assays of BDI-IS1 WT or mutant strains (and QST713 & FZB42) and phytopathogenic bacteria were performed either on REM agar plates (pH 7.0) for temperature effect assessment or on REM agarose plates adjusted to different pH (4.6, 5.0, 6.0, 7.0) to assess the effect of fluctuating initial pH, respectively. The pathogenic bacteria suspension was cautiously spread on the REM square plates and a spot of BDI-IS1 WT or mutant strains (and QST713 & FZB42) cells was deposited on the plates, and the latter were sealed with parafilm paper and incubated at different temperatures (15°C, 20°C, 25°C and 30°C) for seven days and at 30°C for four days to assess the separate effects of temperature and initial pH, respectively. Antibacterial activity was expressed as the diameter of the inhibition zone around the *Bacillus* colony and, where possible, up to two independent experiments were carried out with three technical replicates (n = 6).

2.4. Assessment of bioactive compounds production under different stressful conditions

Analysis of bioactive secondary metabolites of the BDI-IS1 cell-free supernatants generated from different conditions (depending on the experiment) was performed by LC-ESI-qTOF-MS (Agilent 1290 Infinity II coupled with mass detector (Jet Stream ESI-Q-TOF 6530)) in positive mode with the following source parameters: capillary voltage of 3.5kV, nebulizer pressure of 35psi, drying gas of 8 $L.min^{-1}$, gas temperature of 300°C, sheath gas flow rate of 11 $L.min^{-1}$, sheath gas temperature of 350°C, fragmentor voltage of 175V, skimmer voltage of 65V, and octopole radiofrequency of 750V. Accurate mass spectra were recorded in the m/z range of 100 to 1,700 (acquisition rate 2 spectra/s). For an optimal separation, a C18 Acquity UPLC BEH column (2.1 mm; 50 mm; 1.7 µm; Waters) was used at a flow rate of 0.6 mL min^{-1} and a temperature of 40°C (injection volume: 10 µL). A gradient of acidified water (0.1 % formic acid) (solvent A) and acidified acetonitrile (0.1% formic acid) (solvent B) was chosen as the mobile phase, starting at 10 % B, and rising to 100 % B in 20 min. Solvent B was kept at 100 % for 4 min before returning to the initial ratio. Metabolites were identified based on retention time and accurate mass according to the PCDL (Personal Compound Database and Library) generated specifically for BDI-IS1 or QST713 (when used as a control).

The level of production of each metabolite was qualitatively determined by manually integrating the area under the extract ion chromatogram (EIC), which was further normalized to the maximum OD_{600nm} value for that culture. For the effect of temperature and initial pH on BSMs production by BDI-IS1 WT in liquid culture, cell-free supernatants were prepared by microfiltration (0.22 µm of pore size),

whereas for BSMs production by BDI-IS1 upon interaction with pathogenic bacteria on solid media, filtrates (0.22 µm of pore size) from the plug extracts were utilised. To obtain that liquid extract, a mixture (1:1, m/v) of agarose plugs and acetonitrile (50% v/v) was allowed to a continuous shaking over 1h. Agarose plugs were cut within the inhibition zone or around the BDI-IS1 colony for the control.

2.5. BDI-IS1 mutants' construction

Knock-out mutant strains of *B. nakamurai* BDI-IS1 depleted in the production of each of the predicted BSM, of *sfp*-dependent metabolites (*sfp* mutant), and in the production of both *sfp*-dependent metabolites and each of the remaining predicted metabolites (double mutants) were constructed by gene replacement and homologous recombination. The primers used for this purpose are listed in the supplementary material (Table S4). For the single mutants, a cassette containing a chloramphenicol resistance gene flanked by about 1 kb of the upstream and downstream regions of each target gene was constructed by three fragments joining PCR. BDI-IS1 transformation was carried out according to the protocol of Hoff et al. (2021) with minor modifications. Shortly, one colony of BDI-IS1 was grown in LB medium (37°C, 150 rpm) for 4 h, and washed twice with MMG liquid medium (anhydrous K_2HPO_4 19 $g.L^{-1}$, KH_2PO_4 6 $g.L^{-1}$, anhydrous $Na_3C_6H_5O_7$ 1 $g.L^{-1}$, $MgSO_4$ *7 H_2O 0.2 $g.L^{-1}$, Na_2SO_4 2 $g.L^{-1}$, $FeCl_3$ 50 mM, $MnSO_4$ 2 mM, glucose 8 $g.L^{-1}$, and L-glutamic acid 2 $g.L^{-1}$; pH 7.0) and the OD_{600} was adjusted to 0.01. The recombinant cassette (0.5 – 1 µg) was added to 1 mL of the latter prepared suspension and incubated at 37°C for 24 h. Transformed colonies that have integrated the recombinant cassette, were further selected on LBA medium supplemented with chloramphenicol (5 µg/mL). For double mutants, phleomycin-based cassette resistance gene was used for the second transformation whose starting biological material was the already constructed *sfp*-mutant. The same protocol was followed but phleomycin antibiotic (24 µg/L) was used for positive colony selection.

2.6. Data analysis

GraphPad Prism 9.1 was used for all the graph drawings. Comparison of data means (± SD) corresponding to different variables (pH or temperature or strain) was performed with two-way ANOVA coupled to Šídák's or Tukey for multiple comparisons test ($\alpha = 0.05$) in the case of two variables or more, respectively.

3. Results and discussion

3.1. Impact of temperature and pH on growth and biofilm formation

B. nakamurai BDI-IS1was assessed, in comparison with *B. velezensis* QST713 for growth over a range of temperature (15°C-30°C) and medium pH (pH 4.6-7) to better

appreciate its intrinsic ability to cope with low temperature and low pH-derived stresses. Based on the colony expansion assay on solid REM, BDI-IS1 sessile cells showed in general a greater adaptability to the tested conditions than QST713 and were even able to withstand the combined extreme stresses of temperature and pH (15°C & pH 4.6) after six days (Figure 5-2A). BDI-IS1 displayed a particular tolerance to low pH conditions. For instance, upon extreme acidic conditions (pH 4.6), its growth colony occurred within the first 24h of incubation at 20°C,whereas QST713 was unable to grow even at 30°C over the same time course. Growth kinetics over 24h of the two strains, as determined by microplate-based assays mimicking planktonic cell growth, show a similar trend to what observed with sessile cells, with BDI-IS1 exhibiting greater adaptation than QST713. Here again, BDI-IS1 demonstrated a shorter initiation phase than QST713 under all limiting conditions of pH and temperature. However, no significant difference in growth was observed between the two strains under standard conditions (pH 7 and 30°C) (Figure 5-2A). More broadly, we noticed that planktonic cells adapt more rapidily than sessile cells for both strains. This is due to the intrinsic properties of the liquid culture-type with constant agitation, which allow enhanced nutrient availability and easy diffusion of substances. These data do not only demonstrate the critical importance of environmental pH and temperature for bacterial fitness, but they provide also evidence that BDI-IS1 has evolved with a particular inherent adaptive capability to tolerate acidic and cold stresses greater than that of QST713.

Biofilm formation is a trait of crucial importance for the lifestile of plant growth-promoting rhizobacteria, in which it plays a key role in self-protection in this highly competitive environment, as well as in root colonisation (Brokate et al., 2024; Fessia et al., 2022). We therefore wanted to investigate the impact of abiotic factors (pH and temperature) on BDI-IS1's potential to manufacture this valuable phenotypical trait. Our data, obtained from the crystal violet staining test, revealed that temperature is critical to biofilm formation, as the yield at 15°C was significantly lower than at other temperatures, irrespective of pH (Figure 5-2B). On the other hand, pH appeared to have a limited effect on biofilm formation at temperatures of 20°C and above, as no obvious difference was observed between different pH conditions (Figure 5-2B). Moreover, our results show that BDI-IS1 produces more biofilm pellicles than QST713 under limiting conditions of low pH (pH 4.6 & 5) and low temperatures (15°C-20°C) (Figure 5-2B). Indeed, the results obtained with the PGPR *B. pumilus* HR10 are in line with our findings, as they confirm that the tested temperatures (20°C-30°C) and pH range (5.5-7.0) did not significantly affect its ability to form biofilms, although a slight increase was observed at pH 7 (M.-L. Zhu et al., 2020). A recent study also showed that pH stress (pH 5.5) has no major effect on total biofilm formation or exopolysaccharides (Eps) production for different tested *Bacillus* strains (Çam & Badıllı, 2024).

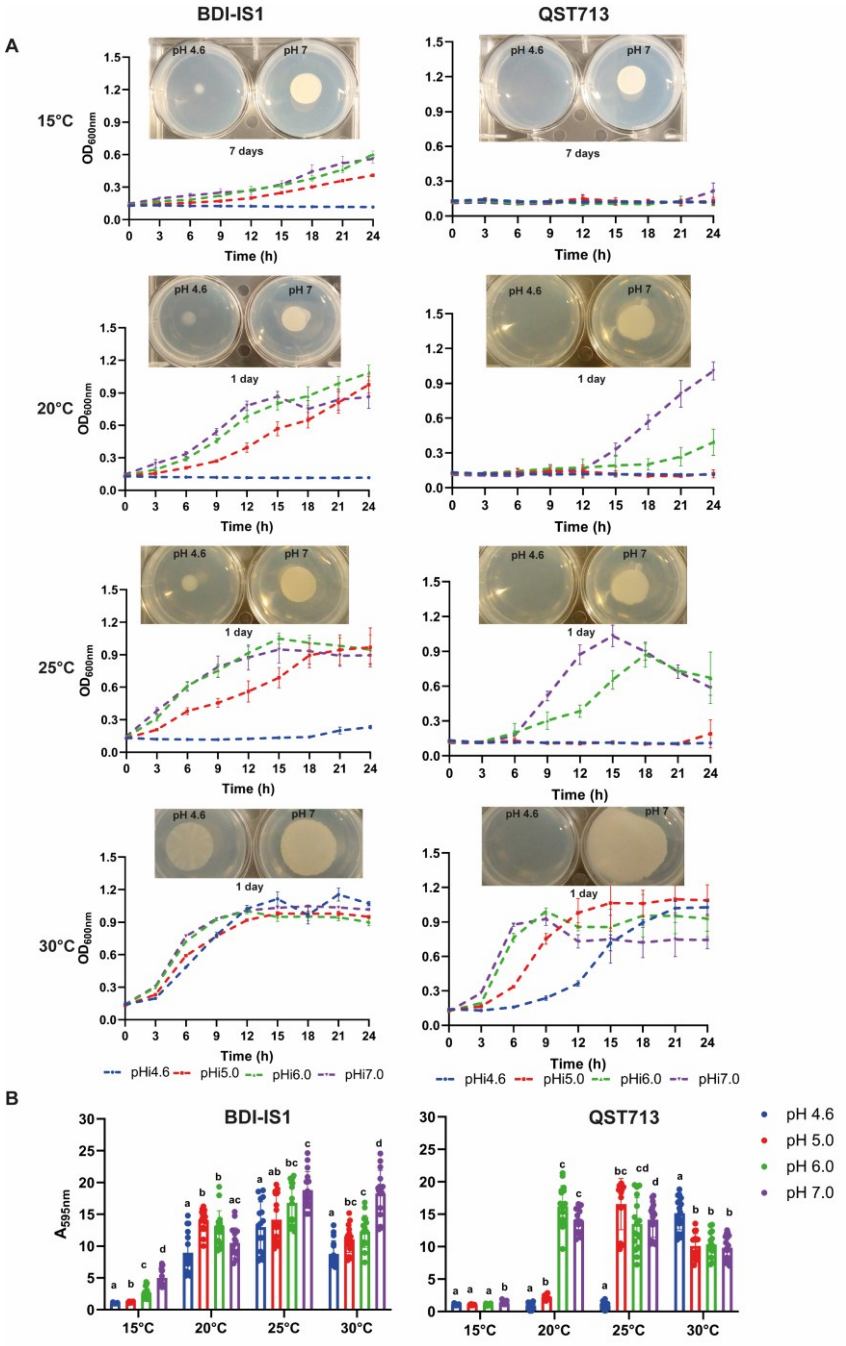


Figure 5-2: Medium pH and temperature impact on the growth and biofilm formation potential of *Bacillus* spp. A. Colony expansion ssay (above) and growth kinetic (below) of BDI-IS1 and QST713 were assessed on gelified REM (pH 4.6 or pH 7) and liquid REM (4.6-5.0-6.0-7.0) and incubated at different temperatures, respectively. Experiments were

performed in triplicates and repeated twice (n = 6). B. Impact of pH and temperature on biofilm formation by BDI-IS1. Total biofilm on 72h-old REM cultures (BDI-IS1 or QST713 at different pH and temperature) was evaluated by the crystal violet staining technique followed by absorbance recording (595 nm) of the glacial acetic acid-dissolved stained biofilm pellicles. Comparison of data means (± SD) were carried out with two-way ANOVA coupled to Tukey for multiple comparisons test ($\alpha = 0.05$). Bars with the same letter imply that the data means are non-significantly different, while bars with distinct letters stand for statistically significant difference between means. The assays were carried in two independent experiments, with triplicates (n = 6) and eight technical replicates (n = 16) for growth assays and biofilm formation assay, respectively.

The improved adaptation of BDI-IS1 to low temperatures and low pH conditions could be explained by the geographical and edaphic parameters of the site in which it was isolated. Indeed, the Isare commune (west of Burundi) is located at an altitude of 1880 m, with an average temperature of 22°C (World Bank Group, 2021) and acidic soils with a pH below 5 (Figure 5-1). Conversely, QST713 originates from Fresno County, California (USA), which is known for its alkaline soils with an average pH of 8 and characterised by an average soil temperature of 23°C (Potter et al., 2003; Zumkeller et al., 2022). This is plausibly the explanation for its poor performance under low pH stress. However, some *Bacillus* isolates can resist acidic environments (Abban et al., 2013; Hazarika et al., 2023) and exhibit an extensive genetic response to cope with low pH stress. These includes the upregulation of stress regulators such as SigX, the overexpression of genes involved in metal export and oxidative stress resistance, as well as and the secretion of acetoin and some enzymes such as dehydrogenases and decarboxylases which consume acids while generating basic amines (e.g. arginine) (Chowdhury, 2020; Goswami et al., 2018; Wilks et al., 2009).

However, it is still unclear whether the strategies employed by stressed *Bacillus* cells in low pH environments are aimed solely for self-protection and successful proliferation in such hostile condition, or whether they also induce external environmental changes that may reshape their ecological interactions. For example, changes in physico-chemical properties of the environment (e.g. pH and nutrient availability) may affect the fitness of other macro/microbiota in the same ecological niche, thereby redefining the interspecies and/or interkingdom relationships (Fuentes et al., 2022; Nzeyimana et al., 2024).

3.2. Medium alkalinization as a strategy to overcome acidic stress

To better understand the mechanisms employed by *B. nakamurai* BDI-IS1 cells to thrive under low pH stress, we monitored biomass and pH dynamics over time in multi-well plates using an automated reader (Biolector). Cells inoculated at pH 4.6 entered their exponential growth phase after 12h, which correlated with an increment in pH to pH 5.0 (Figure 5-3A). After 24h of incubation, BDI-IS1 cells were in their stationary phase, with the pH rising to around pH 7. In contrast, the pH of the control medium remained unchanged, ruling out the plausible artefact that may be due to the

buffering effect of the medium component MOPS (3-(N-morpholino) propanesulfonic acid). This pH correction from pH 4.6 to pH 5.0 during the lag phase implies that BDI-IS1 cells have an intricate mechanism that create favourable environment enabling their proliferation, which would probably benefit the plant host and other members of the holobiont. Indeed, the hydrogen ion pool regulates the availability of the macro- and micro-nutrients necessary for the growth of all the members of the ecological niche (Msimbira & Smith, 2020).

Next, we wanted to understand the factors mediating this medium alkalinisation. To this end, we assessed the final pH of the BDI-IS1 culture in a Biolog-like experiment. Different modified REM media (initial pH 4.6) lacking key ingredients susceptible to influence the overall hydrogen ion pool in the medium (e.g. organic acids, yeast extracts, casamino acids and ammonium sulfate) were prepared and used for the growth assay. The results revealed that the final culture pH of BDI-IS1 grown in modified REM lacking organic acids did not exceed pH 5.2. In contrast, an increase in pH up to around pH 7.3 was observed for the other modified REM media and the control (Figure 5-3B). This indicates that the increase in the pH of the BDI-IS1 culture is partly due to the consumption of the available organic acids in the nutrient substrate. However, the early increment in pH prior to the exponential phase clearly occurs independently of the uptake of organic acids and is likely due to the secretion of other pH-modifying substances such as ammonia, acetoin and carbonate ions, which have been reported to be involved in media alkalinisation during *Bacillus* growth (Allagheny et al., 1996; Ivanova et al., 2023; P. Tran et al., 2024). In parallel, our results highlight the relative negative influence of a lack of yeast extracts on *Bacillus* growth and revealed the critical role played by the readily available inorganic nitrogen source, ammonium sulphate (Figure 5-3B). Indeed, the role of organic acids, amino acids and ammonium salts in bacterial growth has been reported by other researchers (Abhinay & Pan, 2023; L. Chen & Liu, 2024) and their findings are consistent with ours.

Furthermore, we wanted to check whether this ability to adapt to acidic stress through pH correction is specific to BDI-IS1 or common to other beneficial bacteria. Growth and final pH assessment for different PGPRs cultured on REM initially adjusted to pH 4.6 revealed contrasting results. *B. nakamurai* BDI-IS1 grew as fast as *B. velezensis* FZB42 and *B. velezensis* S499 but exhibited more significantly greater growth than other *Bacillus* strains (Figure 5-3C). *B. amyloliquefaciens* DSM7, *B. pumilus* and *P. polymyxa* were most negatively affected by pH stress, with no growth recorded within 24h, followed by *B. subtilis* 168. However, it is worth noting that the level of medium alkalinisation appears similar for strains of the *B. amyloliquefaciens* operational group, including *B. nakamurai* BDI-IS1 and *B. velezensis*, with a final pH greater than 7, except *B. amyloliquefaciens* DSM7 which had a final pH of 6.3 (Figure 5-3C). The plant-beneficial *Pseudomonas* strain tested here adapted and grew relatively fast, outperforming the tested *Bacillus* strains, and its medium alkalinisation effect was comparable to that of their rhizospheric cohabitants bacilli. These contrasting adaptive responses of different PGPRs to low pH stress have also been reported for different biofilm-forming rhizobacteria, where some *Pseudomonas* and

Bacillus isolates were found to be tolerant at low pH, while others were not (M. Haque et al., 2020).

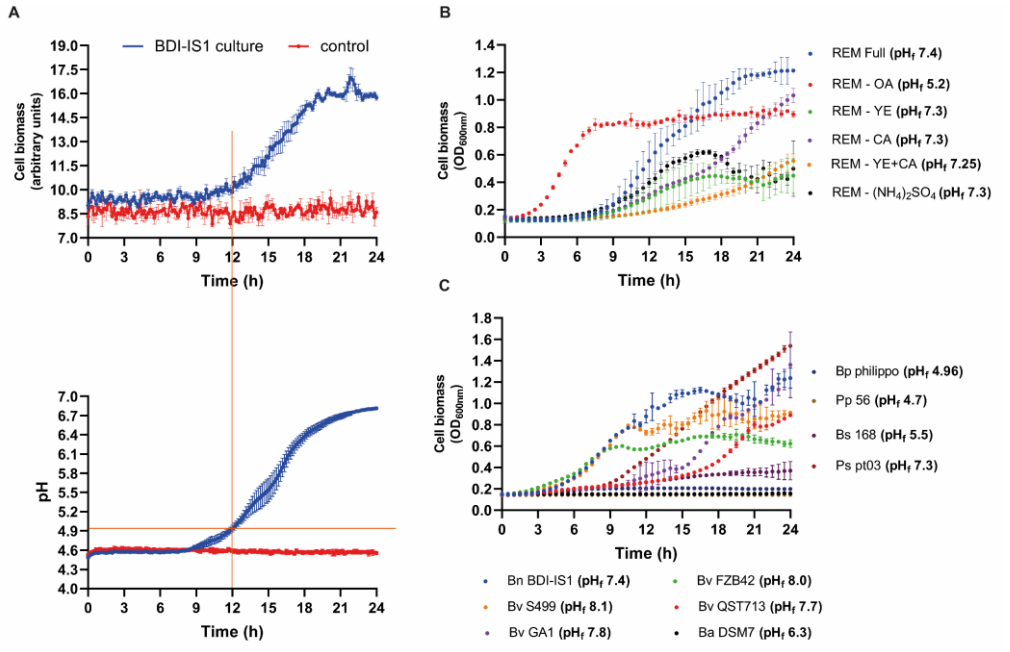


Figure 5-3: Medium alkalinization process during *Bacillus* growth. A. represent the evolution of pH and cell biomass of BDI-IS1 REM culture in Biolector (1500 rpm, 30°C, 24h). The pH and cell biomass (arbitrary units) were automatically recorded each 10min and the assay was performed in triplicates, with three independent repetitions (n = 9). B. Role of medium components into the culture alkalinization by BDI-IS1. Modified REM lacking organic acids (REM-OA), casamino acids (REM-CA), yeast extracts (REM-YE), casamino acids with yeast extracts (REM-(CA+YE)) and ammonium sulphate (REM-$(NH_4)_2SO_4$) adjusted to pH 4.6 was utilized. Optical density at $OD_{600\ nm}$ for bacterial growth was automatically recorded each 30 min and the assay was performed in triplicates (n = 3). The final pH (48h) was checked manually with a pH-meter. C. Assessment of the adaptation ability of different plant beneficial bacteria on low pH. The assay was performed in triplicates (n = 3) and growth ($OD_{600\ nm}$) automatically monitored in TECAN, while the final pH (24h) was determined manually with a pH-meter. Bn BDI-IS1, Bv QST713, Bv FZB42, Bv S499, Bv GA1, Pp 56, Ba DSM7, Bs 168, Bp philippo and Ppt 03 stand for *B. nakamurai* BDI-IS1, *B. velezensis* QST713, *B. velezensis* FZB42, *B. velezensis* S499, *B. velezensis* GA1, *B. amyloliquefaciens* DSM7, *B. subtilis* 168, *B. pumilus* philippo and *Pseudomonas fluorescens* pt03, respectively.

This particular feature of medium alkalinisation during the growth of different bacilli and other root-associated bacteria on root exudates mimicked medium represents an adaptation strategy of these rhizospheric bacteria to thrive in hostile pH conditions, which are characteristic of many farmlands in tropical regions. Although these data still need to be confirmed *in vivo*, they provide interesting insights into the

ecological processes that may happen in acidic agroecosystems and that may influence positively or negatively other members of the holobiont. Soil and rhizosphere, in particular, are a hub for various forms of life, including micro-and macro-organisms (Khade & Sruthi, 2024; Xing et al., 2025), and the pH change in these habitats could lead to significant alterations in existing ecological relationships. Therefore, it is interesting to investigate the ecological outcomes of this physico-chemical modification of the growth medium on the ability of *Bacillus* strains to exert its beneficial properties.

3.3. Abiotic factors modulate the biological activity of BDI-IS1

3.3.1. pH and temperature impact BSM production

BSMs are crucial for pathogen inhibition. So, we wanted to evaluate the impact of low pH and low temperature on their production by BDI-IS1. The relative amounts of BSMs expressed per cell biomass (OD_{600nm} max) were determined at the end of the culture (72h) by the UPLC-MS q-TOF analysis of the cell-free culture supernatant. It was found that the ability of BDI-IS1 to produce BSMs was generally affected by these limiting conditions (Figure 5-4 & S10). For instance, surfactin production was severely impacted by decreasing temperatures at low pH conditions, whereas its production was unaffected by temperature at neutral pH (Figure 5-4 & S10). The production of iturin A, bacillaene, plantazolicin and bacillibactin was found to be negatively affected by acidic stress compared to neutral pH at all tested temperatures. Conversely, bacilysin was secreted in higher amounts at low pH than at neutral pH under all tested conditions of temperature where growth occurred (Figure 5-4 & S10). In addition, the secretion of bacillibactin and plantazolicin was seriously hampered by low temperatures, even at neutral pH (Figure 5-4 & S10). The combined effects of low pH and low temperature prevented BDI-IS1 from growing during the considered incubation period. However, improving either parameter (pH or temperature) enabled this bacterium to recover its full growth and BSMs secretion potential.

These findings are consistent with recent studies that have shown, for instance, that surfactin is produced at constant levels at low and mesophilic temperatures (neutral pH condition) by *B. velezensis* S499 and *B. velezensis* 1B-23.(Li et al., 2021; Moreno-Velandia et al., 2021). However, since the production of certain BSMs is negatively impacted by these abiotic stresses, the overall plant beneficial properties exerted by the bacterium may also be compromised. Therefore, it is interesting to investigate the fate of BDI-IS1 in these limiting conditions through its interaction with other prominent pathogenic competitors. This competitive interaction is considered as the bottleneck of the bacterial establishment in the rhizosphere and thus impacts the bacterium's ability to deliver its valuable services to the plant host.

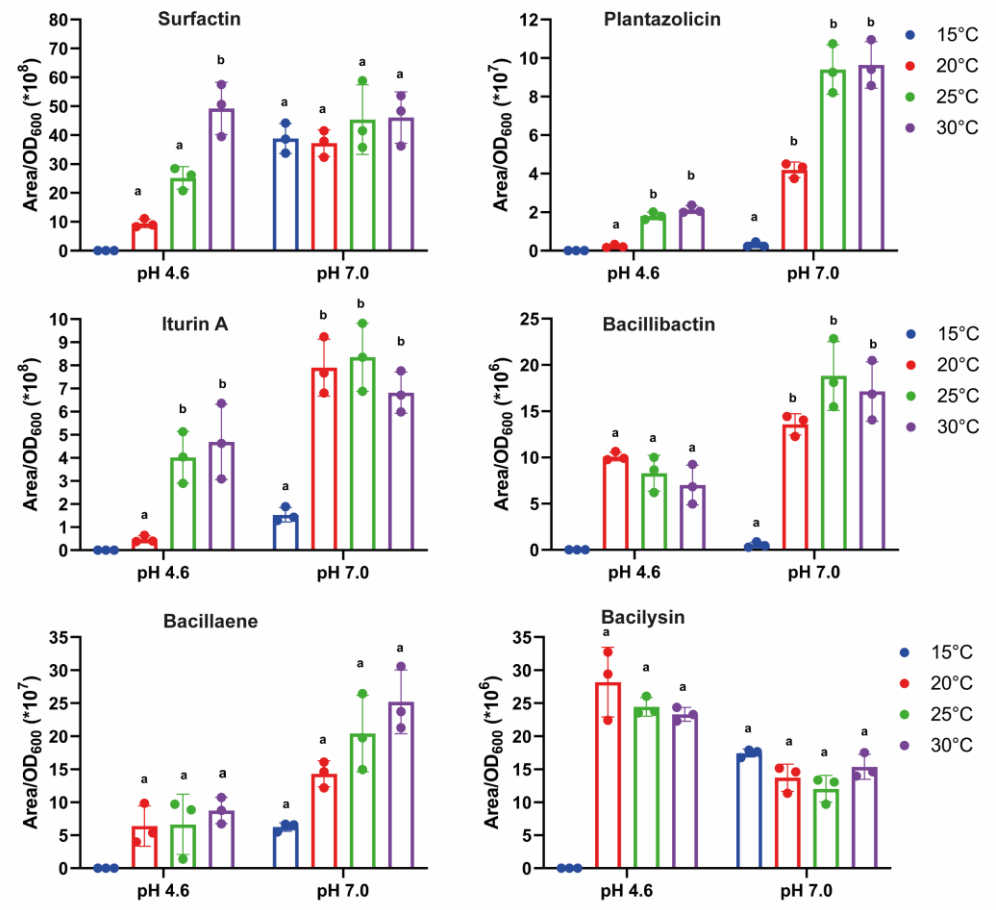


Figure 5-4: Impact of temperature impact on BSMs production by BDI-IS1 at different pH. Cell-free supernatants from cultures (48h, 150 rpm) set in different pH and temperature conditions were analysed by UPLC/MS q-TOF. The plotted data are means (± SD) of the cumulative normalized area under the EIC (Area/$OD_{600\ nm}$ max.) of the different detected chemical variants of each BSM (where applicable). The experiment was carried out in triplicates (n = 3) and comparison of means was performed with two-way ANOVA coupled to Tukey for multiple comparisons test ($\alpha = 0.05$); bars with the same letter implies that the data means are non-significantly different, while bars with distinct letters stand for statistically significant difference between means. Data plots corresponding to combined conditions of 15°C and pH 4.6 were not subject to comparison, since there was no growth.

3.3.2. Modulation of the antibacterial activity of BDI-IS1

We next tested the influence of abiotic parameters (pH and temperature) on the antagonistic potential of BDI-IS1 towards six plant-pathogenic bacteria including *Xanthomonas*, *Clavibacter*, *Rhodococcus*, *Pectobacterium* and two species of *Pseudomonas* through dual confrontation assays.

3.3.2.1. Effect of temperature

The antagonistic activity of BDI-IS1 against bacterial pathogens was strongly affected by low temperature, and two different situations were observed. BDI-IS1 retained its inhibitory potential against *X. campestris*, *C. michiganensis* and *R. fascians*, but lost it completely against *P. carotovorum*, *P. cichorii* and *P. fuscovaginae*. In the latter case, the pathogens inhibited BDI-IS1 cells, as evidenced by a clear halo forming where the *Bacillus* spot was deposited (Figure 5-5A). On one hand, the inhibitory potential of BDI-IS1 against these cold-induced resistant pathogens generally increased with temperature. However, the growth inhibition of *P. carotovorum* was less affected by the temperature change between 20°C and 30°C than that of the two pathogenic *Pseudomonas* (Figure 5-5A). On the other hand, the confrontation between BDI-IS1 and the other group of phytopathogens, whose inhibition was maintained at 15°C, revealed different results depending on the temperature. For instance, antibacterial activity against *Xanthomonas* was similar and slightly higher at 15°C and 30°C than at 20°C and 25°C where no obvious difference was observed. *Rodococcus fascians* manifested enhanced susceptibility to BDI-IS1 at 15°C, whereas its growth inhibition remained constant at the other tested temperatures. However, the growth inhibition of *C. michiganensis* was temperature-dependent and increased with the rise in temperature. These findings demonstrate the critical role of temperature in the interspecies interactions between rhizospheric *Bacillus* and soil-dwelling phytopathogenic bacteria.

Although the mechanisms or factors involved in these temperature-modulated interactions between *Bacillus* and pathogenic bacteria are not yet known, we can hypothesize that the relative differential growth of these microorganisms at low temperature would favour competition for some critical nutrients such as iron. This could therefore contribute significantly to the failure of BDI-IS1 to antagonize the growth of plant pathogens, when outcompeted. Correlatively, we have previously demonstrated that the production of the *Bacillus* siderophore, bacillibactin, is severely affected at 15°C (Figure 5-4). On the other hand, *Pseudomonas* are reported to produce the siderophore pyoverdine (Bultreys et al., 2003; Cezard et al., 2014) and *Pectobacterium* have evolved with the ability to produce some bifunctional molecules such as pectocin with iron-scavenging and antibiotic properties (Grinter et al., 2012; Van Gijsegem et al., 2021).These pathogens also produce toxic compounds, such as the cyclic lipopeptides syringotoxin and fuscopeptin for *Pseudomonas* (Ferrarini et al., 2022), and the bacteriocin pectocin and the β-lactam carbapenem for *Pectobacterium* (Van Gijsegem et al., 2021), which may plausibly have an antagonistic effect against the cold-stressed and frail BDI-IS1 cells.

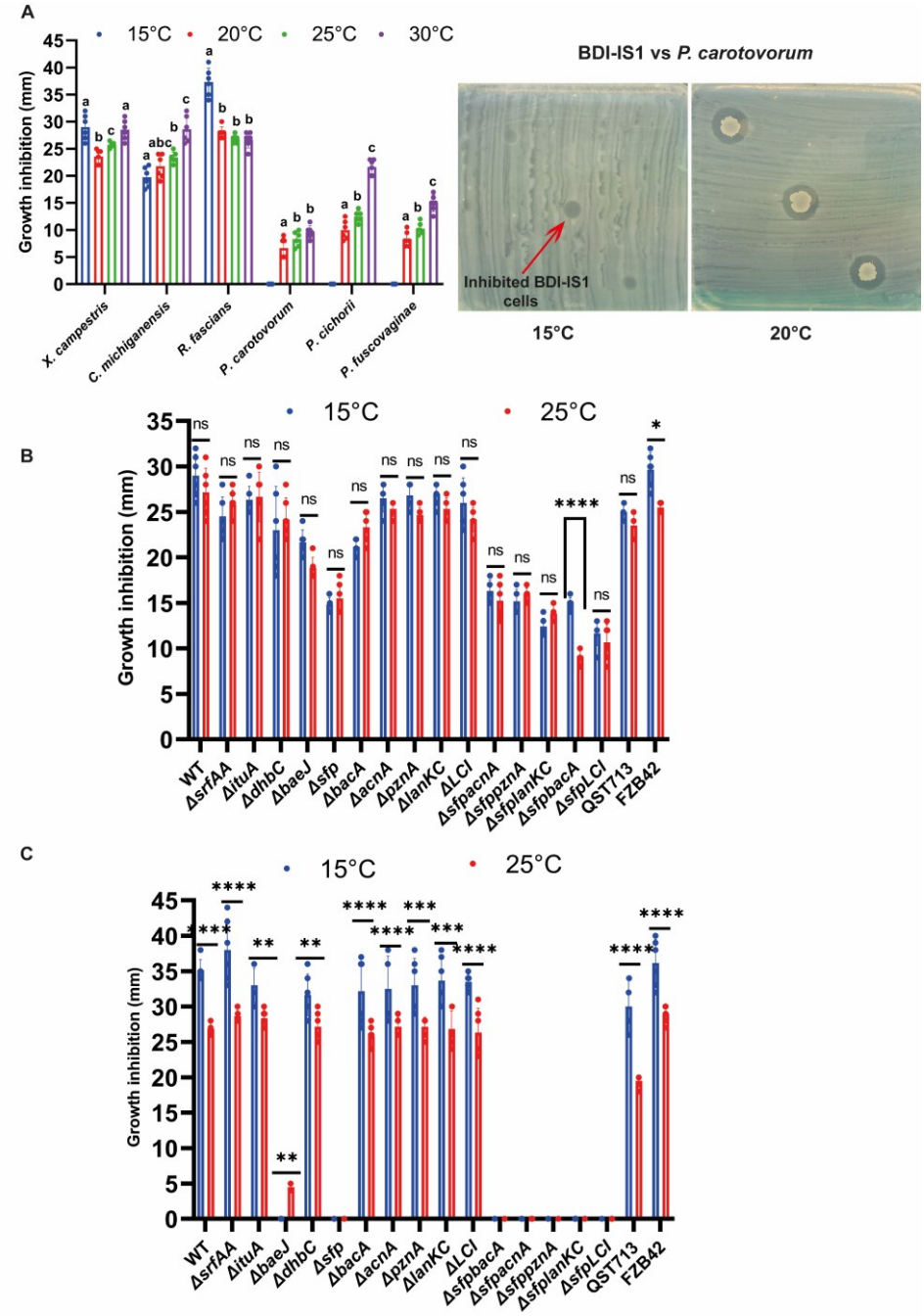


Figure 5-5: Impact of temperature on the antibacterial activity of BDI-IS1. A. shows the effect of differents temperatures on the antagonistic activity of BDI-IS1 against a set of bacterial phytopathogens. C & D denote the impact of temperature (15°C & 20°C) on the

antagonistic activity of BDI-IS1 mutant strains (QST713 and FZB42) against *X. campestris* and *R. fascians*, respectively. Dual confrontation assay between BDI-IS1 WT (or mutant strains) and pathogens was performed on agar REM plates (pH 7) and incubated for 7 days. Plotted data are means (±SD) of inhibition diameter (mm) of three technical replicates from two independent experiments (n = 6). Statistical comparison of means was performed with two-way ANOVA coupled with Šídák's test ($\alpha = 0.05$) and ns represent non-significant difference, while asterisks *, **, ***, **** imply significant differences at $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$, respectively.

We also aimed to decipher the metabolic cues that drive the relative improvement of the antibacterial activity of BDI-IS1 against *R. fascians* and, to a lesser extent, *X. campestris* at low temperature. To this end, we constructed and tested, in confrontation assay, an array of double mutants impaired simultaneously in secretion of both sfp-dependent NRPs and each of the other predicted BSMs. The single mutants constructed previously (see chapter 4, section 3.4) were also tested. Firstly, the results obtained against *X. campestris* revealed that the patterns observed for the wild-type strain were generally preserved at 15°C and 25°C, except for the double mutant *ΔsfpbacA*, which showed a significant decrease of activity at 25°C compared to 15°C (Figure 5-5B). This suggests that some antibacterial compounds produced by BDI-IS1 with masked activity under normal conditions could leverage their potential against these cold-stressed *Xanthomanas* cells. In addition, we suspect a partial contribution of bacilysin under this condition of cold stress, as evidenced by a significant loss of activity for *ΔbacA* at 15°C compared to 25°C (Figure 5-5B).

Secondly, the increment in activity against *R. fascians* observed at 15°C was maintained for all the mutant strains impaired in the secretion of BSMs not involved in antagonism under normal conditions (see chapter 4, section 3.4). However, the residual activity observed at 25°C for *ΔbaeJ* was completely lost at 15°C (Figure 5-5C), suggesting that the secretion of the unknown sfp-dependent metabolite involved in the antagonism of *R. fascians* together with bacillaene (Figure 4-6) is temperature-dependent. Cold stress can hinder the secretion of certain sfp-dependent bioactive secondary metabolites, such as bacillibactin and iturin A (Figure 5-4). Testing dual mutants depleted in the production of both bacillaene and bacillibactin or iturin A against this pathogen at 15°C and 25°C could provide further insights into this temperature-modulated antagonistic activity.

3.3.2.2. Effect of pH

Previous studies have shown that *B. nakamurai* BDI-IS1 and other bacilli can withstand acidity up to pH 4.6. However, none of the tested pathogens were able to grow at such a pH. Only *C. michiganensis* and *P. carotovorum* were able to tolerate the acidic stress of pH 5. The dual confrontation of BDI-IS1 and the pathogens at pH 6 and pH 7, convenient for all the antagonists, showed that the inhibitory potential of BDI-IS1 against most of the pathogens remained unchanged, except against *P. carotovorum* where a slight increase of inhibition was observed at pH 6 (Figure 5-6A). In parallel, the confrontation of *C. michiganensis* and *P. carotovorum* with BDI-IS1 at pH 5.0 resulted in two completely different scenarios. The inhibitory effect of

BDI-IS1 against *C. michiganensis* was decreased by almost 40% compared to pH 6 and pH 7, whereas against *P. carotovorum* it increased three-fold compared to pH 7 (Figure 5-6A).

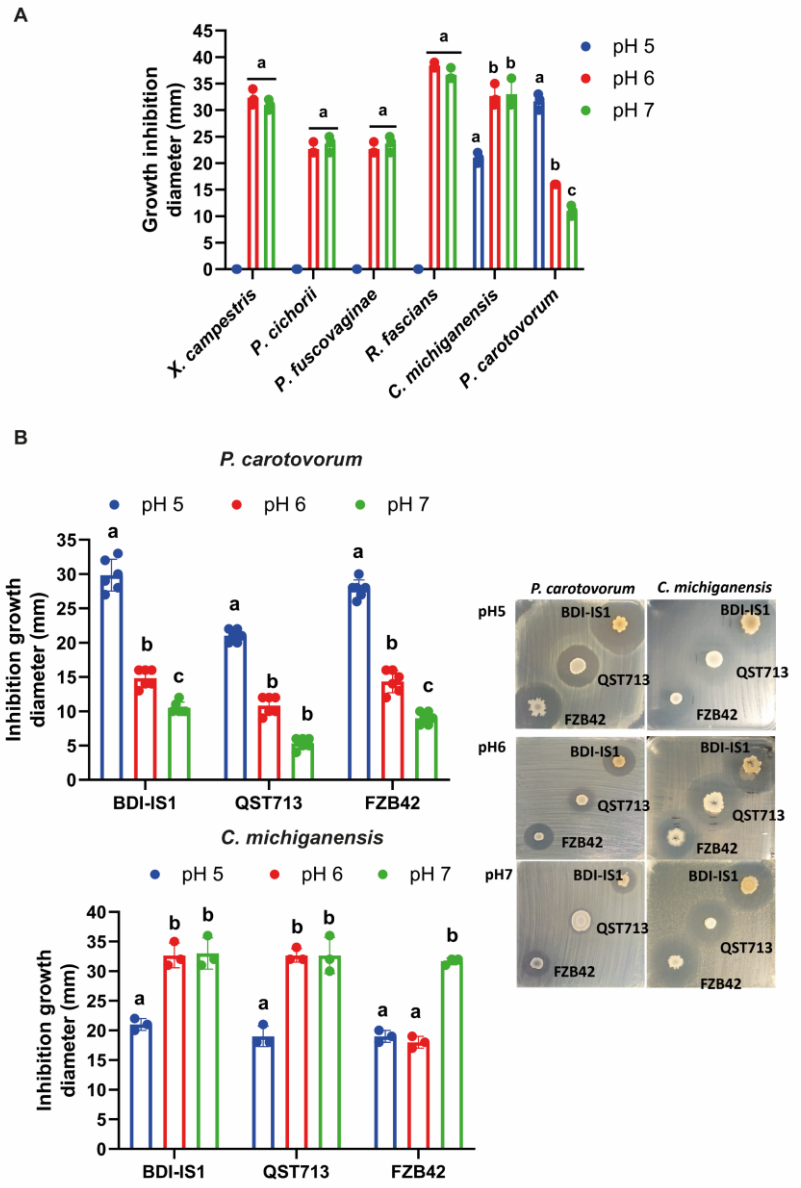


Figure 5-6: pH-mediated modulation of the antagonistic activity of BDI-IS1 against bacterial phytopathogens. A. stands for the antibacterial activity of BDI-IS1 at different pHs, while B. represents the comparative effect of pH on the antagonistic potential of different *Bacillus* strains (BDI-IS1, QST713 and FZB42) against *C. michiganensis* and *P.*

carotovorum. Dual confrontation assay between BDI-IS1 WT (or mutant strains) and pathogens was performed on agarose REM plates (pH 5, pH 6, pH 7) and incubated at 30°C for 72h. Plotted data are means (±SD) of inhibition diameter (mm) of three technical replicates from two independent experiments (n = 6). Experiment was carried out in triplicates with two independent experiment (n = 6), except for the comparative activity of BDI-IS1, QST713 and FZB42 where one experiment was performed in triplicates (n = 3). Comparison of data means (± SD) corresponding to the strains' activities per each pH were performed with two-way ANOVA coupled to Tukey for multiple comparisons test (α = 0.05), bars with the same letter implies that the means are non-significantly different, while bars with distinct letters stand for statistically significant difference between means.

We also wanted to investigate whether this pH-driven controversial situation about the antagonistic activity of BDI-IS1 against *P. carotovorum* and *C. michiganensis* is restricted only to this strain, or if it is conserved in other closely related *Bacillus* strains with known antibacterial activity against these pathogens. Our results showed that the commercial biocontrol agents *B. velezensis* QST713 and *B. velezensis* FZB42 behaved in the same way as BDI-IS1. The enhancement of antagonistic activity observed at low pH against the soft rot-causing pathogen *P. carotovorum* was conserved with the two strains. However, the activity of QST713 was generally inferior to that of BDI-IS1 and FZB42 which exhibited comparable antagonistic activities (Figure 5-6B). However, the decrease in activity against *C. michiganensis* from pH 6 to pH 5 observed with BDI-IS1 and QST713 was not observed with FZB42 (Figure 5-6C).

These findings, coupled with the previously demonstrated differential growth responses of beneficial and pathogenic bacteria at varying pH, show that environmental perturbations can lead to profound ecological changes and redefine the classical and established relationships between soil-dwelling bacteria. However, the triggers of this pH-driven modulation of the antagonistic activity of *Bacillus* spp. against some pathogens, such as *P. carotovorum*, remain unclear. We have previously shown that the ability of BDI-IS1 to secrete most known antibacterial compounds is attenuated at low pH, with the exception of the dipeptide bacilysin. Therefore, exploring the behaviour of mutant strains and the secretion patterns of BSMs upon confrontation would provide further insight into this unusual facet of bacterial interactions shaped by environmental pH.

3.3.2.3. Identification of BSMs responsible for the pH driven-contrasted antagonism of BDI-IS1 against *P. carotovorum*

The increasing in the antagonistic activity against *P. carotovorum* at low pH deserves special attention since this behaviour was unique among the tested phytopathogens. The main anti-*Pectobacterium* compound in BDI-IS1 is bacillaene (or its 2H-form) , the high level of antagonism observed at pH 5 could be correlated to the overproduction of this bioactive compound or it could be due to a synergistic effect with other antibacterial compounds. To test this hypothesis, we screened the bioactive molecules in the acetonitrile extracts generated from agarose plugs sampled in the inhibition zones using UPLC/MS q-TOF. We found out that there was no significant accumulation of bacillaene (and/or dihydrobacillaene) at pH 5, but a

notable accumulation of bacillibactin was observed at all pH conditions. The secretion of other metabolites was generally reduced when compared to the control (BDI-IS1 grown alone). This indicates an apparent competition for iron between the two bacteria, providing *Bacillus* cells a remarkable growth advantage.

Nevertheless, this siderophore was found to be produced less at pH 5 than at other pH points, both in interaction or when BDI-IS1 cultured alone (Figure (5-7A), suggesting that this chelator alone cannot explain the increased antagonistic activity of BDI-IS1 against *P. carotovorum* in acidic conditions. Correspondingly, BDI-IS1 encodes the low pH-induced *efeUOB* (Elemental iron acquisition factor) operon (see chapter 3, section 3.5.2) and would therefore compensate the low production of bacillibactin in these conditions by directly taking up ferrous and/or ferric iron from the medium (Miethke et al., 2013; Roy & Griffith, 2017). Indeed, the balance between the two forms of iron is strongly affected by external cues such as changes in pH and other reducing/oxidative factors (Morrissey & Guerinot, 2009; Schmidt et al., 2020). Testing BDI-IS1 mutants confirmed the previously demonstrated fact that bacillaene is the main inhibitor, under all pH conditions (Figure 5-7B). However, mutant strains impaired in bacinapeptin production exhibited reduced activity at pH 5, indicating that this lanthipeptide may contribute, in this acidic condition, to accelerate the inhibition of *P. carotovorum* cells initiated by bacillaene (and/or dihydrobacillaene) (Figure 5-7B).

In parallel, we observed a high density of *P. carotovorum* cells (high density cells, HDC) around the spot colonies of the non-active BDI-IS1 mutants compared to the low density of the pathogen cells far from the BDI-IS1 colony (low density cells, LDC) (Figure 5-7B). This could be a consequence of the pH correction driven by the BDI-IS1 cells during their growth as shown above. Thus, the fitness of the pathogen including the growth and secretion of virulence factors such as carbapenem, pectocin, hydrolytic enzymes, etc. would probably be compromised in acidic conditions, making the pathogen more vulnerable and more susceptible to antibiotics. Indeed, in liquid growth assay for the pathogen performed at different pH levels showed a significant decrease in growth rate accordingly to the decreasing pH (Figure S11). The secretion of virulence factors by *Pectobacterium* and other pathogenic bacteria is quorum-sensing dependent, and the low pH-driven slender growth may probably not allow the bacterial population to reach required threshold for the expression of QS molecules (Põllumaa et al., 2012; Shyntum et al., 2019). Similarly, the lactonase enzyme predicted in BDI-IS1 (NCBI acc. # MCC9023683.1) could interplay in the degradation of the pathogen-derived hormone acylhomoserine lactone (AHLs) via the quorum quenching (QQ) phenomenon, thereby probably contributing to boost the inhibition of the pH-stressed *Pectobacterium* cells (Noor et al., 2022).

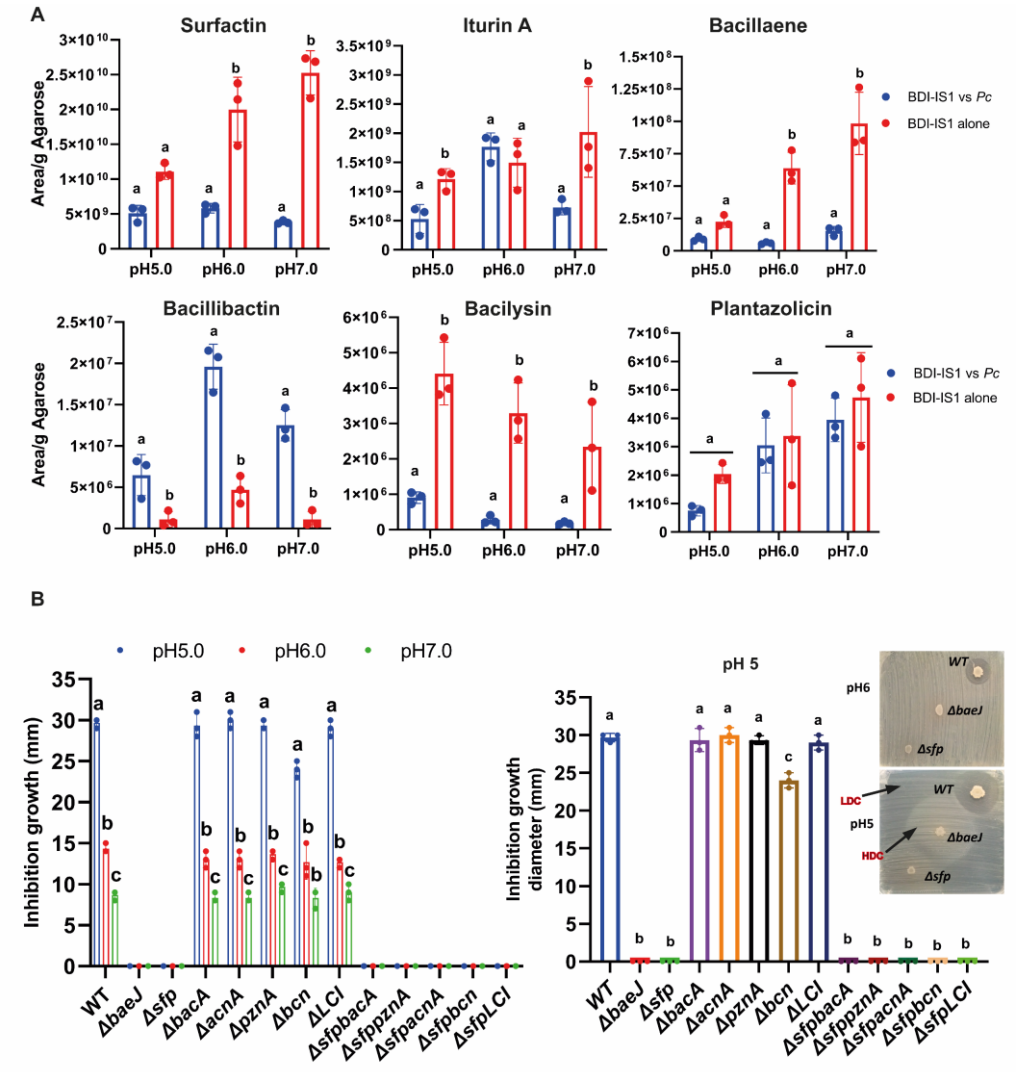


Figure 5-7: Impact of pH on the BSMs production by BDI-IS1 upon confrontation with *P. carotovorum* (Pc). Panels A and B represent the relative production of BSMs by BDI-IS1 in the inhibition zone against *P. carotovorum* and the antibacterial activity (expressed as inhibition diameter (mm)) of a set of BDI-IS1 mutant strains against the latter pathogen at different pH points, respectively. Comparison of data means (± SD) corresponding to BSMs production by BDI-IS1 alone and upon confrontation at each pH at one hand, and comparison of data means (± SD) corresponding to the antibacterial activities of each mutant strain at the three pH points on the other hand, were performed with two-way ANOVA coupled to Šídák's and Tukey, respectively, for multiple comparisons test ($\alpha = 0.05$). Comparison for the different mutants' activities at pH 5 was performed with one-way ANOVA coupled to Tukey for multiple comparisons test ($\alpha = 0.05$). Same letters over the

bars imply that the data means are non-significantly different, whereas distinct letters stand for statistically significant difference between means.

Nevertheless, to better understand the complex and interesting outcome of the *Bacillus-Pectobacterium* interaction in acidic conditions, further research should investigate the response of *Pectobacterium* cells cultured under different pH conditions to BDI-IS1 cell-free supernatants generated under the same pH conditions, or to bacillaene-enriched extracts. It should also consider the level of expression of the bacillaene (*baeJ*), bacinapeptin (*bcn*) and lactonase (*aiiA*) genes in BDI-IS1 when supplemented with *P. carotovorum* cell-free extracts from cultures set at different pH levels.

4. Conclusion

Bacillus nakamurai BDI-IS1 displays an excellent ability to cope with the field-specific abiotic factors, such as low temperature and low pH. *In vitro* studies showed that the bacterium's fitness parameters, including growth and biofilm formation, were not drastically affected by acidic or cold stresses (up to pH 4.6 and 15°C). However, when the two extreme stresses were combined, growth was severely slowed down and and its ability to form biofilm was impaired. Overall, BDI-IS1 exhibited greater adaptation to these constraining conditions than the biocontrol agent *B. velezensis* QST713, which partly explains its superior biocontrol performance compared to QST713, as demonstrated in the previous chapter. Obviously, the bacterium overcame acidic conditions by alkalinizing the medium to reach tolerable conditions of around pH 5 at the start of the exponential phase, and the neutral pH at the beginning of the stationary phase. This alkalinization trait, common to many plant-beneficial bacteria, is primarily due to the consumption acidic nutrients such as organic acids, which are one of the main ingredients of the plant root exudates. Temperature stress negatively affected the secretion of most BSMs, except surfactin. The most affected BSMs by the extreme temperature stress (15°C) were the siderophore bacillibactin and the RiPP plantazolicin. This stress suppressed the antagonistic activity of BDI-IS1 against three phytopathogenic bacteria (*P. carotovorum*, *P. cichorii* and *P. fuscovaginae*) while slightly boosting its inhibition of the leafy gall disease-causing agent *R. fascians* and modulating the BSMs' secretion patterns that mediate this antagonism. Acidic stress hindered BSMs production, expect for bacilysin, for which production is increased. Low pH stress (pH 4.6) was found to be detrimental to the growth of most pathogens, except for *C. michiganensis* and *P. carotovorum*, which grew at pH 5. However, the inhibitory potential of BDI-IS1 against these two low pH-resistant pathogens was found to be modulated by pH. Anti-*Clavibacter* activity decreases with decreasing pH, while anti-*Pectobacterium* activity surprisingly trebles from neutral pH to pH 5. However, no obvious explanation was found for this increase in antibacterial activity at low pH against this soft rot disease-causing agent, since no accumulation of its main inhibitor, bacillaene (and/or dihydrobacillaene), was found in the inhibition zone. The observed compromised pathogen fitness by low pH and high bacillibactin secretion

by BDI-IS1 upon confrontation in acidic conditions could partly explain this increment in anti-*Pectobacterium* activity.

Further investigations should emphasize on understanding this abiotic factors-induced reshaping of bacterial inter-species relationships and expand to assess inter-kingdom interactions in such drastic conditions. In addition, *in planta* experiments that reproduce these stressful conditions are needed to confirm these *in vitro*-based interesting findings. Nevertheless, these results pinpoint the crucial importance of environmental conditions for the fitness of soil-dwelling and rhizosphere microorganisms, whether beneficial or pathogenic, and for their established interactions. This highlights the need to consider these abiotic factors, characteristic to each agro-ecosystem, both for the study of new biocontrol candidates and for the decision-making process that leads to the selection and use of marketed biocontrol agents.

Chapter 6

Formulation of BDI-IS1 on local lignocellulosic substrates

François Nimbeshaho, Gaspard Nihorimbere, Venant Nihorimbere, Anne Legrève and Marc Ongena. Manuscript in preparation. I designed and conducted all the experiments on the bacterial biomass production on lignocellulosic substrates and product formulation. GN and I jointly designed and carried out the experiments on the biocontrol efficacy of the formulated product.

1. Introduction

Bacillus subtilis strains revert many biotechnological applications in various sectors, including as biocontrol and biofertilizer agents in agriculture, as probiotics and sources of enzymes of commercial significance in food and cosmetic industry, as trace metals scavengers and toxic dyes removers in bioremediation strategies, , etc. (Akinsemolu et al., 2024; Ikram et al., 2022; Sanghvi et al., 2016). The production process of bioagents or derived compounds first involves optimizing the growth medium and fermentation type suitable for the given microorganism, and then determining the optimal growth conditions, including pH, aeration, temperature, initial inoculum and incubation time (Teixidó et al., 2022). Once the latter parameters have been fixed to ensure high-yield production of either biomass (vegetative and/or spores) or secondary metabolites, depending on the intended use of the bioproduct, the formulation stage follows. This stage is perceived as the bottleneck of the entire process of producing biocontrol agents. It must guarantee the product stability during the process itself and storage, ease of of handling and use for end-users and efficacy in real field conditions, which are often marked by drastic abiotic factors (Brar et al., 2006; Droby et al., 2016).

Large-scale production of these biogents relies mainly on the classical submerged fermentation (SmF), which requires complex and expensive equipment and technologies, including bioreactors, conventional culture media, anti-foaming agents, freeze-drying machines (Subramaniyam & Vimala, 2012; Valdés-Velasco et al., 2022). This subsequently affects the price of the final product on the market. On the contrary, solid-state fermentation (SSF) has the advantage of utilizing readily available and inexpensive solid substrates made from agro-industrial lignocellulosic waste products such as bagasse, paper pulp, food waste, corncobs and wheat bran, as well as consuming less energy. SSF has evolved as a green and cost-effective alternative to SmF within the context of the circular bioeconomy (Chilakamarry et al., 2022; Oiza et al., 2022; Yafetto, 2022).

Most *Bacillus*-based biocontrol products available on the market are in powder form, saturated with dry endospores and secreted metabolites (Gotor-Vila et al., 2017), and are produced primarily through SmF (Biermann & Beutel, 2023). Conversely, SSF, which is widely adopted in many developing countries (Asia on the top), has also proven to be a promising method in biomass and biometabolites production. For example, high yields of *Bacillus* spores (up to 10^{12} spores/g) and derived-bioactive molecules were achieved when cultivated on various substrates derived from agricultural residues(Berikashvili et al., 2018; Biermann & Beutel, 2023; Valdés-Velasco et al., 2022).

In this chapter, we evaluated the growth and sporulation potential of the Burundi-isolated biocontrol candidate *B. nakamurai* BDI-IS1 when cultured on various lignocellulosic substrates (mostly agricultural residues found in tropical regions), using SmF and SSF. Finally, we proposed an easy, efficient and locally adapted product formulation method. BDI-IS1 based-bioproduct was presented as a dry mixture of bacterial spores, secreted metabolites and the residual substrates that had

supported its growth through SSF. This bioproduct demonstrated also a consistent biocontrol efficacy against northern corn leaf blight.

2. Material and methods

2.1. Lignocellulosic substrates collection and preparation

Four samples of lignocellulosic substrates, including bean haulms (BH), rice straw (RS), maize cobs (MC) and rice husks (RH), were collected in Rugombo, province of Cibitoke, Burundi. The samples were air-dried and grounded into a fine powder with a particle size of 0.5-1 mm before being analysed for different physico-chemical parameters at LASPA (Laboratoire d'Analyse des Sols et des Produits Agricoles), ISABU.

2.2. Physico-chemical analysis of the substrates

2.2.1. pH determination

1g of each sample was weighed and mixed with 10 ml of distilled water. The mixture was stirred and left to stand for 30 minutes, taking care to stir 5 to 6 times during this period. The pH meter was calibrated using pH 7.0 and pH 4.0 buffer solutions. After a final stirring, the electrode was immersed in the mixture and reading was performed once the pH value was stable over 30 seconds (Centre d'expertise en analyse environnementale du Québec, 2003).

2.2.2. Total nitrogen determination

The nitrogen dosage was carried out following Kjeldahl method with minor modifications (Kibiriti et al., 1986). 1g of the sample is weighed and placed in a 500 ml test tube. The mixture of catalysts ($CuSO_4$-K_2SO_4-$FeSO_4$) and powdered selenium is then added and rinsed with demineralised water. After adding 25 ml of concentrated sulphuric acid (H_2SO_4 cc), the tubes were heated in a well-ventilated fume hood until blue colour appeared. The tubes were cooled after the first step of digestion and rinsed carefully with demineralized water to bring all inner walls-bound samples inside the tube. The acid-heat mineralisation went further until the colour of the whole solution became blue.

After the digestion, the tubes were cooled, and 25 ml of demineralised water was added and filtered through a Whatman filter n°41 into a 250 ml volumetric flask. The tube and filter were rinsed thoroughly, and the filtrate was brought to volume with demineralised water. Two blanks were prepared in the same way (demineralised water + catalysts + sulphuric acid).

Ammonia was then produced by steam distillation: 25 ml of the digestated solution is pipetted into a distillation apparatus, along with 25 ml of 50% NaOH. The distilled ammonia is trapped in an Erlenmeyer flask containing 30 ml of 2% boric acid solution (H_3BO_3 2%) with a mixture of indicators. After about 3 min of distillation (after the colour change from red to blue), the flask was set apart and titrated by H_2SO_4 0.05N in presence of phenolphthalein.

$N\ (\%) = (Y\text{-}Blm)*(NH_4)_2SO_4*f*V_1/V_2*100*0.014008/\ P$

Where P is the sample weight (g), V_1 (ml) the volume of the flask (250 ml in this case), V_2 (ml) the taken volume for analysis (25 ml in this case), Y (ml) the added volume of H_2SO_4 0.05N to titrate the sample solution, f the correction factor of H_2SO_4 0.05N and Blm the added volume of H_2SO_4 0.05N to titrate the blank solution. The correction factor (f) is determined by aliquoting 3 ml of Na_2CO_3 0.05N and titrating it by H_2SO_4 0.05N in the presence of phenolphthalein (from red to colourless). If V (ml) is the added volume of H_2SO_4 0.05N as titrant, then f = (0.05 * 3)/(V*0.05).

2.2.3. Carbon content determination

Weigh approximately 0.02g of the substrate on analytical balance. Transfer the quantity into an Erlenmeyer flask (500 ml) and add 10ml of potassium bichromate ($K_2Cr_2O_7$) 1N, 20 ml of sulfuric acid (H_2SO_4), mix by shaking for about 1min and allow to settle down for 30min. Afterwards, add 500 ml of demineralized water, 10 ml of concentrated phosphoric acid (H_3PO_4) and 10 drops of ferroin, the indicator solution. The excess of $K_2Cr_2O_7$ is titrated with the ferrous sulfate solution 0.5N ($FeSO_4.7H_2O$) till to the appearance of brown colour, and the added volume is noted. The blank was prepared in the same way, but without the sample (Centre d'expertise en analyse environnementale du Québec, 2015).

C (%) = (A-B)*10*0.003*100/(P*A)

Where C: Organic carbon content (%), A: Added volume (ml) of the titrant $FeSO_4$. $7H_2O$ for the blank, B: Added volume (ml) of the titrant $FeSO_4$. $7H_2O$ for the sample, 10: Initially added volume of $K_2Cr_2O_7$, 0.003: quantity (g) of C digested by 1ml of $K_2Cr_2O_7$, P: weight (g) of the dried sample, 100: factor allowing results to be expressed in percentage.

2.2.4. Sulphur (S) content determination

Weight approximately 20 g of the dried sample in an Erlenmeyer flask (250 ml) and add 100 ml of distilled water. Mix thoroughly by shaking for 30 min and filter using Whatman filters (45 µm). The filtrate was collected into a beaker (250 ml) and 5 ml of HCl 10% was added before the mixture was treated by heat until boiling. Add 20 ml of barium chloride 10% ($BaCl_2$) and allow the mixture to prolonged boiling for 10 min. Cool down and filtrate (Whatman filter, 45µm) the mixture. Collect the residue on the filter paper and wash it several times with distilled water until no precipitate appears in the reaction between the washing water and silver nitrate ($AgNO_3$). The last washing was carried out with ethanol followed by diethyl-ether and 3ml of HNO_3 was added. The filter paper containing the washed residue was then dried in the oven at 80°C in prior-weighed crucible. The blank was prepared following the same protocol.

S (%) = ([((T+E)-Tc)-Bl]*VT*0.137*100)/(P*V), and S (mg/kg) = %S*10000

Where Tc: weight of the crucible, (T+E): weight of the crucible containing the dried filter paper and sample-derived washed residue, Bl: the weight of crucible containing-dried filter paper and blank-derived washed residue, VT (ml) : Total volume, V (ml):

Utilised volume, 0.137: Correlation factor corresponding to the mass of S per 1 mole of $BaSO_4$.

2.2.5. Minerals content determination

2.2.5.1. Preparation of the sample solution

Weigh approximately 2 g of powdered sample into tared crucible. Place them into an oven previously set at 450°C for 24h. After their removal from the oven, 7 ml of HNO_3 were cautiously added followed by 15 ml of distilled water. The mixture was heated to boil and allowed to cool down afterwards. The mixture was filtered on Whatman filter paper (n° 42) and the filtrate collected into 100 ml volumetric flask. The filter paper and crucible were severally washed with distilled water, and the flask was put to volume (Kibiriti et al., 1986).

2.2.5.2. Dosage of phosphorus

Standard curve determination: 10 ml of the solution containing 1000 ppm P were pipetted and diluted to 100 ml with distilled water into volumetric flask. Close and mix thoroughly. For standard curve determination, 0, 2, 5, and 10 ml of the latter diluted solution (100 ppm P) were pipetted into 100 ml volumetric flask. Rince the pipette with distilled water and add 10 ml of vanado-molybdic solution and bring to volume with distilled water. Close and shake vigorously. Readings are performed at 420 nm in a UV-Vis spectrophotometer 30 min later. Trace the standard curve with the different concentrations (0, 2, 5, 8, 10 ppm P).

Phosphorus content determination: From the sample solution, aliquot 1 ml to the 25 ml volumetric flask and add 2.5 ml (1/10 of the capacity of the flask) of vanado-molybdic solution and bring to volume with distilled water. Close and shake vigorously and perform reading at 420 nm in UV-Visible spectrophotometer, 30 min later. If read values are out of the range of the calibration curve, sample dilutions are carried out.

P (ppm or mg/kg): (X*Y*Z)/A

Where X: concentration (P ppm) on the spectrophotometer, Y: the dilution factor (25), Z: Volume of the prepared sample solution and A: Weight of the initial dried sample. If further dilutions were performed, they have to be considered into the expression of the final result.

2.2.5.3. Dosage of iron

Standard curve determination: 10ml of the solution containing 1000ppm Fe were pipetted and diluted to 100ml with distilled water into volumetric flask. Close and mix thoroughly. For standard curve determination, 0, 2, 5, 10, 15 and 20ml of the latter diluted solution (100ppm Fe) were pipetted into 100ml volumetric flask. Rince the pipette with distilled water and bring to volume with distilled water. Close and shake vigorously. Readings are performed into flame atomic absorption spectrometer PERKIN-ELMER. Trace the standard curve with the different concentrations (0, 2, 5, 8, 10, 15 and 20 ppm Fe).

Fe content determination: From the sample solution, aliquot 1 ml into 25 ml volumetric flasks and bring to volume. Close and shake vigorously and perform reading into flame atomic absorption spectrometer PERKIN-ELMER. If read values are out of the range of the calibration curve, sample dilutions are carried out.

Fe (ppm or mg/kg): (X*Y*Z)/A

Where X: concentration (Fe ppm) on the spectrometer, Y: the dilution factor (25), Z: Volume of the prepared sample solution and A: Weight of the initial dried sample. If further dilutions were performed, they have to be considered into the expression of the final result.

2.2.5.4. Dosage in calcium, potassium and magnesium

Standard curve determination: 10 ml of the solution containing 1000 ppm Ca, K, and Mg were pipetted and diluted to 100 ml with distilled water into volumetric flask. Close and mix thoroughly. For standard curve determination, pipette from the latter diluted solutions 0-2-5-10 ml, 0-1-2-5-10-15 ml and 0-0.2-0.5-1-2 ml for Ca, K and Mg respectively in 100 ml volumetric flasks and bring to volume. Rince the pipette with distilled water, add 10 ml of lanthanum chloride solution 0.5% ($LaCl_3$) and bring to volume with distilled water. Close and shake vigorously. Readings are performed into flame atomic absorption spectrometer PERKIN-ELMER. Trace the standard curve with the different concentrations.

Ca, K and Mg content determination: From the sample solution, pipette 5 ml to the 25 ml volumetric flask, add 2.5 ml (1/10 of the capacity of the flask) of $LaCl_3$ 0.5% and bring to volume with distilled water. Close and shake vigorously and perform reading into flame atomic absorption spectrometer PERKIN-ELMER. If read values are out of the range of the calibration curve, sample dilutions are carried out.

Ca or K or Mg (ppm or mg/kg): (X*Y*Z)/A

Where X: concentration (Ca/K/Mg ppm) on the spectrometer, Y: the dilution factor (5), Z: Volume of the prepared sample solution and A: Weight of the initial dried sample. If further dilutions were performed, they have to be considered into the expression of the final result.

2.2.6. Growth of BDI-IS1 on different lignocellulosic substrates

2.2.6.1. Submerged fermentation

Overnight preculture of BDI-IS1 (and QST713 as reference strain) (LB, 150 rpm, 37°C) were used to prepare the bacterial suspension. Aqueous extracts generated from reflux heating (30 min) of a suspension mixture of 20 g of dried substrate sample and 300 ml of distilled water followed by double filtration on Whatman n°42, were used as liquid medium. After autoclaving at 121°C for 20 min and cooling down, 20 ml (100 ml flask) of aqueous extracts (bean haulms (BH), rice straws (RS), maize cobs (MC) and rice husks (RH)) were inoculated by the bacterial suspension for a starting OD_{600nm} 0.1. The cultures were incubated at 37°C, 150 rpm for 72h. Serial dilution and plate counting allowed the determination of total cells (TC) count (CFU/ml) and spores (Sp) count (CFU/ml) after heat-shock at 80°C for 20 min (Berikashvili et al.

2018). LB was utilized as a positive control and two independent experiments were carried out in triplicates (n = 6).

Sporulation efficiency (%) = ((TC-Sp)/TC)*100

2.2.6.2. Solid state fermentation

10 g of the lignocellulosic substrates supplemented with 30% of RH were humidified with 60 ml (FH), 45 ml (RS), 30 ml (MC) of distilled water in 250 ml flask, and they were sterilized by autoclave at 121°C for 20 min. After cooling, 1 ml of bacterial suspension (OD_{600nm} 0.1) of BDI-IS1 (and QST713 as reference strain) from an overnight preculture (LB, 150 rpm, 37°C) were used to inoculate the substrates. After a thorough mixing of inoculum and the substrates in sterile condition, the mixture was incubated at 30°C for 7days without shaking. The mixture was, however, stirred manually each 24h for proportional aeration. After incubation, 1 g of the wet sample was suspended in 10 ml of sterile distilled water and mixed by vortexing. Another suspension (1 g of wet sample in 10 ml) was heat-treated at 80°C for 20 min. Serial dilution and plate counting were performed to assess the total cell (TC) count (CFU/g) and the spores (Sp) (CFU/g) count in the non-heat-treated and heat-treated sample, respectively (Berikashvili et al. 2018). Two independent experiments were carried out with three repetitions (n = 6).

Sporulation efficiency (%) = ((TC-Sp)/TC)*100

2.2.7. Formulation process and efficacy assessment of the bioproduct

The seven days old-fermented substrates with BDI-IS1 (or QST713), whose spore content was previously determined, were spread on aluminium foil and allowed to oven dry gently at 70°C for 14h. The dried bacterized substrates were reduced manually in small particles, re-assessed for possible spores' loss determination that may be due to the drying process. Formulated product as bacterized lignocellulosic substrates were packaged into plastic bags (30 g) and stored until use.

Bio-efficacy of these bioproducts were evaluated on maize infested with *E. turcicum* in pot experiments set in a tunnel-like facility protected against rain and insect attack at ISABU. The experimental design was made of nine treatments arranged in a completely randomized design, with 12 plants per treatment representing four plants per experimental unit, replicated three times. The nine treatments involve the different formulated products and control: (1) BDI-IS1 + bean haulms (BDI-IS1 BH), (2) BDI-IS1 + rice straw (BDI-IS1 RS), (3) BDI-IS1 + maize cobs (BDI-IS1 MC), (4) QST713 + bean haulms (QST713 BH), (5) QST713 + rice straw (QST713 RS), (6) QST713 + maize cob (QST713 MC), (7) bean haulms (BH), (8) rice straw (RS), and (9) maize cobs (MC). Maize seeds were prior germinated and were grown into 2 L pots with one grain per pot. Each pot received arable soil collected from field and 5 g of the bioproduct or the substrate alone for control. The maize plantlets were inoculated by spraying with a suspension of *E. turcicum* (see chapter 4, section 2.6.3 for preparation) and covered with plastic bags for symptoms development. Disease severity was then scored periodically using the previously defined scale (see Figure S4).

2.2.8. Statistical analysis

Statistical analysis was performed by one-way ANOVA with the multi-comparison of means carried out by Tukey, or with two way-ANOVA supplemented with Šídák test or Tukey test for multiple comparisons of means. The differences between means were considered statistically significant at $p < 0.05$. All these analyses and design of graphs were conducted with GraphPad Prism 9.1 software.

3. Results and discussion

3.1. Physico-chemical characterization of the lignocellulosic agricultural residues

The lignocellulosic agricultural residues used as substrates for bacterial growth in our study are derived from the important crops cultivated in Burundi, such as beans, rice and maize, ensuring their permanent availability. Prior to the bacterial growth assays, the intrinsic physico-chemical parameters of the residues were assessed, including pH and the content in macro and micro-elements, which are crucial for bacterial growth and sporulation. Bean haulms were found to have higher concentrations in most of the tested elements, except for nitrogen and phosphorus which were predominant in rice straw. However, rice husks were found to have the lowest values for the critical elements like carbon, nitrogen and phosphorus (Table 6-1). The pH values ranged from pH 5.5 to pH 7.0, with the lowest value found in rice husks and the highest value in bean haulms. These data demonstrate that these organic wastes have an optimal pH range and nutritional content compatible with microbial growth, meaning they can be recycled and used as potential substrates for the growth, sporulation and BSMs production by plant beneficial bacilli. Additionally, they can serve as an additional source of nutrients for plants as an organic amendment if formulated together with *Bacillus* spores.

Table 6-1: Physico-chemical characterisation of lignocellulosic agricultural residues.

Substrate/ Parameter	Bean haulms (BH)	Rice straw (RS)	Rice husk (RH)	Maize cob (MC)
Carbon (% C)	35.16 ± 0.83	31.03 ± 1.01	28.43 ± 0.59	30.33 ± 1.41
Nitrogen (% N)	0.79 ± 0.01	0.92 ± 0.01	0.37 ± 0.004	0.676 ± 0.01
Phosphorus (mg/kg)	2,083 ± 0.63	5,300 ± 1.76	2,003 ± 0.44	4,489 ± 0.92
Sulfur (mg/kg)	761 ± 0.54	130 ± 1.33	35.6 ± 1.79	10.04 ± 1.26
Calcium (mg/kg)	3,482 ± 1.72	2,728 ± 1.65	1,016 ± 1.21	438 ± 0.84

Magnesium (mg/kg)	2,511 ± 1.25	2,094 ± 1.38	572 ± 0.38	610 ± 1.96
Potassium (mg/kg)	2,6561 ± 0.37	2,1118 ± 0.71	2,0239 ± 0.86	9,380 ± 0.21
Iron (mg/kg)	391.3 ± 1.58	371.7 ± 0.29	382.2 ± 0.38	179.8 ± 1.02
pH	6.04	6.94	5.55	5.72

3.2. Submerged fermentation of BDI-IS1 in aqueous extracts derived from lignocellulosic substrates

For this purpose, the lignocellulosic substrates were subjected to decoction (heat extraction), after which aqueous extracts were generated and tested for growth and sporulation of BDI-IS1 (and QST713) using the submerged fermentation. After 3 days of incubation at 37°C and 150 rpm, the aqueous extracts from bean haulms showed great potential to sustain the growth and sporulation of both BDI-IS1 and QST713 compared to other extracts (Figure 6-1). However, BDI-IS1 generally demonstrated a remarkable adaptation to these liquid media derived from lignocellulosic residues compared to QST713. While QST713 only produced a good yield of total cells and spores on bean haulms-derived medium, BDI-IS1 produced relevant yields of total cells and spores when cultured on rice straw and maize cob-derived aqueous extracts. The lowest spore production and sporulation efficiency for both BDI-IS1 and QST713 were observed in rice husk-derived liquid medium and Luria-Bertani (LB) medium. These contrasting results demonstrate the differential ability of *Bacillus* strains to thrive on different nutrient sources and, most importantly, show that BDI-IS1 can adapt to a variety of nutritional assets. This suggests also that its large-scale production could be achieved using locally available and affordable means. However, the total cell and spore counts achievable with this energy-consuming liquid fermentation are insufficient (10^8) for agricultural application compared to other commercial preparations. In most of the cases, the concentrations of these bioproducts range from 10^{10} to10^{12} spores per ml (e.g. 10^{12} CFU/ml for Serenade® and 10^{10} CFU/ml for RhizoVital® 42) (Bisutti et al., 2017; Tut et al., 2021). This suggests a need to look for other cost-effective methods and culture conditions that would enable high spore concentrations to be attained.

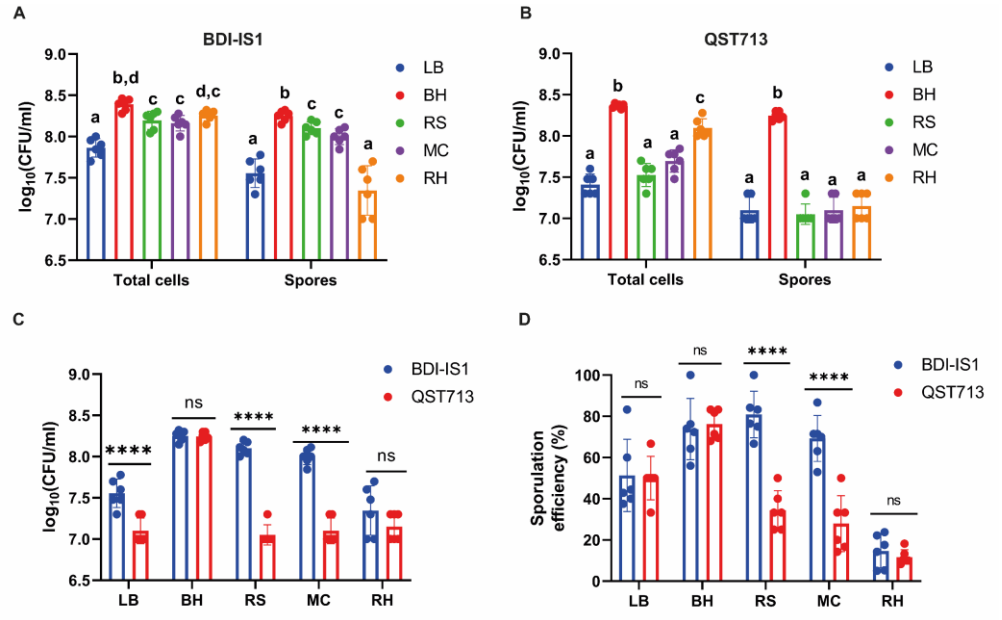


Figure 6-1: Biomass production by BDI-IS1 compared to QST713 using submerged fermentation. A and B denote the total cells and spores' production in different lignocellulosic substrates-derived aqueous extracts for BDI-IS1 and QST713, respectively. C and D show the comparisons of spore production and sporulation efficiency between BDI-IS1 and QST713, respectively, when cultured in different aqueous extracts from lignocellulosic substrates. Comparison of data means (± SD) for A & B was performed with two-way ANOVA coupled to Tukey's test for multiple comparisons test ($\alpha = 0.05$); while for C & D, data means (± SD) comparison was carried out with two-way ANOVA coupled to Šídák's test for multiple comparisons test ($\alpha = 0.05$). Letters above the bars imply statistically non-significant difference or significant difference when they are similar or distinct, respectively. The assay was carried out in triplicates and in two independent experiments (n = 6). BH, RS, MC and RH stand for bean haulms, rice straw, maize cob and rice husks, respectively.

3.3. Solid-state fermentation of BDI-IS1 on different lignocellulosic substrates

Next, we evaluated growth and spore production in solid-state fermentation using the humidified powdered substrates from bean haulms, rice straw and maize cobs (supplemented each with 30% of rice husks) (Figure 6-2). As in the SmF, higher growth and sporulation were observed for BDI-IS1 and QST713 on the bean haulm-based substrates than on the other two substrates. BDI-IS1 adapted better to all three substrates than QST713, producing 40% more spores on average (Figure 6-2C). However, the sporulation efficiency of the two strains did not differ statistically for any of the tested substrates, reaching almost 90% for rice straw, for example (Figure 6-2D). Overall, this SSF greatly improved the spore yield for all the tested substrates

compared to SmF, with an average spore concentration of 10^{13} CFU/g. Comparable biomass and spore yields (up to 10^{11} CFU/g) were also achieved on different lignocellulosic substrates with bacilli, such as maize cobs and wheat bran with *B. amyloliquefaciens* B-1895 and *B. licheniformis*, respectively (Berikashvili et al., 2018; S. Zhao et al., 2008). This technique is not only inexpensive but also suited to developing countries in tropical regions with energy supply issues, since it does not require constant shaking and the ambient temperature can average the optimal growth temperature for many *Bacillus* strains (around 30°C). However, the seven-day incubation period seems too long for industrial-scale production and this process could be optimized in terms of aeration or particle size. Aqueous wetting of solid substrates could be replaced with certain types of liquid organic waste such as fermented human or animal urine and domestic wastewater. This would provide readily available nutrients for an accelerated bacterial growth and degradation of lignocellulosic substrates.

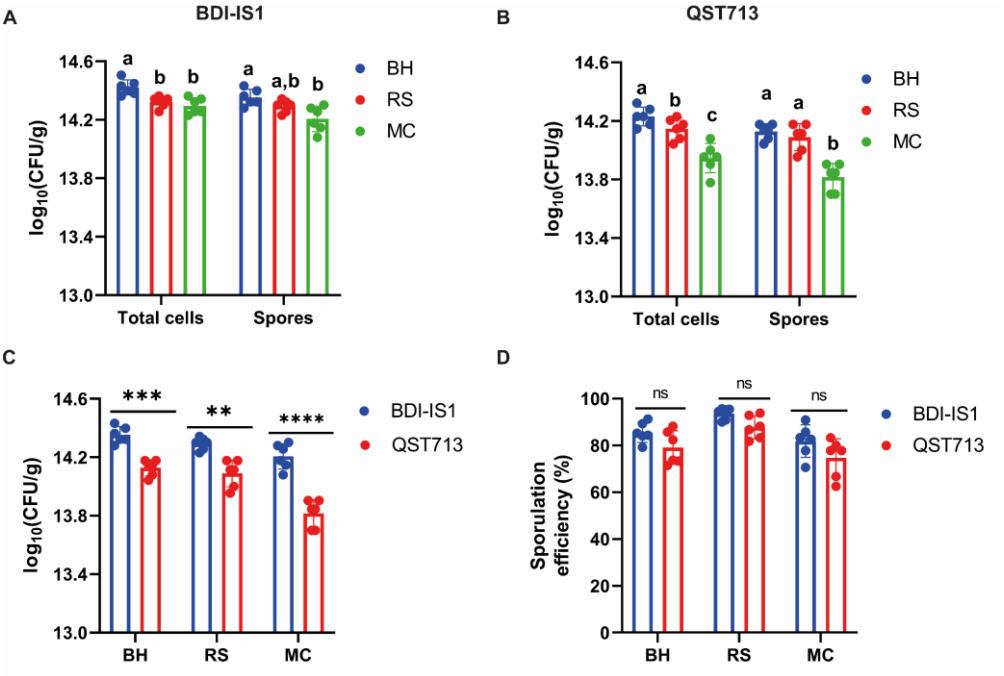


Figure 6-2: Biomass production by BDI-IS1 compared to QST713 using solid-state fermentation. A and B denote the total cells and spores' production in different lignocellulosic substrates for BDI-IS1 and QST713, respectively. C and D show the comparisons of spore production and sporulation efficiency between BDI-IS1 and QST713, respectively, when cultured on different lignocellulosic substrates. Comparison of data means (± SD) for A & B was performed with two-way ANOVA coupled to Tukey's test for multiple comparisons test (α = 0.05); while for C & D, data means (± SD) comparison was carried out with two-way ANOVA coupled to Šídák's test for multiple comparisons test (α = 0.05). Letters above the bars imply statistically non-significant difference or significant

difference when they are similar or distinct, respectively. The assay was carried out in triplicates and in two independent experiments (n = 6).BH, RS and MC stand for bean haulms, rice straw and maize cob, respectively.

3.4. Formulation of BDI-IS1 spores and efficacy assessment of the bioproduct

In addition to biomass production, our aim was to formulate the biocontrol agent candidate BDI-IS1 in a simple yet appropriate manner, adapted to the socio-economic context of many rural farmers in Burundi and other developing countries. The mixture of BDI-IS1 spores and the residual lignocellulosic substrates used for spore production, along with the secreted metabolites, were subjected to soft oven-drying at 70°C for approximately 14h (Figure 6-3A-C). This process avoided the need for complicated downstream processing (i.e. separation of spores and metabolites) and did not lead to significant loss of spore content (Figure 6-3C). This suggests the reliability of the process and the necessity of further studies to validate it at large-scale production level.

The efficacy of the formulated bioproduct was assessed in pot experiments in an open-air, tunnel-like setting that was protected from pest invasion and rain. The maize/*E. turcicum* pathosystem was used for this purpose. The bioproduct was applied to the planting hole, which subsequently received the pre-germinated maize seed, and the two were left to evolve together. The three formulations (based on BH, RS and MC) significantly reduced the disease (by up to 65%) compared to the control, which was treated with non-bacterized substrates (Figure 6-3D). Formulations based on bean haulms or rice straw either for BDI-IS1 or QST713 exhibited slightly improved disease reduction compared to maize cob-based formulations. Here again, products based on BDI-IS1 provided better plant protection than those based on QST713, as shown previously (Figure S6).

This inexpensive technique of producing and formulating spores-based biocontrol agents is mostly used for fungal-based biopesticides (M. Das M & Abdulhameed, 2020). Although some reports do exist at a research level, this technique is not popular for spore-forming bacilli (Berikashvili et al., 2018; El-Bendary & Moharam, 2019; Mizumoto et al., 2006; Su et al., 2019). However, numerous *Bacillus*-based studies have exploited this solid-state fermentation (SSF) technique to produce bioactive secondary metabolites, including lipopeptides (Bouassida et al. 2023; Valdés-Velasco et al. 2022) and various hydrolytic enzymes (El Salamony et al., 2024; Irfan et al., 2016; Mazhar et al., 2023).

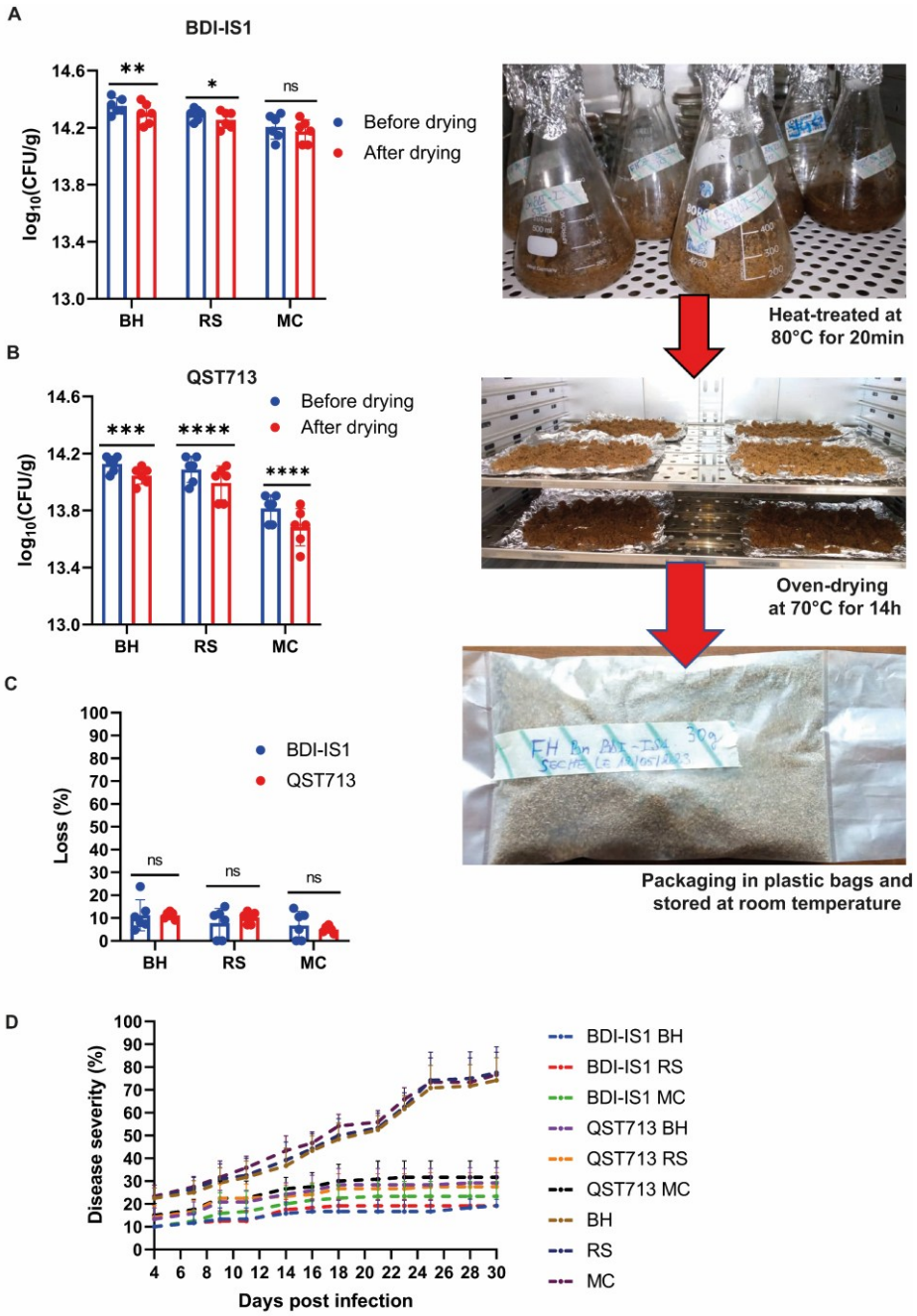


Figure 6-3: Assessment of the formulation process of BDI-IS1 and biocontrol efficacy of the derived-bioproduct. A & B denote the comparisons of the endospores count before and after the oven drying for BDI-IS1 and QST713, respectively. While C shows the comparative effect of oven drying on the spores count (% spore loss) between BDI-IS1 and QST713. D.

illustrates the evolution of the severity of nothern leaf blight disease on maize plants treated with BDI-IS1 and QST713 formulations. Each point on the graph stands as a mean of disease severity values from 12 different plants (n = 12) organized in 3 blocks of 4 plants per treatment, with one trial. Comparison of data means (± SD) for A, B and C were performed with two-way ANOVA coupled to Šídák's test for multiple comparisons test ($\alpha = 0.05$).

Furthermore, this bioproduct formulation approach may enhance treated plants growth owing to the organic substrate matrix mixed with the bacterium (Mizumoto et al., 2006). Taken together with the demonstrated biocontrol properties and putative plant growth promotion potential of BDI-IS1, this bioproduct based on Burundian local bioresources has the potential to revolutionalise the local agricultural systems by improving productivity and reducing chemical inputs. However, further assessment in other pathosystems is still necessary, as well as the establishment of proper framework for its large-scale production and commercialisation and ensure its widespread adoption by local farmers in Burundi or neighbouring countries. Nevertheless, there is a major health concern regarding possible contamination of these organic substrates from conventional agriculture, either by trace metals or pesticide residues. Another quality control issue is the variability of the physico-chemical properties of these agricultural residues, which may differ from batch to batch and between agricultural seasons.

4. Conclusion

Cost-effective methods of producing the biocontrol agent candidate *B. nakamurai* BDI-IS1 were investigated. These methods involved using lignocellulosic substrates, which are abundantly available as agricultural residues in Burundi and other tropical developing countries, through submerged and solid-state fermentation types. Lignocellulosic substrates based on bean haulm, rice straw and maize cobs promoted bacterial growth and ensured high sporulation efficiency in both fermentation techniques. However, solid-state fermentation using wet solid substrates yielded more spores than submerged fermentation using aqueous extracts derived from these biowastes. This high spore-producing fermentation technique offers a particular added-value in terms of production costs, as it does not require expensive facilities and uses affordable locally sourced substrates. It is also energy-effectient compared to submerged fermentation and would therefore guarantee a final product that is relatively affordable to users. This technique also offers a practical way to formulate the bioagent directly with its growth substrate by simply eliminating the humidity through drying. Furthermore, the dry formulated bioproducts provided an excellent protection (up to 65%) of maize against nothern leaf blight disease. Bean haulm-based bioproduct provided enhanced maize protection compared to the two other substrates. Formulated products based on BDI-IS1 were more effective than those based on QST713 in controlling northern corn leaf blight, corroborating the findings with non-formulated bacterial suspensions presented in chapter 4.

These findings pinpoint the potential of alternative approaches of producing biocontrol agents that are environmentally advantageous, innovative, and suited to the

socio-economic circumstances of smallholder farmers in many developing countries (e.g. low income, illiteracy, etc.), while also ensuring effective and sustainable plant growth promotion and crop protection.

Chapter 7

General discussion and perspectives

1. Ecodiversity-guided bioprospection for more efficient *Bacillus*-based biocontrol agents

Over the last decades, he adverse effects of pesticides on human health and the environment have fuelled the search for safe and eco-friendly alternative solutions. This has resulted in rapid growth of the biopesticide market, which now accounts for 10% of the global pesticide market (Marrone, 2024). Ubiquitous, resistant endospore-forming bacilli have been thoroughly documented for their plant beneficial traits, including promoting plant growth and controlling plant diseases and pests. They have gained a firm place among other microbial-based biocontrol alternatives within the framework of integrated pest management (Hamrouni et al., 2025; Serrão et al., 2024). Bioproducts based on strains of the *B. subtilis* clade dedicated to fighting plant diseases are also steadily increasing in popularity, thanks to their proven bio-efficacy, which is mediated by an arsenal of BSMs acting through various synergistic mechanisms, including competition for space and nutrients, antibiosis, and elicitation of plant immunity (A. Ali et al., 2024; G. Nihorimbere et al., 2024).

Nonetheless, these biological products encounter some limitations in terms of the reproducibility of efficacy from laboratory-controlled conditions to natural and complex field conditions (Etesami et al., 2023a), meaning they are not suited to deployment in every agro-ecosystem. This underscores the importance of continued exploration of different agricultural ecosystems to find bioactive native strains that can adapt to local field conditions and successfully control indigenous plant pathogens (Y. Liu et al., 2023). The application of locally-isolated biocontrol agents also reverts ethical implications, as it is less likely to provoke on site-ecological imbalances (Lahlali et al., 2022). In this regard, this work provides a thorough characterisation of a new strain isolated from tropical soils in Burundi, *B. nakamurai* BDI-IS1, and explores its plant beneficial potential, its bioactive secondary metabolome, its ability to adapt to cold and acidic stresses, and the possibility of its utilization as a formulated biocontrol product.

2. *Bacillus nakamurai,* a ubiquitous species with great antimicrobial potential

BDI-IS1, which was isolated from residual roots selected from soil samples collected in a field that had previously been used for potato plantation (at an altitude of 1880 m, Isare, Burundi), was chosen from other *Bacillus*-like isolates due to its strong antagonistic potential against bacterial phytopathogens. These pathogens include two Gram-positive and four Gram-negative bacteria of worldwide agriculture importance. Gram-positive bacteria include *C. michiganensis*, the causal agent of canker disease in *Solanum* crops (Haghverdi et al., 2025; Malliarakis et al., 2023) and *R. fascians*, which causes leafy gall in many ornamental plants (Gordon et al., 2024). The Gram-negative bacteria include *X. campestris*, known to infect several crops (Nakato et al., 2018; Tang et al., 2021), *P. carotovorum*, causing the soft rot disease in many crops (Almasoudi et al., 2024; Osdaghi, 2023), *P. cichorii*, which attacks

various crops (C. J. Huang et al., 2024; Luiz et al., 2024) and *P. fuscovaginae*, the causal agent of rice sheath rot disease (Musonerimana et al., 2020; B. A. Sharma et al., 2023). BDI-IS1 exhibits significant antifungal activity, comparable to QST713, against various fungal phytopathogens that cause severe agricultural yield losses. These include the biotroph *A. niger*,which causes black mould rot disease (Tawfik et al., 2022); the necrotrophs *B. cinerea* and *R. solani*, which target several crops (Senapati et al., 2022; R. Singh et al., 2024), the hemibiotrophs *F. oxysporum*, *Colletotrichum* sp. and *P. oryzae*, which cause vascular wilt, anthracnose and blast diseases, respectively (Baudin et al., 2024; Salotti et al., 2022; Srivastava et al., 2025), and the Burundi-isolated fungal strains *A. solani*, *E. turcicum* and *A. rabiei*, which were collected from diseased tomato, maize and bean leaves, respectively. This illustrates the broad-spectrum antimicrobial potential of BDI-IS1 on a global scale. This should be further substantiated by screening the bacterium against other important phytopathogens, especially those occasioning high pesticide consumption such as *P. infestans*, the causal agent of potato late blight disease (Table 1-1). This would establish BDI-IS1 as a versatile and eco-friendly solution for disease management, and thus, contribute to reduce the indiscriminate reliance on pesticides and the resulting problem of pesticide resistance in agricultural pathogens (Beyari, 2024; Negi et al., 2023).

Phenotypical and molecular identification revealed that BDI-IS1 is a new strain of the recently described species *B. nakamurai* (Dunlap et al., 2016), which is closely related to *B. velezensis*. The two species are phylogenetically clustered into the same *B. amyloliquefaciens* operational group, together with *B. amyloliquefaciens* and *B. siamensis* (X. Xu & Kovács, 2024). The species name "*Bacillus nakamurai*" honours the great microbiologist L. W. Nakamura of the Agricultural Research Service Culture Collection (Peoria, USA), in recognition of his contributions to *Bacillus* taxonomy (Dunlap et al., 2016).

Of the five *B. nakamurai* strains that have been sequenced, BDI-IS1 was the third to be published, following *B. nakamurai* NRRL B-41091 and *B. nakamurai* NRRL B-41092 isolated from soil collected in Tierra del Fuego National Park in Argentina, near Antarctica. The remaining two strains, B-41093 and MZ03-67, originated from soil samples collected in the same National Park, Tierra del Fuego, and from the cave walls of the La Palma lava tubes inSpain, respectively. The geographical diversity of the ecological niches from which these strains were isolated reflects the ubiquitous nature of this species. Furthermore, the eco-diversity of sources of the *B. nakamurai* species can also be evidenced in the origins and matrixes of other non-fully sequenced isolates that were putatively described as *B. nakamurai* strains by the 16s rRNA technique. Some were originated from rhizospheric soil samples (Ahmed Abokoura & Saad, 2019), plant inner parts (Chaouachi et al., 2021), archaeological sites (Yahia Mahfouz & Daigham, 2020), soil sediments from aquatic ecosystem (Shaikh et al., 2023), oil-polluted soil samples (Rasouli et al., 2024), insect guts (MsangoSoko et al., 2022; Usta, 2021), vermicompost (Soltan et al., 2022) and fermented organic materials (Kang et al., 2023; V. Kumar et al., 2022).

The wide ecological distribution of this species would reflect a high level of genetic adaptability, enabling it to cope with various environmental conditions by modulating its physiological, morphological and secretory patterns in accordance to the environment (Etesami et al., 2023b; Y. Huang et al., 2023; Polonca, 2020). Correlatively, our data revealed that BDI-IS1 is equipped with more genes related to stress resistance than its closely related strains of the *B. subtilis* clade. These genes are involved in the synthesis of cold and heat chaperones, a set of oxidative enzymes, osmoprotectant molecules and PCB-degrading enzymes. In addition, BDI-IS1 possesses a panoply of lytic enzymes that can degrade different types of polymeric nutrient, as well as a secondary iron uptake system (EfeUOB) and heme/hemin efflux systems, which were absent in other *Bacillus* strains considered for comparison. Furthermore, BDI-IS1 and the two other strains, NRRL B-41091 and NRRL B-41092, produce UV- and oxidative stress- alleviating melanin-like pigments (see Chapter 3, Section 3.2). This black pigment, which is biosynthesized through the action of tyrosinases and/or laccases (two copper-containing polyphenol oxidases) with hydroxylated aromatic compounds as substrates, has also been described in some *Bacillus* strains, but most often in actinobacteria and proteobacteria (Ghadge et al., 2020; Pettinari et al., 2023). These environmental adaptive traits would confer to BDI-IS1, and probably other *B. nakamurai* strains, an excellent ability to colonise several ecological niches, as well as enhanced competitiveness at the expense of their congeners. This would, thus, ensure successful establishment and persistence upon application.

However, extensive investigations into the significance of these predicted anti-stress genes in real experimental conditions have yet to be undertaken. Nevertheless, our findings constitute a good basis for any biotechnological project intended to valorise this bacterium. Most of the available studies involving *B. nakamurai* strains (or the putative ones) showcase their potential to secrete an array of economically important enzymes (X.-M. Kang et al., 2023; Shaikh et al., 2023; Sorde & Ananthanarayan, 2019; Yan et al., 2022). However, rare reports on their agricultural relevance briefly highlight their antagonistic potential against the phytopathogens *E. amylovora*, *Fusarium* spp. and *B. cinerea*, which cause fire blight, head blight and gray mould diseases, respectively, in several important crops (Chaouachi et al., 2021; Leathers et al., 2020; Zanon et al., 2024), as well as their ability to promote the growth of wheat plants (Ahmed Abokoura & Saad, 2019). Concurrently, their closely related *Bacillus* strains, belonging to the *B. amyloliquefaciens* operational group, are widely utilised as plant beneficial bioinoculants (Keshmirshekan et al., 2024; W. Sun & Shahrajabian, 2025) owing their potential to secrete a panoply of broad-range BSMs. Therefore, it is noteworthy to unravel the full capacity of this species to produce bioactive compounds, their role in observed antagonistic properties, as well as their significance in the biocontrol.

3. Biocontrol performance: adaptation to abiotic conditions beyond the richness in BSMs

This work also showed that the observed antagonistic activities of BDI-IS1 against a wide range of bacterial and fungal plant pathogens are mediated by soluble secreted metabolites rather than volatiles. This prompted us to explore the secondary metabolome of the strain and define its contribution in inhibiting pathogens. Bioinformatics-based prediction of BGCs coupled with analytical metabolomics revealed that BDI-IS1 can secrete two lipopeptides (surfactin and iturin A), one polyketide (bacillaene and its variant, dihydrobacillaene), the siderophore bacillibactin, bacilysin, and several RiPPs (plantazolicin, amylocyclicin, LCI and bacinapeptin). Moreover, comparative metabolomics revealed that the core-metabolome peculiar to *B. nakamurai* strains consists of non-ribosomal peptides (surfactin, iturin A, bacillaene, bacillibactin and bacilysin), a circular and a class III lanthipeptide, and this can be considered as a fingerprint of plausible taxonomic relevance at species level.

Using reverse genetics, we discovered that bacillaene (and/or dihydrobacillaene) is the main inhibitor of *P. carotovorum* and *R. fascians* and also play a role in the antagonism toward other bacterial phytopathogens. However, the main inhibitor of *P. fuscovaginae* was found to be bacilysin. Iturin A was the main antifungal compound that restricted the growth of *A. niger*, *R. solani* and *E. turcicum*, and contributed moderately against *A. solani* and *B. cinerea*. As no obvious metabolite was primarily responsible for inhibiting the growth of *X. campestris*, *C. michiganensis*, *P. cichorii*, *B. cinerea* and *A. solani*, we hypothesize that some of the predicted antibiotic compounds that were not detected may interplay solely or synergistically with other metabolites to exert the overall antagonism of BDI-IS1. Indeed, the four RiPPs predicted in BDI-IS1 are potent inhibitors of various pathogenic bacteria, through disruption of cell envelop by hindering peptidoglycan synthesis and/or through interfering with protein synthesis by damaging DNA structures (L. Cao et al., 2021; Chang et al., 2024; Molohon et al., 2016; D. Xue et al., 2023). The LCI-like compound APC_2 has also recently been reported to play a role in inhibiting some fungi, such as *F. solani* (R. Wang et al., 2021). While most RiPPs are secreted in relatively low quantities under laboratory conditions (Y. Zhang et al., 2018), they may contribute to the overall bioactivity of the producer strains by acting synergistically. In this regard, it can be hypothesized that these antibiotic compounds are produced more efficiently in mutant strains, resulting in a plausible enhanced biological activity. For instance, it has been demonstrated that the production of adenosine, an anti-arrhythmic agent, is encreased in the *B. subtilis* mutant impaired in the secretion of another type of nucleoside, guanosine (B. Li et al., 2019). In addition, this boost in the secretion of metabolites may be more significant in confrontation settings between bacilli and pathogens, as demonstrated in the case of *B. velezensis* S499 against *Rhizomucor variabilis* (Kulimushi et al., 2017). Another possible explanation is the synthesis of cryptic metabolites or the induction of silent genes expression specifically upon confrontation, which could have an additive effect on antagonism against targeted

pathogens (Rigolet, 2023). The predicted silent BGC T_3PKS has been reported to mediate the biosynthesis of diacetylphloroglucinol in *Pseudomonas* sp. and flaviolin in *Streptomyces* sp., which are described as having plausible antifungal and antibacterial activities, respectively (Biessy & Filion, 2021; S. I. Lee et al., 2024). The predicted pyrroloquinoline quinone D (PqqD), a RRE-like protein, is part of the *pqq*ABCDE operon, which is mostly found in Gram-negative bacteria. In addition to its co-factor role and involvement in phosphorus solubilisation, it may also be implicated in synthesizing the antifungal compound 3-methylpropanoic acid, which is active against *Magnaporte oryzae* (Carreño-López et al., 2019).

Furthermore, BDI-IS1 has the potential to produce other biological compounds that may interplay in the observed antagonistic activity. The array of lytic enzymes putatively produced by our strain and other bacilli are known to contribute to antifungal activities by degrading the target' cell wall (Ajuna et al., 2023). In addition, quorum quenching enzymes, i.e. lactonase, amidase and oxidoreductase, which are encoded in BDI-IS1, may contribute to the antibacterial activity by interfering with the quorum sensing molecules of pathogens. Quorum sensing signals and pathogen virulence factors, such as homoserine lactones (AHLs), can be degraded by cleaving the lactone ring (lactonase), the amide bond (amidase or acylase), or by reducing the 3-oxo-substitued AHLs or oxidizing the acyl chain (oxidoreductases) (Grandclément et al., 2015). The antibacterial activity through quorum quenching phenomenon has been demonstrated, for example, against *P. carotovorum*, *P. syringae* and *X. campestris* (Roca et al., 2024; T. Ye et al., 2020). On the other hand, the quorum sensing-associated pheromone *ComX*, which is common to bacilli, was found to be the main biometabolite mediating the antifungal activity of *B. licheniformis* against the aflatoxin producer *A. flavus* (Esmaeilishirazifard et al., 2018). Furthermore, the melanin-like black pigment secreted by BDI-IS1 and some other bacilli has been linked to antagonistic activity against the phytopathogen *X. campestris* and the marine pathogen *Alteromonas macleodi* (Ghadge et al., 2020). Untargeted metabolomics could help to decipher the identity and the relative abundance of these unknown bioactive molecules, that support the observed antagonistic activities (Andrić et al., 2023; Schmid et al., 2023). This can be coupled with transcriptomics, such as RNA-seq and RT-qPCR, to untangle the bioactive compounds, for which an upregulation of their cognate genes is observed in the presence of the pathogen or its extracts (X. Li et al., 2022; Tian et al., 2021). Additionally, knock-out mutant strains impaired in the secretion of one or more of the suspected bioactive compounds can be constructed using either the classical homologous recombination technique or CRISPR-Cas system (Y. Cao et al., 2018; S. Liu et al., 2025). Testing these strains against the pathogens of interest may contribute to identifying potential biochemicals that mediate the antagonistic activities of BDI-IS1.

We also demonstrated the biocontrol potential of BDI-IS1, which provides efficient protection against tomato early blight and northern maize leaf blight of up to 35% and 65%, respectively, under greenhouse conditions. The bacterium was active upon treatment of either the leaves or the roots, suggesting modes of action involving either direct antagonism or ISR, respectively. An *in vitro* assay revealed that iturin A is the

main inhibitor of *E. turcicum*, suggesting a role in direct antagonism upon leaf treatment. However, greenhouse experiments carried out with *ΔituA* did not clearly confirm iturin A's implication in maize protection after leaf treatment, as no significant difference in disease reduction was observed compared to the wild-type BDI-IS1 strain (G. Nihorimbere, 2025). Meanwhile, the BDI-IS1 maize protection against NLB upon root treatment was found to be partially mediated by the well-known ISR elicitor surfactin, although the disease reduction levels were not significantly different between *ΔsrfA* and the wild-type strain (G. Nihorimbere, 2025). Furthermore, investigating the expression of ISR molecular markers in maize plants pre-treated with BDI-IS1 on roots and infected with *E. turcicum* revealed an upregulation of some ISR-related genes, including *PAL* and *AOS1* genes (G. Nihorimbere, 2025). The latter genes are involved in the biosynthesis of bioactive phenolic compounds and phytoalexins, and in jasmonic acid biosynthesis, respectively (Morales et al., 2023). In addition, the biocontrol activity of BDI-IS1 was confirmed in field conditions against NLB (up to 34%) (G. Nihorimbere, 2025), as well as protecting bean plants against the angular leaf spot disease caused by *Pseudocercospora griseola* (Mbabou Mbianzoue, 2023).

Though not widely reported and the underlying mechanisms not yet fully elucidated, some phytohormone-like molecules predicted to be produced by BDI-IS1 such as polyamines (spermidine, spermine and putrescine), may enhance its disease protection abilities. Polyamines are small, polycationic molecules found in all living organisms (T. Roy et al., 2024) and have been shown to protect eggplants from *Ralstonia solanacearum* infection and tomato plants against viral infections and diseases caused by *Fusarium* and *Alternaria* pathogens (Kaur & Das, 2023; Naguib, Nabil, et al., 2020). The accumulation of spermidine following the treatment of plants with *Streptomyces* and the antifungal molecule potassium phosphide has been shown to confer resistance to *P. syringae* and *P. infestans*, respectively (Cassanelli et al., 2025; Lobato et al., 2024). Therefore, apart from their prominent role as a biomarker in plant immunity studies alongside well-studied hormones such as jasmonic acid, salicylic acid and ethylene, the role of these molecules in *Bacillus*-plant interactions merits further investigation since they are co-secreted by both the plant and associated microorganisms.

Furthermore, our data demonstrate the superior biocontrol effectiveness of BDI-IS1 compared to the commercial strain QST713 in the two pathosystems, under both controlled and field conditions. QST713 naturally produces a huge diversity of known bioactive metabolites including the antifungal fengycin and the antibacterials difficidin and macrolactin, which are not produced by BDI-IS1. However, QST713 is less effective at protecting tomato against *A. solani* when applied onto the leaves compared to BDI-IS1, although the two bacilli exert an *in vitro* similar good inhibition against this fungus (Chapter 3, section 3.1). This suggests that QST713 may not be well adapted to the tomato phyllosphere. on the other hand, this overall BDI-IS1's superior performance can be attributed to its enhanced ability to withstand stressful abiotic conditions, and this may influence the efficiency of colonisation, bioactive compounds secretion, and ultimately, plant protection (Sagar et al., 2022; Saleem,

2021). Indeed, our *in vitro* data revealed that BDI-IS1 exhibits a significant fitness advantage over QST713 under stressful conditions, characterised by rapid growth and enhanced biofilm formation at low pH and low temperature (See Chapter 5, section 3.1). In acidic conditions (≤ pH 5), which prevail in many natural arable soils (Gurmessa, 2021; X. Huang et al., 2022), QST713 was unable to grow within 24h at temperatures below 25°C. BDI-IS1 was also found to host additional anti-oxidant genes (lactoylglutathione lyase and alkyl hydroperoxide reductase) and acidic pH-adapted iron sequestration systems (EfeUOB). These features may enhance BDI-IS1's ability to adapt to oxidative stress, while preserving its capacity to sequester environmental iron (Lyng et al., 2024; Sudharsan et al., 2023). In addition, BDI-IS1 produces melanin, which is renowned for its anti-UV and anti-oxidative properties (Idris et al., 2024; Muñoz-Torres et al., 2024). This may ensure bacterial survival and performance on the plant surfaces under fluctuating natural light conditions, as was recently demonstrated for *B. subtilis* and *B. velezensis*, which were isolated from the maize phyllosphere and active against *E. turcicum* (Fessia et al., 2024). While the population size in the rhizosphere and phyllosphere, as well as the level of stress gene expression *in planta*, were not determined in our study, we hypothesize that BDI-IS1, isolated from sunny tropical highlands coped well with the *in situ* conditions and quickly colonized the plant roots and aerial parts (in the case of tomato) to provide better protection to the host plant (J. Chen et al., 2022; Y. Liu, Xu, et al., 2024).

B. nakamurai secretes a lower diversity of BSMs than QST713 (Adamo et al., 2024) but still, is equally or even more efficient than the commercial strain QST713 in terms of biocontrol activity. This suggests that the BSMs-based identity card, which is generally used for screening putative biocontrol candidates, should not be the only golden criterion. This should pave the way for rethinking existing research and registration guidelines for the assessment of potential biocontrol and plant growth promotion agent candidates (Kenfaoui et al., 2024). Ultimately, they should consider the ability to adapt to fluctuating environmental parameters as one of the prime criteria (Valencia-Marin et al., 2025).

4. pH and temperature modulate BDI-IS1 antagonistic interactions

All inhabitants of a specific ecological niche sense an abiotic stress simultaneously and depending on the intrinsic adaptation ability of each member, their interaction may be impacted. By focusing on the antagonistic interactions established between BDI-IS1 (and other bacilli) and soil-dwelling phytopathogenic bacteria (putative competitors), our data revealed that environmental temperature and pH dictate the outcome of these bacterial relationships.

Chilling stress (15°C) reversed the nature of the interaction established between BDI-IS1 and three plant pathogens, namely the Gram-negative bacteria *P. carotovorum*, *P. cichorii* and *P. fuscovaginae*. The inhibitory potential of BDI-IS1 was suppressed, resulting in inhibition of the antagonist. Conversely, this cold stress enhanced the antagonistic potential of BDI-IS1 against *R. fascians* and *X. campestris*

and it has been found that it modulates the secretion of the anti-*Rhodococcus* metabolites that act in synergy with bacillaene (and/or dihydrobacillaene). Competition for space and iron was postulated as the main mechanism underlying this loss of activity, since the production of bacillibactin by BDI-IS1 is drastically reduced at 15°C (Chapter 5, section 3.3.1). All of these pathogens are also known to secrete siderophores and bioactive metabolites that may restrict the growth of outcompeted cells (Cezard et al., 2014; Ferrarini et al., 2022; Shyntum et al., 2019). To our knowledge, this is the first report of the chilling stress-induced suppression of the antagonistic activity in biocontrol agent candidates against normally susceptible bacterial pathogens. Although these data are based on *in vitro* studies and still need to be confirmed *in planta*, they demonstrate the importance of this fluctuating environmental factor to the survival and plant beneficial properties of these bioagents in the real natural conditions, where a fierce battle for space and nutrients is constantly occurring. Some rare studies on the effect of temperature on the antagonistic potential of plant beneficial bacteria have focused on antifungal activity and have shown that a decrease in temperature (from 30°C to15°C) negatively impacts the inhibitory potential of *Bacillus* spp., *Pseudomonas* spp. and *Paenibacillus* sp. against *Fusarium oxysporum* (Landa et al., 2004; Moreno-Velandia et al., 2021), while no significant effect was observed for *B. amyloliquefaciens* against *Botrytis cinerea* (15°C-30°C) (Ahlem et al., 2012).

On the other hand, the extent to which BDI-IS1 withstands acid stress (pH 4.6) was detrimental to the growth of all the tested pathogens,with only *C. michiganensis* and *P. carotovorum* able to tolerate conditions of pH 5. This implies that pH possibly exerts a form of pure natural selection on soil microbiota. The antagonistic interaction between BDI-IS1 and these two pathogens under this acidic condition (pH 5) was significantly impacted. The inhibitory effect on *C. michiganensis* decreased in intensity, whereas it increased three-fold against *P. carotovorum* compared to pH 7. This contrasting effect of low pH on antibacterial activity highlights the complexity of these inter-species interactions once again. The increase in anti-*Pectobacterium* activity is totally strange, as the main inhibitor, bacillaene (and/or dihydrobacillaene), was not accordingly secreted in higher quantities, leaving an open door to further investigation. Our data demonstrated that the growth of *P. carotovorum* was gradually reduced with the decrease of initial medium pH, but this cannot alone explain this particular increment of susceptibility. We hypothesize that the secretion of pathogen's defensive traits, such as the quorum sensing-dependent AHLs, carbapenem, and lytic enzymes, may also be compromised by the low pH, rendering the pathogen cells defenseless and more susceptible to antibiotics. Indeed, it has been shown that the induction of the virulence factors, such as the cellulolytic enzymes in *Pectobacterium*, is insignificant at pH 5 compared to the optimal pH 7 (Agyemang et al., 2020). However, adequate experiments are needed to determine the effect of BDI-IS1 extracts generated from different conditions of initial pH (pH 7- pH 5) on the viability of *Pectobacterium* cells cultured at various pH conditions (pH 7 to pH 5). This pH-driven modulation of antagonistic activity depends heavily on the ability of each bacterial competitor to cope with acidic stress. The inhibitory potential of *B. cereus*

and *P. fluorescens* against the bacterial wilt pathogen, *Ralstonia solanacearum*, was completely suppressed by acidic stress (pH 4.5 - 5.5), favouring the pathogen growth (S. Li et al., 2017).

However, the fact that most of pathogenic bacteria do not thrive at extreme low pH levels is consistent with the previous reports of low bacterial abundance compared to fungi in acidic soils, but also lower bacterial counts in such soils than what found in neutral or alkaline soils (E. Kang et al., 2021; Y. Ni et al., 2021; W. Wan et al., 2020). Although fungi are more tolerant of low pH than bacteria and often responsible for environmental acidification as a means of competitive warfare (Richter et al., 2024; Rousk et al., 2009; C. Wang & Kuzyakov, 2024a), low pH-tolerant bacilli such as *B. amyloliquefaciens* MBNC maintained the antifungal activity against *Fusarium* spp. and *A. rolfsii* at an initial medium pH 4.5 (Chowdhury et al., 2022). In this regard, the medium alkalinization strategy developed by BDI-IS1 and other plant beneficial bacilli during growth could probably contribute to the neutralisation of the fungal-derived acidification that would jeopardize their antagonistic effect (Goswami et al., 2018b). Soil alkalinisation has been reported as one of the mechanisms employed by a bacterial consortium consisting of *B. tequilensis* and *Streptomyces fradiae* to effectively reduce the incidence of the soil-borne pathogen *R. solanacearum*, which causes tomato bacterial wilt (X. Zheng et al., 2024). Further studies should evaluate the effect of low pH stress on the antifungal activity of BDI-IS1, as well as investigating the alkalinisation effect of BDI-IS1 (alone or in consortia with others) on soil in real conditions. This could serve as an interesting, eco-friendly and cost-effective alternative to liming.

This contrasting outcome of bacterial interaction in response to abiotic factors pinpoints the importance of the surrounding environment in natural ecological processes, even reshaping the existing relationships between members of the same ecological niche. This laboratory-scale study clearly illustrates how dynamic the competitive interactions between beneficial bacteria and pathogens can be in the natural world due to changes in the expression of certain abiotic parameters, particularly in the current context of climate change. It also provides a plausible explanation for the variability in the efficacy of biocontrol agents when applied in different cultural seasons and fields against the same pathogen and crop variety. For example, the protection of maize against NLB varied for about 20% in the two assays conducted in the same field but in different seasons (G. Nihorimbere, 2025). Indeed, the antagonism of *F. graminearum* by the extracts of *B. velezensis* was found to depend on the pH of the medium, with better restriction at near-neutral conditions (pH 6 – 7) (S. Wang et al., 2020). However, it woud be unwise to appreciate the impact of changing environmental conditions on the overall efficacy of bioproducts by only considering antagonistic interactions between plant beneficial microorganisms and phytopathogens. All the whole members of the holobiont are affected, including the host plant and its associated microbiome (both beneficial and pathogenic). The beneficial effect on the plant will depend on its own stress tolerance, that of its associated microbiome, and the status of their cross-interactions in such stressful conditions (Lund et al., 2020; Nadeem et al., 2023). Further research is needed to

improve our understanding of the behaviour of different plant beneficial microbes and the nature of their interspecific and interkingdom relationships (e.g. *Bacillus* with *Rhizobium*, *Pseudomonas*, *Streptomyces*, arbuscular mycorrhiza fungi (AMF), *Trichoderma*, etc.) in response to various abiotic stresses. This would facilitate the appropriate selection of biocontrol agents for specific conditions. It may also contribute to the better design of synthetic microbial communities (SynComs) or consortia with increased resilience to abiotic/biotic stresses that are capable of providing stable beneficial services to plants (Northen et al., 2024; Shayanthan et al., 2022).

5. Leveraging the plant growth promotion potential of *Bacillus nakamurai*

In addition to its ability to protect plants against diseases, genomic investigation revealed that BDI-IS1 has the genetic potential to promote plant growth and development. These *in silico* data evidenced the presence of genes associated with bacterial motility and biofilm formation, which are the prerequisites for a successful root colonization (Y. Cai et al., 2025). We demonstrated that biofilm formation is maintained under diverse stressful conditions of pH and temperature. Maize root colonisation assay with the BDI-IS1 green fluorescent protein (GFP) mutant, carried out by our colleagues from Université Catholique de Louvain (UCL), showed that this bacterium was localized to the rhizoplane and did not migrate inside the different internal plant tissues (G. Nihorimbere, 2025), constituting additional evidence for its rhizospheric pattern. Furthermore, genomic analysis revealed that the BDI-IS1 proteome comprises pectate lyases (Pel enzymes), which are devoted to the degradation of homogalacturonan-the root pectin backbone (J. Li et al., 2024). These enzymes are conserved in the strains of the *B. subtilis* complex, well-known for their root colonisation potential, and are absent from the *B. cereus* complex and they have been shown to play a crucial role in bacterial fitness and root colonization (Boubsi et al., 2023). This suggests that the *B. nakamurai* species has the potential to be considered as a root-associated bacteria. However, as previously discussed, some putative *B. nakamurai* strains have been isolated from upper plant tissues, suggesting that these strains can also be endophytes (Chaouachi et al., 2021). This indicates that the same bacterium may be capaable of colonising the entire plant, from the rhizosphere to above-ground tissues, depending on the specificities of the bacterium and plant, and their dedicated genetic capabilities (Riseh et al., 2025).

Genomic inspection also revealed that this bacterium has the potential to produce the phytohormones (auxins and cytokinins), volatile organic compounds, and polyamines, which are indirectly implicated in boosting plant growth, development, and stress tolerance by modulating the expression of plant developmental genetic traits. It also possesses all the necessary genes for the solubilization of inorganic and organic phosphates and organic nitrogen sources, which directly benefit plants as biofertilizer. A huge number of strains of the *B. subtilis* group have been reported to promote plant growth and alleviate abiotic stress through the secretion of these

hormones and/or chemicals, and by enhancing nutrient uptake (Cheng et al., 2024; W. Li et al., 2024; X. Wu et al., 2025). The phytohormones auxin (represented by indole-3-acetic acid) and cytokinin are two important hormones or signals that are involved in a plant's early developmental processes, and their supplementation from plant-associated bacteria greatly improves plant fitness. Their cross-talk is either complementary or antagonistic, depending on the developmental stage of the plant. Complentarity occurs, for example, in modulating of the early reactions that define root meristem size, with auxin enhancing cell division and cytokinins promoting cell differentiation (Navarro-Cartagena & Micol, 2023). Conversely, antagonism occurs in later developmental stages of development, with auxin promoting root development and restraining shoot growth, and while the cytokinins do the opposite (Kurepa & Smalle, 2022). These two hormones are also implicated in the plant resistance mechanisms against abiotic stresses (Kosakivska et al., 2022; Waadt et al., 2022). For instance, the positive interaction between auxins and cytokinins promotes optimal growth of *Arabidopsis* under low-temperature stress by limiting DNA impairment in root stem cells through interdependent self-regulation of their concentrations (Tiwari et al., 2023).

BDI-IS1 has also been shown to harbour genes essential for the secretion of acetoin and 2,3-butanediol, two important volatiles produced by root-associated bacilli. In addition to their putative key role as chemical messenger between the members of the same ecological niche, they have been reported to enhance the plant root development, induce plant immunity under abiotic and biotic stress, and directly inhibit the growth of soil-borne plant pathogens (Laller et al., 2023; Song et al., 2022; T. Yang et al., 2022). For example, a mixture of VOCs emitted by *B. vallismortis* EXTN-1, including 2,3-butanediol, has been shown to stimulate tobacco seedling growth via priming (Dutta et al., 2025). Another study revealed that *B. zantholaxyli*-derived VOCs play a role in improving the salt and heat tolerance of cabbage and cucumber plants by enhancing the secretion and activity of anti-oxidant enzymes (Barghi & Jung, 2024).

Polyamines, i.e. spermine, spermidine and putrescine, potentially produced by BDI-IS1, have been reported to regulate general cellular and developmental processes, such as cellular translation (Blázquez, 2025). Indeed, the spermidine-derived hypusine is a substrate in the post-translational modification of the eukaryotic translation factor 5A (eIF5A), a process known as hypusination. This process is crucial for normal plant development and in stress-related responses (Pálfi et al., 2021). Polyamines have also been shown to modulate seed germination, flowering and fruiting, boosting these processes as demonstrated in *Arabidopsis* and rice. Conversely, plant senescence has been found to be delayed by the addition of exogenous polyamines (Blázquez, 2025). Polyamines also play an important role in helping plants to mitigate various abiotic stresses, including oxidative stress, drought, UV stress, hypoxia, heavy metals stress, salinity and cold stress (Maurya et al., 2025), and their cytosolic pool is usually elevated in stressed plants (Tyagi et al., 2023). In the case of oxidative stress, for example, it has been suggested that polyamines act either directly as ROS scavengers or indirectly by inducing strong expression of the plant's innate anti-oxidant systems, thereby maintaining ROS pool at beneficial levels (Kaur & Das, 2023; P. Wang et al.,

2024). *Bacillus megaterium* polyamines production has been shown to enhance drought stress tolorance in *Arabidopsis* plants by improving endogenous spermidine and abscisic acid levels compared to control plants (Zhou et al., 2016). Furthermore, polyamines have been reported to play a role in establishing the symbiosis between rhizobia and legumes, where bacterial polyamines act as recognition patterns for the plant symbiont and plant polyamines generate H_2O_2 and NO, which are detected by rhizobia as signals enabling the early stages of symbiosis establishment (Hidalgo-Castellanos et al., 2022).

Bacillus-based plant biofertilisation relies on the intrinsic ability of many rhizospheric bacilli to sequester from soil and inorganic or organic inputs key plant nutritional elements such as phosphorus (P), nitrogen (N) and potassium (K). BDI-IS1 and other bacilli have the potential to secrete urease (B. Roy et al., 2024; Sreekala et al., 2025). Urease aids in the biodegradation of excess of amended urea into NH_4^+ and CO_2, as part of general nitrogen recycling, and as a strategy to limit urea-driven soil toxicity, susceptible to cause ecological disturbances (Coelho et al., 2025; Mekonnen et al., 2021; Motasim et al., 2024). BDI-IS1 possesses the genetic potential to solubilise insoluble phosphorus sources, either organic (phytate) via enzymatic digestion or inorganic via enzymatic and organic acids-aided solubilisation. This trait is important for promoting plant growth, as phosphates are critical to life processes and must be provided externally. *Bacillus* strains and other PGPR have been extensively investigated for this property and have been shown to clearly improve phosphorus uptake and thus an enhance plant growth (Bakki et al., 2024; Z. Iqbal et al., 2024; D. Shao et al., 2025). The genetic potential of BDI-IS1 to aid plants in the uptake of these key nutrient elements, stimulate plant growth and development, and improve tolerance to abiotic stresses underscores the need to evaluate the significance of these properties *in planta* (Abdelkefi et al., 2024; Siddika et al., 2024).

6. Towards a new biocontrol product based on *Bacillus nakamurai* BDI-IS1

BDI-IS1 has proven its antagonistic potential *in vitro* against an array of phytopathogens and demonstrated its biocontrol efficacy against three plant diseases of economic importance in Burundi, i.e. tomato early blight, maize nothern leaf blight and bean angular leaf disease. Although the different bioassays relied on the utilization of bacterial suspensions, large-scale production (as bacterial suspension) is not feasible in Burundi and many developing countries due to socio-economic constraints. The biotechnological processes required for this purpose are expensive and necessitate stable and permanent electricity and maintenance, which cannot be guaranteed in these countries. Inspired by the solid-state fermentation strategy utilising lignocellulosic agricultural residues as substrates for growth of fungal biocontrol agents (Duré et al., 2025), we attempted to multiply the bacterium and prepare a BDI-IS1-based formulated product suitable for the local context in Burundi. BDI-IS1 grew well and produced high endospore counts (10^{13} CFU/g) on inexpensive and readily available agricultural residues from three major cultivated crops, including

bean haulms (BH), rice straw (RS) and maize cobs (MC). The resulting fermented bacterized substrates were subsequently dried to produce a powdered product consisting of residual solid substrate mixed with BDI-IS1 endospores and secreted bioactive metabolites. The BDI-IS-based bioproducts formulated using the three substrates i.e. BH, RS and MC, respectively, provided significant protection (60-67%) to maize plants against NLB in pot experiments. An open field experiment using the BH-based product provided 26% protection to maize against NLB (Tasiaux, 2024). Once again, this BDI-IS1-based product exhibited better disease reduction than the QST713-based product prepared under the same conditions (Figure 6-3), confirming the previously obtained results exalting the biocontrol potential of BDI-IS1 over QST713. In addition to its low production cost, this product offers practical advantages such as ease of use for the end-user, who is often illiterate. The application of the bioproduct mimics the familiar method used by farmers for applying manure or compost, which consists of depositing the bioproduct/compost in the seedbed before sowing the seed.

Nevertheless, studies evaluating bioproduct stability over time (shelf-life), the quality between batches due to variable physico-chemical properties of used lignocellulosic substrates (seasonal and regional disparities) and efficacy in other pathosystems have yet to be undertaken. This bioproduct based on a locally adapted strain and that relies on available lignocellulosic substrates, has the potential to revolutionize agricultural practices in Burundi and other tropical countries. It also represents a significant contribution to the management of organic solid waste, which is chaotic in these countries (Ntagisanimana et al., 2021; Z. Zhang et al., 2024). These agricultural residues constitute the main part (69%) of the municipal solid waste produced in Burundi and can thereby pollute the atmosphere by emitting greenhouse gases and aquatic systems by infiltration or runoff from uncontrolled landfill sites (N. Manirakiza et al., 2023; Ndikumana et al., 2024). This approach of recycling agricultural by-products, from waste-to-value concept, is key to the circular economy, ensuring a sustainable agricultural system with minimised production costs, waste generation, and thus reducing ecosystem pollution (F. Haque et al., 2023; Velasco-Muñoz et al., 2022).

This BDI-IS1-based biocontrol product is therefore an innovative tool that can contribute simultaneously to the mitigation of two public health issues related to agriculture and waste management, thus helping the attainment of the UN Sustainable Development Goals. Burundi and many other tropical developing countries face the problems of high disease prevalence, limited purchasing power for proposed chemical control methods and the toxicity of the main utilized pesticides (e.g. mancozeb, imidacloprid) (G. Nihorimbere et al., 2024). The increase in cancer cases in recent years (Jimbere, 2019; Niyongabo, 2023), even if not yet reported, may be a consequence of mismanagement in the pesticide sector. Local officials should therefore regulary review the list of commercialized pesticides, banning toxic ones and promoting the use of safe and eco-friendly biocontrol agents in line with the holistic approach of integrated pest management. The international community should

also take restrictive measures against the deliberate production and commercialisation of hazardous pesticides (that are banned in the EU) in the third-world countries.

However, a well-organised framework is needed to ensure the widespread dissemination of this product and its efficient use by the rural farmers (Figure 7-1). At national level, the supervising body of the entire structure would be a governmental institution involved in agricultural research, such as ISABU (Institut des Sciences Agronomiques du Burundi). This institution would seek for the bioproduct's registration by competent authorities, ensure the proper storage of the bacterium and preparation of the starting inoculum in sterile conditions. This inoculum would then be sold at an affordable price to local farmers' cooperatives. Each cooperative should have a well-trained unit by researchers of ISABU in how to carry out large-scale multiplication of the bacterium (solid-state fermentation), formulation and packaging of the product. Rural smallholder farmers would then purchase the bioproduct from their cooperatives at an advantageous price. These farmers must be accompanied by the trained unit to ensure the proper use of the product, and the unit must provide reports to ISABU about any complaints from users. This would facilitate quality control throughout the process and enable the prompt addressing of raised issues.

The *B. thuringiensis*-based bioinsecticide is the only biological product registered in Burundi, and efforts should be made to register new biofungicides such as the formulated BDI-IS1-based product, which has proven its effectiveness in local pathosystems. This would help to reduce the use of dangerous pesticides, such as mancozeb, for which the residues are found daily on tomato fruits in the markets of Bujumbura (Figure 1-1). Furthermore, further works should focus on the prospecting of muti-purpose plant beneficial bacteria that combine both insecticidal, nematicidal, fungicidal, antibacterial and growth promoting properties. Often, pathogens and pests affect crops simultaneously, with one acting as a vector or spreader for the other, as is the case with viruses and some pathogens (Masoudi et al., 2024; Yigezu Wendimu & Kassaye Gurmu, 2024). Interestingly, BDI-IS1 could probably be active against plant pests, in addition to the biocontrol potential against phytopathogens and plausible plant growth promotion virtues. Preliminary assays showed interesting insecticidal activity against the fall army warm *Spodoptera frugiperda* (https://youtu.be/0_s6-lI70HM), a devastating pest of maize plantations and other cereals. In fact, a honeybee gut-derived isolate, which was putatively identified as a *B. nakamurai* strain, was found to encode the Cry1 and Cry3 toxin genes (Usta, 2021). Additionally, moderate nematicidal activity has been observed with plantazolicin, a lanthipeptide produced by *B. nakamurai* strains and other related *Bacillus* species (Scholz et al., 2011). Further investigations are needed to better evaluate this important insecticidal potential. For example, PCR amplication of different *Cry* genes inside the DNA of BDI-IS1 would help to fast-screen the presence of these genes and *in vivo* laboratory testing should then follow to assess the spectrum and intensity of insecticidal activity against pests of agricultural importance (Murphy et al., 2025).

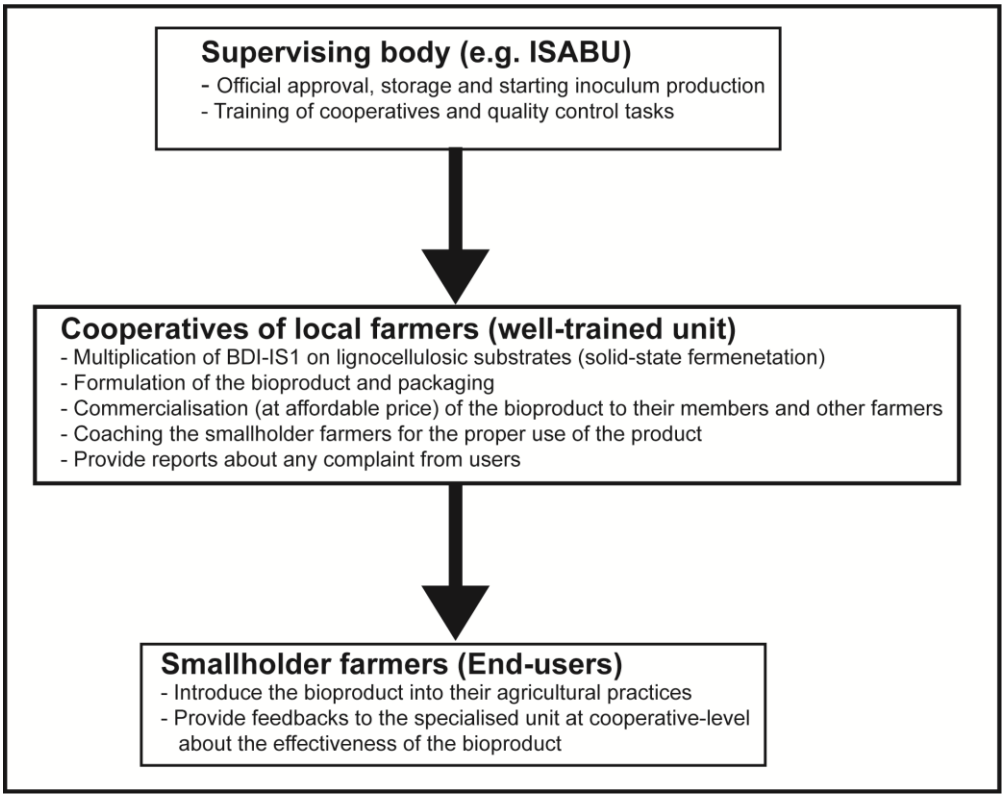


Figure 7-1: Schematic overview of the framework for the wide dissemination of BDI-IS1-based bioproduct

General conclusion

In this work, we prospected for a new plant beneficial *Bacillus*-based product adapted to the contrasting abiotic conditions prevailing in Burundi and many tropical countries, and which would meet the need of local agriculture. Developing countries in tropical regions are prone to high incidence of plant diseases, and the limited proposed solutions rely on highly toxic pesticides, which exacerbate existing health concerns. In order to contribute for the search for safe and eco-friendly alternatives, *Bacillus nakamurai* BDI-IS1 was isolated from Burundian soil samples. It demonstrated, *in vitro*, an excellent antagonistic activity against many bacterial and fungal pathogens of worldwide agricultural relevance, mediated by some of its soluble secreted BSMs. Its biocontrol efficacy was proven *in planta* against tomato early blight and northern corn leaf blight, two threatening plant diseases in Burundi and tropical regions, caused by the pathogens *A. solani* and *E. turcicum*, respectively. Its effectiveness was either by foliar application or by root application, suggesting two modes of action including antibiosis and systemic resistance induction (ISR), respectively. Through *in vitro* studies, BDI-IS1 exhibited a consistent tolerance to low pH and temperature conditions typical to many tropical farmlands. However, these stresses were shown to modulate its antagonistic activities against bacterial pathogens, either increasing or reducing its inhibition level depending on the pathogen. BDI-IS1 exhibited comparable or better performance than QST713 in terms of biocontrol and adaptation to abiotic stresses properties. Additionally, BDI-IS1 possesses the genetic ability to promote plant growth and development, as well as alleviate plant abiotic stress. Solid-state fermentation using locally sourced lignocellulosic substrates derived from agricultural residues was found to be a promising way of multiplying the bacterium, offering an easy and inexpensive method of biomass production and further formulation. A simple formulated product, consisting of a dry mixture of BDI-IS1 spores and secreted metabolites embedded within the residual fermented substrates, was proposed and found to be more effective than QST713-based product in protecting maize against northern leaf blight disease. Consequently, this BDI-IS1-based bioproduct represents an innovative tool for enabling more productive agriculture that is respectful of human health and the environment, as well as improving local organic waste management. Nevertheless, additional experimental assays are still needed to ensure its readiness for public use. Firstly, these include fundamental studies aimed at assessing the innocuity of the bacterium towards other plant beneficial microorganisms sharing the same ecological niche (e.g. *Rhizobium*, *Pseudomonas*, arbuscular mycorrhiza fungi (AMF) and entomopathogenic fungi), as well as evaluating *in planta* its antagonistic and biocontrol fate under abiotic stress conditions in light of the *in vitro* findings. Secondly, the stability of the formulated bioproduct should be assessed throughout the different batches (depending on the substrate quality) and during storage (shelf-life). The bioproduct should also be evaluated for its overall efficacy in other pathosystems. This would pave the way for further administrative formalities regarding its registration and the establishment of a framework ensuring its widespread dissemination.

References

Abban, S., Brimer, L., Abdelgadir, W. S., Jakobsen, M., & Thorsen, L. (2013). Screening for *Bacillus subtilis* group isolates that degrade cyanogens at pH4.5-5.0. *International Journal of Food Microbiology*, *161*(1), 31–35. https://doi.org/10.1016/j.ijfoodmicro.2012.11.019

Abdelaziz, A. M., Hashem, A. H., El-Sayyad, G. S., El-Wakil, D. A., Selim, S., Alkhalifah, D. H. M., & Attia, M. S. (2023). Biocontrol of soil borne diseases by plant growth promoting rhizobacteria. *Tropical Plant Pathology*, *48*(2), 105–127. https://doi.org/10.1007/s40858-022-00544-7

Abdelmoteleb, A., Moreno-Ramírez, L., Valdez-Salas, B., Seleiman, M. F., El-Hendawy, S., Aldhuwaib, K. J., Alotaibi, M., & González-Mendoza, D. (2023). New *Bacillus subtilis* strains isolated from *Prosopis glandulosa* rhizosphere for suppressing *Fusarium* spp. and enhancing growth of *Gossypium hirsutum* L. *Biology*, *12*(1), 73. https://doi.org/10.3390/biology12010073

Abd-Elsalam, K. A., & Mohamed, H. I. (2023). Synthesis and application of bacterial secondary metabolites in agroecosystems: A note from the editors. In K. A. Abd-Elsalam & H. I. Mohamed (Eds.), *Bacterial secondary metabolites: Synthesis and applications in agroecosystem* (pp. 1–14). Elsevier. https://doi.org/10.1016/B978-0-323-95251-4.00020-X

Abhinay, M., & Pan, I. (2023). Growth analysis of bacteria when subjected to ammonium salts ammonium persulphate, ammonium sulfate, ammonium ferrous sulfate as nitrogen sources by measuring optical density. *AIP Conference Proceedings*, *2587*(1), 140022. https://doi.org/10.1063/5.0150925

Adamo, I., Acin-Albiac, M., Röttjers, S., de Prado, D. R., Benito, B. M., Zamora, J., Godara, R., García-Jiménez, B., Jiang-Rempel, P., Cline, L. C., & Acedo, A. (2024). Short impact on soil microbiome of a *Bacillus amyloliquefaciens* QST713 based product that correlates with higher potato yield across USA. *Frontiers in Plant Science*, *15*, 1332840. https://doi.org/10.3389/fpls.2024.1332840

Adams, I. P., Harju, V. A., Hodges, T., Hany, U., Skelton, A., Rai, S., Deka, M. K., Smith, J., Fox, A., Uzayisenga, B., Ngaboyisonga, C., Uwumukiza, B., Rutikanga, A., Rutherford, M., Ricthis, B., Phiri, N., & Boonham, N. (2014). First report of maize lethal necrosis disease in Rwanda. *New Disease Reports*, *29*(1), 22–22. https://doi.org/10.5197/j.2044-0588.2014.029.022

Afridi, M. S., Fakhar, A., Kumar, A., Ali, S., Medeiros, F. H. V., Muneer, M. A., Ali, H., & Saleem, M. (2022). Harnessing microbial multitrophic interactions for rhizosphere microbiome engineering. *Microbiological Research*, *265*, 127199. https://doi.org/10.1016/j.micres.2022.127199

Agyemang, P. A., Kabir, M. N., Kersey, C. M., & Dumenyo, C. K. (2020). The bacterial soft rot pathogens, pectobacterium carotovorum and p. Atrosepticum, respond to different classes of virulence-inducing host chemical signals. *Horticulturae*, *6*(1). https://doi.org/10.3390/horticulturae6010013

Ahlem, H., Mohammed, E., Badoc, A., & Ahmed, L. (2012). Effect of pH, temperature and water activity on the inhibition of *Botrytis cinerea* by *Bacillus amyloliquefaciens* isolates. *African Journal of Biotechnology*, *11*(9), 2210–2217. https://doi.org/10.5897/ajb11.645

Ahmed Abokoura, H., & Saad, M. M. (2019). Isolation and identification of N-fixing, phosphate and potassium solubilizing rhizobacteria and their effect on root colonization of wheat plant. *International Journal of Microbiological Research*, *10*(2), 62–76. https://doi.org/10.5829/idosi.ijmr.2019.62.76

Ajouz, S., Walker, A. S., Fabre, F., Leroux, P., Nicot, P. C., & Bardin, M. (2011). Variability of *Botrytis cinerea* sensitivity to pyrrolnitrin, an antibiotic produced by biological control agents. *BioControl*, *56*, 353–363. https://doi.org/10.1007/s10526-010-9333-7

Ajuna, H. B., Lim, H. I., Moon, J. H., Won, S. J., Choub, V., Choi, S. I., Yun, J. Y., & Ahn, Y. S. (2023). The Prospect of hydrolytic enzymes from *Bacillus* species in the biological control of pests and diseases in forest and fruit tree production. *International Journal of Molecular Sciences*, *24*, 16889. https://doi.org/10.3390/ijms242316889

Ajuna, H. B., Lim, H. I., Moon, J. H., Won, S. J., Choub, V., Choi, S. I., Yun, J. Y., & Ahn, Y. S. (2024). The prospect of antimicrobial peptides from *Bacillus* species with biological control potential against insect pests and diseases of economic importance in agriculture, forestry and fruit tree production. *Biotechnology and Biotechnological Equipment*, *38*(1), 2312115. https://doi.org/10.1080/13102818.2024.2312115

Akinsemolu, A. A., Onyeaka, H., Odion, S., & Adebanjo, I. (2024). Exploring *Bacillus subtilis*: Ecology, biotechnological applications, and future prospects. *Journal of Basic Microbiology*, *64*, 2300614. https://doi.org/10.1002/jobm.202300614

Alajlani, M. M. (2022). Characterization of subtilosin gene in wild type *Bacillus* spp. and possible physiological role. *Scientific Reports*, *12*, 10521. https://doi.org/10.1038/s41598-022-13804-y

Alcock, B. P., Huynh, W., Chalil, R., Smith, K. W., Raphenya, A. R., Wlodarski, M. A., Edalatmand, A., Petkau, A., Syed, S. A., Tsang, K. K., Baker, S. J. C., Dave, M., Mccarthy, M. C., Mukiri, K. M., Nasir, J. A., Golbon, B., Imtiaz, H., Jiang, X., Kaur, K., … Mcarthur, A. G. (2023). CARD 2023: Expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. *Nucleic Acids Research*, *51*, D690–D699. https://doi.org/10.1093/nar/gkac920

Aleti, G., Sessitsch, A., & Brader, G. (2015). Genome mining: Prediction of lipopeptides and polyketides from *Bacillus* and related Firmicutes. *Computational and Structural Biotechnology Journal*, *13*, 192–203. https://doi.org/10.1016/j.csbj.2015.03.003

Ali, A., Ahmed, T., Ibrahim, E., Rizwan, M., Chong, K. P., & Yong, J. W. H. (2024). A review on mechanisms and prospects of endophytic bacteria in biocontrol of plant pathogenic fungi and their plant growth-promoting activities. *Heliyon*, *10*(11). https://doi.org/10.1016/j.heliyon.2024.e31573

Ali, N., Pang, Z., Wang, F., Xu, B., & El-Seedi, H. R. (2022). Lipopeptide biosurfactants from *Bacillus* spp.: Types, production, biological activities, and applications in food. *Journal of Food Quality*, *2022*, 3930112. https://doi.org/10.1155/2022/3930112

Ali, S. A. M., Sayyed, R. Z., Mir, M. I., Khan, M. Y., Hameeda, B., Alkhanani, M. F., Haque, S., Mohammad Al Tawaha, A. R., & Poczai, P. (2022). Induction of systemic resistance in maize and antibiofilm activity of surfactin from *Bacillus velezensis* MS20. *Frontiers in Microbiology*, *13*, 879739. https://doi.org/10.3389/fmicb.2022.879739

Allagheny, N., Obanu, Z. A., Campbell-Platt ', G., & Owens, J. D. (1996). Control of ammonia formation during *Bacillus subtilis* fermentation of legumes. *International Journal of Food Microbiology*, *29*(2–3), 32–333. https://doi.org/10.1016/0168-1605(95)00069-0

Almasoudi, N. M., Al-Qurashi, A. D., Elsayed, M. I., & Abo-Elyousr, K. A. M. (2024). Native bacterial bioagents for management of potato soft rot disease caused by *Pectobacterium carotovorum* subsp. *carotovorum*. *Egyptian Journal of Biological Pest Control*, *34*, 31. https://doi.org/10.1186/s41938-024-00794-4

Almeida, O. A. C., de Araujo, N. O., Mulato, A. T. N., Persinoti, G. F., Sforça, M. L., Calderan-Rodrigues, M. J., & Oliveira, J. V. de C. (2023). Bacterial volatile organic compounds (VOCs) promote growth and induce metabolic changes in rice. *Frontiers in Plant Science*, *13*, 1056082. https://doi.org/10.3389/fpls.2022.1056082

Al-Mutar, D. M. K., Noman, M., Alzawar, N. S. A., Qasim, H. H., Li, D., & Song, F. (2023). The extracellular lipopeptides and volatile organic compounds of *Bacillus subtilis* DHA41 display broad-spectrum antifungal activity against soil-borne phytopathogenic fungi. *Journal of Fungi*, *9*, 797. https://doi.org/10.3390/jof9080797

Aloo, B. N., Tripathi, V., Makumba, B. A., & Mbega, E. R. (2022). Plant growth-promoting rhizobacterial biofertilizers for crop production: The past, present, and future. *Frontiers in Plant Science*, *13*, 1002448. https://doi.org/10.3389/fpls.2022.1002448

Altaf, M. M., Ahmad, I., Khan, M. S. A., & Grohmann, E. (2017). *Bacillus* biofilms and their role in plant health. In I. Ahmad & M. F. Husain (Eds.), *Biofilms in plant and soil health* (1st ed., pp. 55–67). John Wiley & Sons. https://doi.org/10.1002/9781119246329.ch4

Ambrico, A., & Trupo, M. (2017). Efficacy of cell free supernatant from *Bacillus subtilis* ET-1, an iturin A producer strain, on biocontrol of green and gray mold. *Postharvest Biology and Technology*, *134*(2017), 5–10. https://doi.org/10.1016/j.postharvbio.2017.08.001

Amiri, H., Banakar, M. H., & Hemmati Hassan Gavyar, P. (2024). Polyamines: New plant growth regulators promoting salt stress tolerance in plants. *Journal of Plant Growth Regulation*, *43*, 4923–4940. https://doi.org/10.1007/s00344-024-11447-z

Anastassiadou, M., Arena, M., Auteri, D., Brancato, A., Bura, L., Carrasco Cabrera, L., Chaideftou, E., Chiusolo, A., Crivellente, F., De Lentdecker, C., Egsmose, M., Fait, G., Greco, L., Ippolito, A., Istace, F., Jarrah, S., Kardassi, D., Leuschner, R., Lostia, A., … Villamar-Bouza, L. (2021). Peer review of the pesticide risk assessment of

the active substance *Bacillus amyloliquefaciens* strain QST 713 (formerly *Bacillus subtilis* strain QST 713). *EFSA Journal*, *19*(1), 6381. https://doi.org/10.2903/j.efsa.2021.6381

Anckaert, A., Arias, A. A., Hoff, G., Calonne-Salmon, M., Declerck, S., & Ongena, M. (2021). The use of *Bacillus* spp. as bacterial biocontrol agents to control plant diseases. In J. Köhl & R. Willem (Eds.), *Microbial bioprotectants for plant disease management* (1st Edition, pp. 247–300). Burleigh Dodds Science Publishing. https://doi.org/10.19103/AS.2021.0093.10

Andrić, S., Rigolet, A., Argüelles Arias, A., Steels, S., Hoff, G., Balleux, G., Ongena, L., Höfte, M., Meyer, T., & Ongena, M. (2023). Plant-associated *Bacillus* mobilizes its secondary metabolites upon perception of the siderophore pyochelin produced by a *Pseudomonas* competitor. *ISME Journal*, *17*(2), 263–275. https://doi.org/10.1038/s41396-022-01337-1

Anita, Mathur, N., & Shekhawat, G. S. (2024). Hemin in Plants: Biosynthesis and role in ROS detoxification during oxidative stress. In M. Faizan & S. Hayat (Eds.), *Plant growth regulators: Resilience for sustainable agriculture* (pp. 135–146). Springer Nature. https://doi.org/10.1007/978-981-97-2918-0_8

Ansari, M. M., Bisht, N., Singh, T., & Chauhan, P. S. (2024). Symphony of survival: Insights into cross-talk mechanisms in plants, bacteria, and fungi for strengthening plant immune responses. *Microbiological Research*, *285*, 127762. https://doi.org/10.1016/j.micres.2024.127762

Antoshina, D. V., Balandin, S. V., Tagaev, A. A., Potemkina, A. A., & Ovchinnikova, T. V. (2024). Biotechnological production of the recombinant two-component lantibiotic lichenicidin in a bacterial expression system. *Russian Journal of Bioorganic Chemistry*, *50*(4), 1150–1161. https://doi.org/10.1134/S1068162024040459

Anzaldi, L. L., & Skaar, E. P. (2010). Overcoming the heme paradox: Heme toxicity and tolerance in bacterial pathogens. *Infection and Immunity*, *78*(12), 4977–4989. https://doi.org/10.1128/IAI.00613-10

ARECO-Rwanda Nziza. (2020). *Highly hazardous pesticides (HHPs): Rwanda situation report*. https://ipen.org/documents/highly-hazardous-pesticides-rwanda-situation-report

Arguelles Arias, A., Ongena, M., Devreese, B., Terrak, M., Joris, B., & Fickers, P. (2013). Characterization of amylolysin, a novel lantibiotic from *Bacillus amyloliquefaciens* GA1. *PLoS ONE*, *8*(12), e83037. https://doi.org/10.1371/journal.pone.0083037

Arguelles-Arias, A., Ongena, M., Halimi, B., Lara, Y., Brans, A., Joris, B., & Fickers, P. (2009). *Bacillus amyloliquefaciens* GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens. *Microbial Cell Factories*, *8*, 1–12. https://doi.org/10.1186/1475-2859-8-63

Arkhipov, A., Carvalhais, L. C., & Schenk, P. M. (2023). PGPR control *Phytophthora capsici* in tomato through induced systemic resistance, early hypersensitive response

and direct antagonism in a cultivar-specific manner. *European Journal of Plant Pathology*, *167*, 811–832. https://doi.org/10.1007/s10658-023-02734-8

Arkhipova, T. N., Veselov, S. Y., Melent'ev, A. I., Martynenko, E. V., & Kudoyarova, G. R. (2006). Comparison of effects of bacterial strains differing in their ability to synthesize cytokinins on growth and cytokinin content in wheat plants. *Russian Journal of Plant Physiology*, *53*(4), 507–513. https://doi.org/10.1134/S1021443706040121

Arnaouteli, S., Bamford, N. C., Stanley-Wall, N. R., & Kovács, Á. T. (2021). *Bacillus subtilis* biofilm formation and social interactions. *Nature Reviews Microbiology*, *19*(9), 600–614. https://doi.org/10.1038/s41579-021-00540-9

Asari, S., Tarkowská, D., Rolčík, J., Novák, O., Palmero, D. V., Bejai, S., & Meijer, J. (2017). Analysis of plant growth-promoting properties of *Bacillus amyloliquefaciens* UCMB5113 using *Arabidopsis thaliana* as host plant. *Planta*, *245*(1), 15–30. https://doi.org/10.1007/s00425-016-2580-9

Assié, L. K., Deleu, M., Arnaud, L., Paquot, M., Thonart, P., Gaspar, C., & Haubruge, E. (2002). Insecticide activity of surfactins and iturins from a biopesticide *Bacillus subtilis* Cohn (S499 strain). *Mededelingen van de Faculteit Landbouwkundige En Toegepaste Biologische Wetenschappen*, *67*(3), 647–655.

Awan, Z. A., Shoaib, A., Schenk, P. M., Ahmad, A., Alansi, S., & Paray, B. A. (2023). Antifungal potential of volatiles produced by *Bacillus subtilis* BS-01 against *Alternaria solani* in *Solanum lycopersicum*. *Frontiers in Plant Science*, *13*, 1089562. https://doi.org/10.3389/fpls.2022.1089562

Ayaz, M., Li, C. H., Ali, Q., Zhao, W., Chi, Y. K., Shafiq, M., Ali, F., Yu, X. Y., Yu, Q., Zhao, J. T., Yu, J. W., Qi, R. De, & Huang, W. K. (2023). Bacterial and fungal biocontrol agents for plant disease protection: Journey from lab to field, current status, challenges, and global perspectives. *Molecules*, *28*(18), 6735. https://doi.org/10.3390/molecules28186735

Aziz, R. K., Bartels, D., Best, A., DeJongh, M., Disz, T., Edwards, R. A., Formsma, K., Gerdes, S., Glass, E. M., Kubal, M., Meyer, F., Olsen, G. J., Olson, R., Osterman, A. L., Overbeek, R. A., McNeil, L. K., Paarmann, D., Paczian, T., Parrello, B., … Zagnitko, O. (2008). The RAST Server: Rapid annotations using subsystems technology. *BMC Genomics*, *9*, 75. https://doi.org/10.1186/1471-2164-9-75

Azulay, N. D., Spaeker, O., Ghrayeb, M., Wilsch-Bräuninger, M., Scoppola, E., Burghammer, M., Zizak, I., Bertinetti, L., Politi, Y., & Chai, L. (2022). Multiscale X-ray study of *Bacillus subtilis* biofilms reveals interlinked structural hierarchy and elemental heterogeneity. *PNAS*, *119*(4), e2118107119. https://doi.org/10.1073/pnas.2118107119/-/DCSupplemental

Babasaki, K., Takao, T., Shimonishi, Y., & Kurahashi, K. (1985). Subtilosin A, a new antibiotic peptide produced by *Bacillus subtilis* 168: Isolation, structural analysis, and biogenesis. *J. Biochem*, *98*(3), 585–603. https://doi.org/10.1093/oxfordjournals.jbchem.a135315

Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., & Smith, D. L. (2018). Plant growth-promoting rhizobacteria:

Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Frontiers in Plant Science*, *9*, 1473. https://doi.org/10.3389/fpls.2018.01473

Báez-Astorga, P. A., Cázares-Álvarez, J. E., Cruz-Mendívil, A., Quiroz-Figueroa, F. R., Sánchez-Valle, V. I., & Maldonado-Mendoza, I. E. (2022). Molecular and biochemical characterisation of antagonistic mechanisms of the biocontrol agent *Bacillus cereus* B25 inhibiting the growth of the phytopathogen *Fusarium verticillioides* P03 during their direct interaction *in vitro*. *Biocontrol Science and Technology*, *32*(9), 1074–1094. https://doi.org/10.1080/09583157.2022.2085662

Bakki, M., Banane, B., Marhane, O., Esmaeel, Q., Hatimi, A., Barka, E. A., Azim, K., & Bouizgarne, B. (2024). Phosphate solubilizing *Pseudomonas* and *Bacillus* combined with rock phosphates promoting tomato growth and reducing bacterial canker disease. *Frontiers in Microbiology*, *15*, 1289466. https://doi.org/10.3389/fmicb.2024.1289466

Balasha, A. M., Mulume, D. A., Mwisha, S. W., Fyama, J. N. M., & Kalumbu, J. T. (2023). Utilisation des pesticides en cultures maraîchères sur l'île d'Idjwi à l'Est de la République Démocratique du Congo : Connaissances et pratiques des agriculteurs. *Cahiers Agricultures*, *32*, 5. https://doi.org/10.1051/CAGRI/2022033

Balleux, G., Höfte, M., Arguelles-Arias, A., Deleu, M., & Ongena, M. (2024). *Bacillus* lipopeptides as key players in rhizosphere chemical ecology. *Trends in Microbiology*, *33*(1), 80–95. https://doi.org/10.1016/j.tim.2024.08.001

Banerjee, S., & Hansen, J. N. (1988). Structure and expression of a gene encoding the precursor of subtilin, a small protein antibiotic. *The Journal of Biological Chemistry*, *263*(19), 9508–9514.

Bararyenya, A., Nahayo, P. C., Nduwimana, A., Niyonzima, P., Nyawakira, D., Sindihebura, J.-P., Vyizigiro, E., Ndayishimiye, V., & Haverkort, J. A. (2018). Assessment of opportunities for Burundian small-scale potato farmers to increase productivity and income. *Potato Research*, *61*, 73–88. https://doi.org/10.1007/s11540-018-9359-2

Barbosa, J. C., Caetano, T., & Mendo, S. (2015). Class I and Class II lanthipeptides produced by *Bacillus* spp. *Journal of Natural Products*, *78*(11), 2850–2866. https://doi.org/10.1021/np500424y

Barbosa, J. C., Silva, Í. C., Caetano, T., Mösker, E., Seidel, M., Lourenço, J., Süssmuth, R. D., Santos, N. C., Gonçalves, S., & Mendo, S. (2022). Assessing the potential of the two-peptide lantibiotic lichenicidin as a new generation antimicrobial. *World Journal of Microbiology and Biotechnology*, *38*, 18. https://doi.org/10.1007/s11274-021-03196-y

Barger, S. R., Hoefler, B. C., Cubillos-Ruiz, A., Russell, W. K., Russell, D. H., & Straight, P. D. (2012). Imaging secondary metabolism of *Streptomyces* sp. Mg1 during cellular lysis and colony degradation of competing *Bacillus subtilis*. *Antonie van Leeuwenhoek*, *102*, 435–445. https://doi.org/10.1007/s10482-012-9769-0

Barghi, A., & Jung, H. W. (2024). Insights into *Bacillus zanthoxyli* HS1-mediated systemic tolerance: multifunctional implications for enhanced plant tolerance to

abiotic stresses. *Physiologia Plantarum*, *176*(4), e14458. https://doi.org/10.1111/ppl.14458

Barrera-Ortiz, S., Balderas-Ruíz, K. A., López-Bucio, J. S., López-Bucio, J., Flores, C., Galindo, E., Serrano-Carreón, L., & Guevara-García, Á. A. (2023). A *Bacillus velezensis* strain improves growth and root system development in *Arabidopsis thaliana* through cytokinin signaling. *Rhizosphere*, *28*, 100815. https://doi.org/10.1016/j.rhisph.2023.100815

Bassily, N. (2008, January). Burundi: Toxic pesticides threaten health and environment. *Syfia Grands Lacs*. https://wire.farmradio.fm/farmer-stories/3-burundi-toxic-pesticides-threaten-health-and-environment-syfia-grands-lacs/

Basu, A., Prasad, P., Das, S. N., Kalam, S., Sayyed, R. Z., Reddy, M. S., & Enshasy, H. El. (2021). Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: Recent developments, constraints, and prospects. *Sustainability*, *13*, 1140. https://doi.org/10.3390/su13031140

Bayebila, M. T., Dufour, P., Pirard, C., Nsangu, J., Mufusama, J. P., Mbinze, K. J., Marini, D. R., & Charlier, C. (2021). Bio-surveillance of environmental pollutants in the population of Kinshasa, Democratic Republic of Congo (DRC): A small pilot study. *Archives of Public Health*, *79*(1), 1–15. https://doi.org/10.1186/s13690-021-00717-x

Begley, M., Cotter, P. D., Hill, C., & Ross, R. P. (2009). Identification of a novel two-peptide lantibiotic, lichenicidin, following rational genome mining for LanM proteins. *Applied and Environmental Microbiology*, *75*(17), 5451–5460. https://doi.org/10.1128/AEM.00730-09

Behan-Bush, R. M., Liszewski, J. N., Schrodt, M. V., Vats, B., Li, X., Lehmler, H. J., Klingelhutz, A. J., & Ankrum, J. A. (2023). Toxicity impacts on human adipose mesenchymal stem/stromal cells acutely exposed to aroclor and non-aroclor mixtures of polychlorinated biphenyl. *Environmental Science and Technology*, *57*(4), 1731–1742. https://doi.org/10.1021/acs.est.2c07281

Belete, T., Kurtulus Bastas, K., Francesconi, S., & Balestra, G. M. (2021). Biological effectiveness of *Bacillus subtilis* on common bean bacterial blight. *Journal of Plant Pathology*, *103*, 249–258. https://doi.org/10.1007/s42161-020-00727-8/Published

Belga. (2020, February 27). La RDC à son tour touchée par les criquets ravageurs, une première depuis 1944. *RTBF*. https://www.rtbf.be/article/la-rdc-a-son-tour-touchee-par-les-criquets-ravageurs-une-premiere-depuis-1944-10443038

Beneduzi, A., Ambrosini, A., & Passaglia, L. M. P. (2012). Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. *Genetics and Molecular Biology*, *35*(4), 1044–1051. https://doi.org/10.1590/S1415-47572012000600020

Berikashvili, V., Sokhadze, K., Kachlishvili, E., Elisashvili, V., & Chikindas, M. L. (2018). *Bacillus amyloliquefaciens* spore production under solid-state fermentation of lignocellulosic residues. *Probiotics and Antimicrobial Proteins*, *10*(4), 755–761. https://doi.org/10.1007/s12602-017-9371-x

Berlanga-Clavero, M. V., Molina-Santiago, C., Caraballo-Rodríguez, A. M., Petras, D., Díaz-Martínez, L., Pérez-García, A., de Vicente, A., Carrión, V. J., Dorrestein, P. C., & Romero, D. (2022). *Bacillus subtilis* biofilm matrix components target seed oil bodies to promote growth and anti-fungal resistance in melon. *Nature Microbiology*, *7*, 1001–1015. https://doi.org/10.1038/s41564-022-01134-8

Bhadrecha, P., Singh, S., & Dwibedi, V. (2023). A plant's major strength in rhizosphere: The plant growth promoting rhizobacteria. *Archives of Microbiology*, *205*, 165. https://doi.org/10.1007/s00203-023-03502-2

Biermann, R., & Beutel, S. (2023). Endospore production of *Bacillus* spp. for industrial use. *Engineering in Life Sciences*, *23*, e2300013. https://doi.org/10.1002/elsc.202300013

Biessy, A., & Filion, M. (2021). Phloroglucinol derivatives in plant-beneficial *Pseudomonas* spp.: Biosynthesis, regulation, and functions. *Metabolites*, *11*(3), 182. https://doi.org/10.3390/metabo11030182

Bigirimana, S., Barumbanze, P., Ndayihanzamaso, P., Shirima, R., & Legg, J. P. (2011). First report of cassava brown streak disease and associated Ugandan cassava brown streak virus in Burundi . *New Disease Reports*, *24*(1), 26–26. https://doi.org/10.5197/j.2044-0588.2011.024.026

Bigirimana, S., Barumbanze, P., Obonyo, R., & Legg, J. P. (2004). First evidence for the spread of East African cassava mosaic virus - Uganda (EACMV-UG) and the pandemic of severe cassava mosaic disease to Burundi. *Plant Pathology*, *53*(2), 231–231. https://doi.org/10.1111/J.0032-0862.2004.00971.X

Bindraban, P. S., Dimkpa, C. O., & Pandey, R. (2020). Exploring phosphorus fertilizers and fertilization strategies for improved human and environmental health. *Biology and Fertility of Soils*, *56*(3), 299–317. https://doi.org/10.1007/s00374-019-01430-2

Biruma, M., Pillay, M., Tripathi, L., Blomme, G., Abele, S., Mwangi, M., Bandyopadhyay, R., Muchunguzi, P., Kassim, S., Nyine, M., Turyagyenda, L., Eden-Green, S., Kiggundu, A., Pillay, M., Viljoen, A., Gold, C., Tushemereirwe, W., & Kunert, K. (2007). Banana *Xanthomonas* wilt: A review of the disease, management strategies and future research directions. *African Journal of Biotechnology*, *6*(8), 953–962.

Bisutti, I. L., Pelz, J., Büttner, C., & Stephan, D. (2017). Field assessment on the influence of RhizoVital® 42 fl. and Trichostar® on strawberries in the presence of soil-borne diseases. *Crop Protection*, *96*, 195–203. https://doi.org/10.1016/j.cropro.2017.02.004

Biswas, S., Wu, C., & Van Der Donk, W. A. (2021). The antimicrobial activity of the glycocin sublancin is dependent on an active phosphoenolpyruvate-sugar phosphotransferase system. *ACS Infectious Diseases*, *7*(8), 2402–2412. https://doi.org/10.1021/acsinfecdis.1c00157

Bjornlund, V., Bjornlund, H., & Rooyen, A. F. Van. (2020). Why agricultural production in sub-Saharan Africa remains low compared to the rest of the world - a historical perspective. *International Journal of Water Resources Development*, *36*(S1), S20–S53. https://doi.org/10.1080/07900627.2020.1739512

Blázquez, M. A. (2025). Polyamines: Their role in plant development and stress. *Annual Review of Plant Biology*, *75*, 95–117. https://doi.org/10.1146/annurev-arplant-070623

Blin, K., Shaw, S., Augustijn, H. E., Reitz, Z. L., Biermann, F., Alanjary, M., Fetter, A., Terlouw, B. R., Metcalf, W. W., Helfrich, E. J. N., Van Wezel, G. P., Medema, M. H., & Weber, T. (2023). AntiSMASH 7.0: New and improved predictions for detection, regulation, chemical structures and visualisation. *Nucleic Acids Research*, *51*(W1), W46–W50. https://doi.org/10.1093/nar/gkad344

Boloy, F. N., Nkosi, B. I., Losimba, J. K., Bungamuzi, C. L., Siwako, H. M., Walunkonka Balowe, F., Lohaka, J. W., Dhed'a Djailo, B., Lepoint, P., Sivirihauma, C., & Blomme, G. (2014). Assessing incidence, development and distribution of banana bunchy top disease across the main plantain and banana growing regions of the Democratic Republic of Congo. *African Journal of Agricultural Research*, *9*(34), 2611–2623. https://doi.org/10.5897/AJAR2014.8751

Bonaterra, A., Badosa, E., Daranas, N., Francés, J., Roselló, G., & Montesinos, E. (2022). Bacteria as biological control agents of plant diseases. *Microorganisms*, *10*(9), 1759. https://doi.org/10.3390/microorganisms10091759

Boopathi, S., Vashisth, R., Mohanty, A. K., Jia, A. Q., Sivakumar, N., & Arockiaraj, J. (2022). *Bacillus subtilis* BR4 derived stigmatellin Y interferes Pqs-PqsR mediated quorum sensing system of *Pseudomonas aeruginosa*. *Journal of Basic Microbiology*, *62*(7), 801–814. https://doi.org/10.1002/jobm.202200017

Borriss, R. (2020). Phytostimulation and biocontrol by the plant-associated *Bacillus amyloliquefaciens* FZB42: An update. In M. Kumar, V. Kumar, & R. Prasad (Eds.), *Phyto-microbiome in stress regulation. Environmental and microbial biotechnology* (pp. 1–20). Springer. https://doi.org/10.1007/978-981-15-2576-6_1

Bosak, T., Losick, R. M., & Pearson, A. (2008). A polycyclic terpenoid that alleviates oxidative stress. *PNAS*, *105*(18), 6725–6729. https://doi.org/10.1073/pnas.0800199105

Bouassida, M., Mnif, I., Hammami, I., Triki, M. A., & Ghribi, D. (2023). *Bacillus subtilis* SPB1 lipopeptide biosurfactant: Antibacterial efficiency against the phytopathogenic bacteria *Agrobacterium tumefaciens* and compared production in submerged and solid state fermentation systems. *Food Science and Biotechnology*, *32*(11), 1595–1609. https://doi.org/10.1007/s10068-023-01274-5

Boubsi, F., Hoff, G., Arguelles Arias, A., Steels, S., Andrić, S., Anckaert, A., Roulard, R., Rigolet, A., van Wuytswinkel, O., & Ongena, M. (2023). Pectic homogalacturonan sensed by *Bacillus* acts as host associated cue to promote establishment and persistence in the rhizosphere. *IScience*, *26*(10), 107925. https://doi.org/10.1016/j.isci.2023.107925

Brar, S. K., Verma, M., Tyagi, R. D., & Valéro, J. R. (2006). Recent advances in downstream processing and formulations of *Bacillus thuringiensis* based biopesticides. *Process Biochemistry*, *41*(2), 323–342. https://doi.org/10.1016/j.procbio.2005.07.015

Bremer, E., Hoffmann, T., Dempwolff, F., Bedrunka, P., & Bange, G. (2022). The many faces of the unusual biofilm activator RemA. *BioEssays*, *44*(5), 2200009. https://doi.org/10.1002/bies.202200009

Brettin, T., Davis, J. J., Disz, T., Edwards, R. A., Gerdes, S., Olsen, G. J., Olson, R., Overbeek, R., Parrello, B., Pusch, G. D., Shukla, M., Thomason, J. A., Stevens, R., Vonstein, V., Wattam, A. R., & Xia, F. (2015). RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Scientific Reports*, *5*, 8365. https://doi.org/10.1038/srep08365

Brokate, O., Papenbrock, J., & Turcios, A. E. (2024). Biofilm-forming microorganisms in the rhizosphere to improve plant growth: Coping with abiotic stress and environmental pollution. *Applied Soil Ecology*, *202*, 105591. https://doi.org/10.1016/j.apsoil.2024.105591

Bultreys, A., Gheysen, I., Wathelet, B., Maraite, H., & De Hoffmann, E. (2003). High-performance liquid chromatography analyses of pyoverdin siderophores differentiate among phytopathogenic fluorescent *Pseudomonas* species. *Applied and Environmental Microbiology*, *69*(2), 1143–1153. https://doi.org/10.1128/AEM.69.2.1143-1153.2003

Burpee, L. L. (1990). The influence of abiotic factors on biological control of soilborne plant pathogenic fungi. *Canadian Journal of Plant Pathology*, *12*(3), 308–317. https://doi.org/10.1080/07060669009501005

Busogoro, J. P., Jijakli, M. H., & Lepoivre, P. (1999). Identification of a novel source of resistance to angular leaf spot disease of common bean within the secondary gene pool. *Plant Breeding*, *118*(5), 417–423. https://doi.org/10.1046/J.1439-0523.1999.00413.X

Caetano, T., Barbosa, J., Möesker, E., Süssmuth, R. D., & Mendo, S. (2014). Bioengineering of lanthipeptides in *Escherichia coli*: Assessing the specificity of lichenicidin and haloduracin biosynthetic machinery. *Research in Microbiology*, *165*(7), 600–604. https://doi.org/10.1016/j.resmic.2014.07.006

Caetano, T., Krawczyk, J. M., Mösker, E., Süssmuth, R. D., & Mendo, S. (2011). Heterologous expression, biosynthesis, and mutagenesis of type II lantibiotics from *Bacillus licheniformis* in *Escherichia coli*. *Chemistry and Biology*, *18*(1), 90–100. https://doi.org/10.1016/j.chembiol.2010.11.010

Cai, L., Guo, H. T., Zheng, G. Di, Wang, X. Y., & Wang, K. (2022). Metagenomic analysis reveals the microbial degradation mechanism during kitchen waste biodrying. *Chemosphere*, *307*, 135862. https://doi.org/10.1016/j.chemosphere.2022.135862

Cai, Y., Tao, H., Gaballa, A., Pi, H., & Helmann, J. D. (2025). The extracytoplasmic sigma factor σX supports biofilm formation and increases biocontrol efficacy in *Bacillus velezensis* 118. *Scientific Reports*, *15*(1), 5315. https://doi.org/10.1038/s41598-025-89284-7

Çam, S., & Badıllı, İ. (2024). The effect of NaCl, pH, and phosphate on biofilm formation and exopolysaccharide production by high biofilm producers of *Bacillus* strains. *Folia Microbiologica*, *69*(3), 613–624. https://doi.org/10.1007/s12223-023-01101-8

Camatti, G., Mulinari dos Santos, F., Luis dos Santos Rodrigues Júnior, G., Pereira Camargo, D., Stefanello Manfio, G., Rodrigo Pereira Santos, J., & Carlos Pereira da Silva, J. (2023). *Bacillus*-and *Trichoderma*-based products control the spiral nematode *Helicotylenchus dihystera* in soybean. *Rhizosphere*, *27*, 100717. https://doi.org/10.1016/j.rhisph.2023.100717

Cao, L., Do, T., & James Link, A. (2021). Mechanisms of action of ribosomally synthesized and posttranslationally modified peptides (RiPPs). *Journal of Industrial Microbiology and Biotechnology*, *48*(3–4), kua005. https://doi.org/10.1093/jimb/kuab005

Cao, Y., Pi, H., Chandrangsu, P., Li, Y., Wang, Y., Zhou, H., Xiong, H., Helmann, J. D., & Cai, Y. (2018). Antagonism of two plant-growth promoting *Bacillus velezensis* isolates against *Ralstonia solanacearum* and *Fusarium oxysporum*. *Scientific Reports*, *8*, 4360. https://doi.org/10.1038/s41598-018-22782-z

Cao, Y., Zhang, Z., Ling, N., Yuan, Y., Zheng, X., Shen, B., & Shen, Q. (2011). *Bacillus subtilis* SQR 9 can control *Fusarium* wilt in cucumber by colonizing plant roots. *Biology and Fertility of Soils*, *47*(5), 495–506. https://doi.org/10.1007/s00374-011-0556-2

Carreño-López, R., Alatorre-Cruz, J. M., & Marín-Cevada, V. (2019). Pyrroloquinoline quinone (PQQ): Role in plant-microbe interactions. In B. H. Singh, C. Keswami, M. S. Reddy, E. Sansinenea, & C. García-Estrada (Eds.), *Secondary metabolites of plant growth promoting rhizomicroorganisms: Discovery and applications* (pp. 169–184). Springer Nature. https://doi.org/10.1007/978-981-13-5862-3_9

Casinga, C. M., Shirima, R. R., Mahungu, N. M., Tata-Hangy, W., Bashizi, K. B., Munyerenkana, C. M., Ughento, H., Enene, J., Sikirou, M., Dhed'a, B., Monde, G., Kumar, P. L., & Legg, J. P. (2021). Expansion of the cassava brown streak disease epidemic in eastern Democratic Republic of Congo. *Plant Disease*, *105*(8), 2177–2188. https://doi.org/10.1094/PDIS-05-20-1135-RE

Cassanelli, S., Bellameche, F., Caradonia, F., Cortiello, M., Perez Fuentealba, S., & Giovanardi, D. (2025). Foliar application of *Streptomyces* sp. DLS2013 induces transcriptional changes on tomato plants and confers resistance to *Pseudomonas syringae* pv. *tomato*. *Journal of Plant Diseases and Protection*, *132*, 19. https://doi.org/10.1007/s41348-024-01027-4

Caulier, S., Gillis, A., Colau, G., Licciardi, F., Liépin, M., Desoignies, N., Modrie, P., Legrève, A., Mahillon, J., & Bragard, C. (2018). Versatile antagonistic activities of soil-borne *Bacillus* spp. and *Pseudomonas* spp. against *Phytophthora infestans* and other potato pathogens. *Frontiers in Microbiology*, *9*, 143. https://doi.org/10.3389/fmicb.2018.00143

Caulier, S., Nannan, C., Gillis, A., Licciardi, F., Bragard, C., & Mahillon, J. (2019). Overview of the antimicrobial compounds produced by members of the *Bacillus*

subtilis group. *Frontiers in Microbiology*, *10*, 302. https://doi.org/10.3389/fmicb.2019.00302

Cellini, A., Spinelli, F., Donati, I., Ryu, C. M., & Kloepper, J. W. (2021). Bacterial volatile compound-based tools for crop management and quality. *Trends in Plant Science*, *26*(9), 968–983. https://doi.org/10.1016/j.tplants.2021.05.006

Centre d'expertise en analyse environnementale du Québec. (2003). *Détermination du pH à l'eau et du pH tampon dans les sols agricoles : méthode électrométrique*.

Centre d'expertise en analyse environnementale du Québec. (2015). *Détermination du carbone organique total dans les solides : dosage par titrage.*

Cezard, C., Farvacques, N., & Sonnet, P. (2014). Chemistry and biology of pyoverdines, *Pseudomonas* primary siderophores. *Current Medicinal Chemistry*, *22*(2), 165–186. https://doi.org/10.2174/09298673216661410111946 24

Chadhary, P., Singh, S., Chaudhary, A., Sharma, A., & Kumar, G. (2022). Overview of biofertilizers in crop production and stress management for sustainable agriculture. *Frontiers in Plant Science*, *13*, 930340. https://doi.org/10.3389/fpls.2022.930340

Chakraborty, K., Kizhakkekalam, V. K., Joy, M., & Dhara, S. (2021). Difficidin class of polyketide antibiotics from marine macroalga-associated *Bacillus* as promising antibacterial agents. *Applied Microbiology and Biotechnology*, *105*(16–17), 6395–6408. https://doi.org/10.1007/s00253-021-11390-z

Chakraborty, U., Chakraborty, B., & Basnet, M. (2006). Plant growth promotion and induction of resistance in *Camellia sinensis* by *Bacillus megaterium*. *Journal of Basic Microbiology*, *46*(3), 186–195. https://doi.org/10.1002/jobm.200510050

Chan, W. C., Bycroft, B. W., Leyland, M. L., Liant, L.-Y., & Robertst, G. C. K. (1993). A novel post-translational modification of the peptide antibiotic subtilin: Isolation and characterization of a natural variant from *Bacillus subtilis* ATCC 6633. *Biochemistry Journal*, *291*(1), 23–27. https://doi.org/10.1042/bj2910023

Chang, X., Yun, L., Liu, Z., Shen, Y., Feng, S., Yang, G., & Meng, X. (2024). Antagonistic Effects and the underlying mechanisms of *Bacillus velezensis* and its antibacterial peptide LCI against *Aeromonas hydrophila* infection in largemouth bass. *Probiotics and Antimicrobial Proteins*. https://doi.org/10.1007/s12602-024-10329-w

Chaouachi, M., Marzouk, T., Jallouli, S., Elkahoui, S., Gentzbittel, L., Ben, C., & Djébali, N. (2021). Activity assessment of tomato endophytic bacteria bioactive compounds for the postharvest biocontrol of *Botrytis cinerea*. *Postharvest Biology and Technology*, *172*, 111389. https://doi.org/10.1016/j.postharvbio.2020.111389

Chatterjee, S., Chatterjee, S., Lad, S. J., Phansalkar, M. S., Rupp, R. H., Ganguli, B. N., Fehlhaber, H.-W., & Kogler, H. (1992). Mersacidin, a new antibiotic from *Bacillus* fermentation, isolation, purification and chemical characterization. *The Journal of Antibiotics*, *45*(6), 832–838. https://doi.org/10.7164/antibiotics.45.832

Chen, H., Xiao, X., Wang, J., Wu, L., Zheng, Z., & Yu, Z. (2008). Antagonistic effects of volatiles generated by *Bacillus subtilis* on spore germination and hyphal growth of the plant pathogen, *Botrytis cinerea*. *Biotechnology Letters*, *30*(5), 919–923. https://doi.org/10.1007/s10529-007-9626-9

Chen, K., Tian, Z., Luo, Y., Cheng, Y., & Long, C. A. (2018). Antagonistic activity and the mechanism of *Bacillus amyloliquefaciens* DH-4 against citrus green mold. *Phytopathology*, *108*(11), 1253–1262. https://doi.org/10.1094/PHYTO-01-17-0032-R

Chen, L., & Liu, Y. (2024). The function of root exudates in the root colonization by beneficial soil rhizobacteria. *Biology*, *13*(2), 95. https://doi.org/10.3390/biology13020095

Chen, L., Wu, Y. D., Chong, X. Y., Xin, Q. H., Wang, D. X., & Bian, K. (2020). Seed-borne endophytic *Bacillus velezensis* LHSB1 mediate the biocontrol of peanut stem rot caused by *Sclerotium rolfsii*. *Journal of Applied Microbiology*, *128*(3), 803–813. https://doi.org/10.1111/jam.14508

Chen, M. C., Wang, J. P., Zhu, Y. J., Liu, B., Yang, W. J., & Ruan, C. Q. (2019). Antibacterial activity against *Ralstonia solanacearum* of the lipopeptides secreted from the *Bacillus amyloliquefaciens* strain FJAT-2349. *Journal of Applied Microbiology*, *126*(5), 1519–1529. https://doi.org/10.1111/jam.14213

Chen, M., Wang, J., Liu, B., Zhu, Y., Xiao, R., Yang, W., Ge, C., & Chen, Z. (2020). Biocontrol of tomato bacterial wilt by the new strain *Bacillus velezensis* FJAT-46737 and its lipopeptides. *BMC Microbiology*, *20*(1), 160. https://doi.org/10.1186/s12866-020-01851-2

Chen, M. Y., Teng, W. K., Zhao, L., Hu, C. X., Zhou, Y. K., Han, B. P., Song, L. R., & Shu, W. S. (2021). Comparative genomics reveals insights into cyanobacterial evolution and habitat adaptation. *ISME Journal*, *15*(1), 211–227. https://doi.org/10.1038/s41396-020-00775-z

Chen, X. H., Scholz, R., Borriss, M., Junge, H., Mögel, G., Kunz, S., & Borriss, R. (2009). Difficidin and bacilysin produced by plant-associated *Bacillus amyloliquefaciens* are efficient in controlling fire blight disease. *Journal of Biotechnology*, *140*, 38–44. https://doi.org/10.1016/j.jbiotec.2008.10.015

Chen, X. H., Vater, J., Piel, J., Franke, P., Scholz, R., Schneider, K., Koumoutsi, A., Hitzeroth, G., Grammel, N., Strittmatter, A. W., Gottschalk, G., Süssmuth, R. D., & Borriss, R. (2006). Structural and functional characterization of three polyketide synthase gene clusters in *Bacillus amyloliquefaciens* FZB42. *Journal of Bacteriology*, *188*(11), 4024–4036. https://doi.org/10.1128/JB.00052-06

Chen, X.-H., Koumoutsi, A., Scholz, R., & Borriss, R. (2009). More than anticipated – production of antibiotics and other secondary metabolites by *Bacillus amyloliquefaciens* FZB42. *Microbial Physiology*, *16*(1–2), 14–24. https://doi.org/10.1159/000142891

Chen, Y., Gozzi, K., Yan, F., & Chai, Y. (2015). Acetic acid acts as a volatile signal to stimulate bacterial biofilm formation. *MBio*, *6*(3), e00392-15. https://doi.org/10.1128/mBio.00392-15

Chen, Y., Wang, J., Li, G., Yang, Y., & Ding, W. (2021). Current advancements in sactipeptide natural products. *Frontiers in Chemistry*, *9*, 595991. https://doi.org/10.3389/fchem.2021.595991

Chen, Y., Yan, F., Chai, Y., Liu, H., Kolter, R., Losick, R., & Guo, J. (2013). Biocontrol of tomato wilt disease by *Bacillus subtilis* isolates from natural environments depends on conserved genes mediating biofilm formation. *Environmental Microbiology*, *15*(3), 848–864. https://doi.org/10.1111/j.1462-2920.2012.02860.x

Cheng, C., Su, S., Bo, S., Zheng, C., Liu, C., Zhang, L., Xu, S., Wang, X., Gao, P., Fan, K., He, Y., Zhou, D., Gong, Y., Zhong, G., & Liu, Z. (2024). A *Bacillus velezensis* strain isolated from oats with disease-preventing and growth-promoting properties. *Scientific Reports*, *14*(1), 12950. https://doi.org/10.1038/s41598-024-63756-8

Chilakamarry, C. R., Mimi Sakinah, A. M., Zularisam, A. W., Sirohi, R., Khilji, I. A., Ahmad, N., & Pandey, A. (2022). Advances in solid-state fermentation for bioconversion of agricultural wastes to value-added products: Opportunities and challenges. *Bioresource Technology*, *343*, 126065. https://doi.org/10.1016/j.biortech.2021.126065

Choby, J. E., & Skaar, E. P. (2016). Heme synthesis and acquisition in bacterial pathogens. *Journal of Molecular Biology*, *428*(17), 3408–3428. https://doi.org/10.1016/j.jmb.2016.03.018

Chopra, L., Singh, G., Kumar Jena, K., & Sahoo, D. K. (2015). Sonorensin: A new bacteriocin with potential of an anti-biofilm agent and a food biopreservative. *Scientific Reports*, *5*(1), 13412. https://doi.org/10.1038/srep13412

Chowdhury, N. (2020). *Study on acid stress response in* Bacillus amyloliquefaciens. Assam Agricultural University.

Chowdhury, N., Hazarika, D. J., Goswami, G., Sarmah, U., Borah, S., Boro, R. C., & Barooah, M. (2022). Acid tolerant bacterium *Bacillus amyloliquefaciens* MBNC retains biocontrol efficiency against fungal phytopathogens in low pH. *Archives of Microbiology*, *204*(2), 124. https://doi.org/10.1007/s00203-021-02741-5

Cokola, M. C., Ndjadi, S. S., Bisimwa, E. B., Ahoton, L. E., & Francis, F. (2021). First report of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on Onion (*Allium cepa* L.) in South Kivu, Eastern DR Congo. *Revista Brasileira de Entomologia*, *65*(1), e20200083. https://doi.org/10.1590/1806-9665-rbent-2020-0083

Conijn, J. G., Bindraban, P. S., Schröder, J. J., & Jongschaap, R. E. E. (2018). Can our global food system meet food demand within planetary boundaries? *Agriculture, Ecosystems and Environment*, *251*, 244–256. https://doi.org/10.1016/j.agee.2017.06.001

Contesini, F. J., Melo, R. R. de, & Sato, H. H. (2018). An overview of *Bacillus* proteases: From production to application. *Critical Reviews in Biotechnology*, *38*(3), 321–334. https://doi.org/10.1080/07388551.2017.1354354

Corvey, C., Stein, T., Düsterhus, S., Karas, M., & Entian, K. D. (2003). Activation of subtilin precursors by *Bacillus subtilis* extracellular serine proteases subtilisin (AprE), WprA, and Vpr. *Biochemical and Biophysical Research Communications*, *304*(1), 48–54. https://doi.org/10.1016/S0006-291X(03)00529-1

Couvin, D., Bernheim, A., Toffano-Nioche, C., Touchon, M., Michalik, J., Néron, B., Rocha, E. P. C., Vergnaud, G., Gautheret, D., & Pourcel, C. (2018). CRISPRCasFinder, an update of CRISRFinder, includes a portable version,

enhanced performance and integrates search for Cas proteins. *Nucleic Acids Research*, *46*(W1), W246–W251. https://doi.org/10.1093/nar/gky425

Coyne, D. L., Cortada, L., Dalzell, J. J., Claudius-Cole, A. O., Haukeland, S., Luambano, N., & Talwana, H. (2018). Plant-parasitic nematodes and food security in Sub-Saharan Africa. *Annual Review of Phytopathology*, *56*, 381–403. https://doi.org/10.1146/annurev-phyto-080417-045833

Crouzet, J., Arguelles-Arias, A., Dhondt-Cordelier, S., Cordelier, S., Pršić, J., Hoff, G., Mazeyrat-Gourbeyre, F., Baillieul, F., Clément, C., Ongena, M., & Dorey, S. (2020). Biosurfactants in plant protection against diseases: Rhamnolipids and lipopeptides case study. *Frontiers in Bioengineering and Biotechnology*, *8*, 1014. https://doi.org/10.3389/FBIOE.2020.01014/BIBTEX

Cubry, P., Vigouroux, Y., & François, O. (2017). The empirical distribution of singletons for geographic samples of DNA sequences. *Frontiers in Genetics*, *8*, 139. https://doi.org/10.3389/fgene.2017.00139

Cui, Q., Beiyuan, J., Chen, Y., Li, M., Qiu, T., Zhao, S., Zhu, X., Chen, H., & Fang, L. (2024). Synergistic enhancement of plant growth and cadmium stress defense by *Azospirillum brasilense* and plant heme: Modulating the growth–defense relationship. *Science of the Total Environment*, *946*, 174503. https://doi.org/10.1016/j.scitotenv.2024.174503

Czajkowski, R., Rabalski, L., Kosinski, M., de Neergaard, E., & Harding, S. (2021). High-quality complete genome resource of plant-pathogenic bacterium *Pectobacterium atrosepticum* strain Green1 isolated from potato (*Solanum tuberosum* L.) in greenland. *Molecular Plant-Microbe Interactions*, *34*(11), 1328–1333. https://doi.org/10.1094/MPMI-06-21-0130-A

da Silva Junior, A. L., Borges, Á. V., da Silva, H. A. O., Leite, I. C. H. L., Alves, K. S., de Medeiros, L. S., & Abreu, L. M. de. (2023). Lipopeptide-enriched extracts of *Bacillus velezensis* B157 for controlling tomato early blight. *Crop Protection*, *172*, 106317. https://doi.org/10.1016/j.cropro.2023.106317

Dalvan do Nascimento, D., Rodrigues, M., Junior Ferreira, R., Marchioro, V., Macedo da Silva, E., Alberto Silva Junior, C., Cristina Kupper, K., Antonio Polanczyk, R., & Luiz Martins Soares, P. (2022). Soybean growth-promotion and *Heterodera glycines* suppression in two application methods of *Bacillus* strains. *Biological Control*, *175*, 105039. https://doi.org/10.1016/j.biocontrol.2022.105039

Danevčič, T., Spacapan, M., Dragoš, A., Kovács, Á. T., & Mandic-Mulec, I. (2023). DegQ is an important policing link between quorum sensing and regulated adaptative traits in *Bacillus subtilis*. *Microbiology Spectrum*, *11*(5), 1–14. https://doi.org/10.1128/spectrum.00908-23

Danilova, I., & Sharipova, M. (2020). The practical potential of bacilli and their enzymes for industrial production. *Frontiers in Microbiology*, *11*, 1782. https://doi.org/10.3389/fmicb.2020.01782

Darbandi, A., Asadi, A., Mahdizade Ari, M., Ohadi, E., Talebi, M., Halaj Zadeh, M., Darb Emamie, A., Ghanavati, R., & Kakanj, M. (2022). Bacteriocins: Properties and

potential use as antimicrobials. *Journal of Clinical Laboratory Analysis*, *36*(1), e24093. https://doi.org/10.1002/jcla.24093

Das M, M., & Abdulhameed, S. (2020). Agro-processing residues for the production of fungal bio-control agents. In A. Z. Zakaria, N. C. Aguilar, D. R. Kusumaningtyas, & P. Binod (Eds.), *Valorisation of agro-industrial residues-Non-biological approaches, Applied environmental science and engineering for a sustainable future: Vol. II* (pp. 107–126). Springer Nature. https://doi.org/10.1007/978-3-030-39208-6_5

de O. Nunes, P. S., de Medeiros, F. H. V., de Oliveira, T. S., de Almeida Zago, J. R., & Bettiol, W. (2023). *Bacillus subtilis* and *Bacillus licheniformis* promote tomato growth. *Brazilian Journal of Microbiology*, *54*(1), 397–406. https://doi.org/10.1007/s42770-022-00874-3

de Sousa Ramos, G. K., Vivas, M., Pinho, D. B., de Almeida, R. N., de Andrade Junior, M. S., Vivas, J. M. S., Mussi-Dias, V., & Gonçalves, J. M. (2024). Characterization of *Exserohilum* isolates associated with northern corn leaf blight in Brazil. *Indian Phytopathology*, *77*(2), 447–455. https://doi.org/10.1007/s42360-024-00742-0

Deb, C. R., & Tatung, M. (2024). Siderophore producing bacteria as biocontrol agent against phytopathogens for a better environment: A review. *South African Journal of Botany*, *165*, 153–162. https://doi.org/10.1016/j.sajb.2023.12.031

Delcambe, L., & Devignat, R. (1957). L'iturine, nouvel antibiotique d'origine congolaise. *Acad. Roy. Sci. Coloniales*, *6*, 1–77. https://www.kaowarsom.be/

Denaxa, N. K., Tsafouros, A., Ntanos, E., & Roussos, P. A. (2023). Role of glycine betaine in the protection of plants against environmental stresses. In M. Ghorbanpour & M. Adnan Shahid (Eds.), *Plant stress mitigators: Types, techniques and functions* (pp. 127–158). Academic Press, Elsevier. https://doi.org/10.1016/B978-0-323-89871-3.00009-4

Dertz, E. A., Xu, J., Stintzi, A., & Raymond, K. N. (2006). Bacillibactin-mediated iron transport in *Bacillus subtilis*. *Journal of the American Chemical Society*, *128*(1), 22–23. https://doi.org/10.1021/ja055898c

Dey, S., Guchhait, K. C., Jana, D., Majumder, S., Patra, A., Panda, A. K., & Ghosh, C. (2023). Biosynthesis of lantibiotics. In S. Joshi, K. R. Kar, D. Lahiri, & M. Nag (Eds.), *Lantibiotics as alternative therapeutics* (pp. 43–63). Academic Press, Elsevier. https://doi.org/10.1016/B978-0-323-99141-4.00014-X

Dhankhar, P., Dalal, V., Mahto, J. K., Gurjar, B. R., Tomar, S., Sharma, A. K., & Kumar, P. (2020). Characterization of dye-decolorizing peroxidase from *Bacillus subtilis*. *Archives of Biochemistry and Biophysics*, *693*, 108590. https://doi.org/10.1016/j.abb.2020.108590

Di Francesco, A., Milella, F., Mari, M., & Roberti, R. (2017). A preliminary investigation into *Aureobasidium pullulans* as a potential biocontrol agent against *Phytophthora infestans* of tomato. *Biological Control*, *114*, 144–149. https://doi.org/10.1016/J.BIOCONTROL.2017.08.010

Di, Y. ning, Kui, L., Singh, P., Liu, L. feng, Xie, L. yan, He, L. lian, & Li, F. sheng. (2023). Identification and characterization of *Bacillus subtilis* B9: A diazotrophic

plant growth-promoting endophytic bacterium isolated from sugarcane root. *Journal of Plant Growth Regulation*, *42*(3), 1720–1737. https://doi.org/10.1007/s00344-022-10653-x

Dimkić, I., Janakiev, T., Petrović, M., Degrassi, G., & Fira, D. (2022). Plant-associated *Bacillus* and *Pseudomonas* antimicrobial activities in plant disease suppression via biological control mechanisms-A review. *Physiological and Molecular Plant Pathology*, *117*, 101754. https://doi.org/10.1016/j.pmpp.2021.101754

Dimopoulou, A., Theologidis, I., Benaki, D., Koukounia, M., Zervakou, A., Tzima, A., Diallinas, G., Hatzinikolaou, D. G., & Skandalis, N. (2021). Direct antibiotic activity of bacillibactin broadens the biocontrol range of *Bacillus amyloliquefaciens* MBI600. *MSphere*, *6*(4), e0037621. https://doi.org/10.1128/msphere.00376-21

Dischinger, J., Josten, M., Szekat, C., Sahl, H. G., & Bierbaum, G. (2009). Production of the novel two-peptide lantibiotic lichenicidin by *Bacillus licheniformis* DSM 13. *PLoS ONE*, *4*(8), e6788. https://doi.org/10.1371/journal.pone.0006788

Do Prado Mattos, A., Rissato, B. B., Itako, A. T., Júnior, J. B. T., & Estrada, K. R. F. S. (2023). *Bacillus amyloliquefaciens* PKM16 acts as an antagonist of white mold and an inducer of defense enzymes in tomato plants. *Acta Scientiarum - Agronomy*, *45*, e59586. https://doi.org/10.4025/actasciagron.v45i1.59586

Dobrzyński, J., Jakubowska, Z., & Dybek, B. (2022). Potential of *Bacillus pumilus* to directly promote plant growth. *Frontiers in Microbiology*, *13*, 1069053. https://doi.org/10.3389/fmicb.2022.1069053

Droby, S., Wisniewski, M., Teixidó, N., Spadaro, D., & Jijakli, M. H. (2016). The science, development, and commercialization of postharvest biocontrol products. *Postharvest Biology and Technology*, *122*, 22–29. https://doi.org/10.1016/j.postharvbio.2016.04.006

Duanis-Assaf, D., Steinberg, D., Chai, Y., & Shemesh, M. (2016). The LuxS based quorum sensing governs lactose induced biofilm formation by *Bacillus subtilis*. *Frontiers in Microbiology*, *6*, 1517. https://doi.org/10.3389/fmicb.2015.01517

Dunbar, K. L., Melby, J. O., & Mitchell, D. A. (2012). YcaO domains use ATP to activate amide backbones during peptide cyclodehydrations. *Nature Chemical Biology*, *8*(6), 569–575. https://doi.org/10.1038/nchembio.944

DunhamTrimmer. (2018). Biological products market around the world. *Bioproducts Industry Alliance Spring Meeting and International Symposium*, 28. http://www.bpia.org/wp-content/uploads/2018/03/Biological-Products-Markets-Around-The-World.pdf

DunhamTrimmer. (2023). *DunhamTrimmer® Global Biocontrol Report: Market Overview, Trends, Drivers and Insights*. https://dunhamtrimmer.com/reports/global-biocontrol-market-report/

Dunlap, C. A. (2019). Taxonomy of registered *Bacillus* spp. strains used as plant pathogen antagonists. *Biological Control*, *134*, 82–86. https://doi.org/10.1016/j.biocontrol.2019.04.011

Dunlap, C. A., Bowman, M. J., & Rooney, A. P. (2019). Iturinic lipopeptide diversity in the *Bacillus subtilis* species group- Important antifungals for plant disease biocontrol

applications. *Frontiers in Microbiology*, *10*, 1794. https://doi.org/10.3389/fmicb.2019.01794

Dunlap, C. A., Saunders, L. P., Schisler, D. A., Leathers, T. D., Naeem, N., Cohan, F. M., & Rooney, A. P. (2016). *Bacillus nakamurai* sp. nov., a black-pigment-producing strain. *International Journal of Systematic and Evolutionary Microbiology*, *66*(8), 2987–2991. https://doi.org/10.1099/ijsem.0.001135

Dunn, M. F., & Becerra-Rivera, V. A. (2023). The biosynthesis and functions of polyamines in the interaction of plant growth-promoting rhizobacteria with plants. *Plants*, *12*(14), 2671. https://doi.org/10.3390/plants12142671

Dunyashev, T. P., Laptev, G. Yu., Yildirim, E. A., Ilina, L. A., Filippova, V. A., Tiurina, D. G., Dubrovin, A. V., Tarlavin, N. V., Bikonya, S. N., Brazhnik, E. A., Melikidy, V. H., & Platonov, A. V. (2021). Identification of genes associated with the synthesis of siderophores by the *Bacillus subtilis*. *Journal of Livestock Science*, *12*(4), 287–291. https://doi.org/10.33259/jlivestsci.2021.287-291

Duré, L. M. M., Mascarin, G. M., & Bettiol, W. (2025). Optimization of endospore production by solid and liquid fermentation for the development of effective formulations of *Bacillus velezensis*-based products. *Brazilian Journal of Microbiology*. https://doi.org/10.1007/s42770-025-01626-9

Dushimirimana, S., Gasogo, A., Kazitsa, E.-G., & Hance, T. (2016). Dynamics and seasonal variability of *Bemesia tabaci* colonies in cassava. *Modern Agriclutural Science and Technology*, *2*(1), 26–32. https://doi.org/10.15341/mast(2375-9402)/01.02.2016/004

Dutt, S., Hamza, I., & Bartnikas, T. B. (2022). Molecular mechanisms of iron and heme metabolism. *Annual Reviews of Nutrition*, *42*, 311–335. https://doi.org/10.1146/annurev-nutr-062320

Dutta, S., Balaraju, K., Oh, S.-Y., Lee, M.-H., Lee, S. W., Lee, Y. H., & Park, K. (2025). Plant growth promotion via priming with volatile organic compounds emitted from *Bacillus vallismortis* strain EXTN-1. *Frontiers in Microbiology*, *15*, 1524888. https://doi.org/10.3389/fmicb.2024.1524888

Ejaz, U., Sohail, M., & Ghanemi, A. (2021). Cellulases: From bioactivity to a variety of industrial applications. *Biomimetics*, *6*(3), 44. https://doi.org/10.3390/biomimetics6030044

El Aichar, F., Muras, A., Parga, A., Otero, A., & Nateche, F. (2022). Quorum quenching and anti-biofilm activities of halotolerant *Bacillus* strains isolated in different environments in Algeria. *Journal of Applied Microbiology*, *132*(3), 1825–1839. https://doi.org/10.1111/jam.15355

Elazouni, I., Abdel-Aziz, S., & Rabea, A. (2019). Microbial efficacy as biological agents for potato enrichment as well as bio-controls against wilt disease caused by *Ralstonia solanacearum*. *World Journal of Microbiology and Biotechnology*, *35*(3), 30. https://doi.org/10.1007/s11274-019-2596-y

El-Bendary, M. A., & Moharam, M. E. (2019). Formulation of spore toxin complex of *Bacillus thuringiensis* and *Lysinibacillus sphaericus* grown under solid state

fermentation. *Biological Control*, *131*, 54–61. https://doi.org/10.1016/j.biocontrol.2019.01.005

Elnahal, A. S. M., El-Saadony, M. T., Saad, A. M., Desoky, E. S. M., El-Tahan, A. M., Rady, M. M., AbuQamar, S. F., & El-Tarabily, K. A. (2022). The use of microbial inoculants for biological control, plant growth promotion, and sustainable agriculture: A review. *European Journal of Plant Pathology*, *162*(4), 759–792. https://doi.org/10.1007/s10658-021-02393-7

El-Saadony, M. T., Saad, A. M., Soliman, S. M., Salem, H. M., Ahmed, A. I., Mahmood, M., El-Tahan, A. M., Ebrahim, A. A. M., Abd El-Mageed, T. A., Negm, S. H., Selim, S., Babalghith, A. O., Elrys, A. S., El-Tarabily, K. A., Abuqamar, S. F., Vassilev, N., Singh, J., & Castaldi, S. (2022). Plant growth-promoting microorganisms as biocontrol agents of plant diseases: Mechanisms, challenges and future perspectives. *Frontiers in Plant Science*, *13*, 923880. https://doi.org/10.3389/fpls.2022.923880

Enebe, M. C., & Babalola, O. O. (2018). The influence of plant growth-promoting rhizobacteria in plant tolerance to abiotic stress: A survival strategy. *Applied Microbiology and Biotechnology*, *102*(18), 7821–7835. https://doi.org/10.1007/s00253-018-9214-z

Erega, A., Stefanie, P., Dogsa, I., Danevčič, T., Simunovic, K., Klančnik, A., Možina, S. S., & Mulec, I. M. (2021). Bacillaene mediates the inhibitory effect of *Bacillus subtilis* on *Campylobacter jejuni* biofilms. *Applied and Environmental Microbiology*, *87*(12), e02955-20. https://doi.org/10.1128/AEM.02955-20

Ertekin, O., Kutnu, M., Taşkin, A. A., Demir, M., Karataş, A. Y., & Özcengiz, G. (2020). Analysis of a *bac* operon-silenced strain suggests pleiotropic effects of bacilysin in *Bacillus subtilis*. *Journal of Microbiology*, *58*(4), 297–313. https://doi.org/10.1007/s12275-020-9064-0

Eslami, S. M., & van der Donk, W. A. (2024). Proteases involved in leader peptide removal during RiPP Biosynthesis. *ACS Bio & Med Chem Au*, *4*(1), 20–36. https://doi.org/10.1021/acsbiomedchemau.3c00059

Esmaeilishirazifard, E., Dariush, A., Moschos, S. A., & Keshavarz, T. (2018). A novel antifungal property for the *Bacillus licheniformis* ComX pheromone and its possible role in inter-kingdom cross-talk. *Applied Microbiology and Biotechnology*, *102*(12), 5197–5208. https://doi.org/10.1007/s00253-018-9004-7

Essiedu, J. A., Adepoju, F. O., & Ivantsova, M. N. (2020). Benefits and limitations in using biopesticides: A review. *AIP Conference Proceedings*, *2313*, 080002. https://doi.org/10.1063/5.0032223

Etesami, H. (2020). Plant-microbe interactions in plants and stress tolerance. In K. D. Tripathi, P. V. Singh, K. D. Chauhan, S. Sharma, M. S. Prasad, K. N. Dubey, & N. Ramawat (Eds.), *Plant life under changing environment: Responses and management* (pp. 355–396). Academic Press, Elsevier. https://doi.org/10.1016/B978-0-12-818204-8.00018-7

Etesami, H., Jeong, B. R., & Glick, B. R. (2023a). Biocontrol of plant diseases by *Bacillus* spp. *Physiological and Molecular Plant Pathology*, *126*, 102048. https://doi.org/10.1016/j.pmpp.2023.102048

Etesami, H., Jeong, B. R., & Glick, B. R. (2023b). Potential use of *Bacillus* spp. as an effective biostimulant against abiotic stresses in crops - a review. *Current Research in Biotechnology*, *5*, 100128. https://doi.org/10.1016/j.crbiot.2023.100128

EU Food Safety. (2023). *EU Pesticides Database*. https://food.ec.europa.eu/plants/pesticides/eu-pesticides-database_en

FAAPA. (2021). *Le secteur agricole contribue à 36 % dans la formation du PIB en RDC*. http://www.faapa.info/blog/le-secteur-agricole-contribue-a-36-dans-la-formation-du-pib-en-rdc/

Fan, B., Wang, C., Ding, X., Zhu, B., Song, X., & Borriss, R. (2019). AmyloWiki: An integrated database for *Bacillus velezensis* FZB42, the model strain for plant growth -promoting bacilli. *Database*, *2019*, baz071. https://doi.org/10.1093/database/baz071

Fan, B., Wang, C., Song, X., Ding, X., Wu, L., Wu, H., Gao, X., & Borriss, R. (2018). *Bacillus velezensis* FZB42 in 2018: The Gram-positive model strain for plant growth promotion and biocontrol. *Frontiers in Microbiology*, *9*, 2491. https://doi.org/10.3389/fmicb.2018.02491

FAO. (2022). *FAO employs innovative plant pest control solutions in Eastern Africa*. https://reliefweb.int/report/burundi/fao-employs-innovative-plant-pest-control-solutions-eastern-africa

FAO. (2023a). *En Afrique centrale, environ 42,7 millions de personnes sont en situation d'insécurité alimentaire et nutritionnelle*. https://www.fao.org/africa/news/detail-news/fr/c/1273669/

FAO. (2023b). *FAOSTAT*. https://www.fao.org/faostat/en/#data/RP

FAO & WHO. (2014). *The International Code of Conduct on Pesticide Management*. https://www.who.int/publications/i/item/9789251085493

Farag, M. A., Song, G. C., Park, Y. S., Audrain, B., Lee, S., Ghigo, J. M., Kloepper, J. W., & Ryu, C. M. (2017). Biological and chemical strategies for exploring inter- and intra-kingdom communication mediated via bacterial volatile signals. *Nature Protocols*, *12*(7), 1359–1377. https://doi.org/10.1038/nprot.2017.023

Farrow, A., & Muthoni-Andriatsitohaina, R. (2020). *Atlas of common bean production in Africa* (A. Farrow & R. Muthoni-Andriatsitohaina, Eds.; 2nd ed.). Pan Africa Bean Research Alliance (PABRA); International Center for Tropical Agriculture (CIAT).

Fasim, A., More, V. S., & More, S. S. (2021). Large-scale production of enzymes for biotechnology uses. *Current Opinion in Biotechnology*, *69*, 68–76. https://doi.org/10.1016/j.copbio.2020.12.002

Fazle Rabbee, M., & Baek, K.-H. (2020). Antimicrobial activities of lipopeptides and polyketides of *Bacillus velezensis* for agricultural applications. *Molecules*, *25*(21), 4973. https://doi.org/10.3390/molecules25214973

Feng, H., Zhang, N., Du, W., Zhang, H., Liu, Y., Fu, R., Shao, J., Zhang, G., Shen, Q., & Zhang, R. (2018). Identification of chemotaxis compounds in root exudates and their sensing chemoreceptors in plant-growth-promoting rhizobacteria *Bacillus*

amyloliquefaciens SQR9. *Molecular Plant-Microbe Interactions*, *31*(10), 995–1005. https://doi.org/10.1094/MPMI-01-18-0003-R

Feng, H., Zhang, N., Fu, R., Liu, Y., Krell, T., Du, W., Shao, J., Shen, Q., & Zhang, R. (2019). Recognition of dominant attractants by key chemoreceptors mediates recruitment of plant growth-promoting rhizobacteria. *Environmental Microbiology*, *21*(1), 402–415. https://doi.org/10.1111/1462-2920.14472

Feng, Y., Zhang, Y., Shah, O. U., Luo, K., & Chen, Y. (2023). Isolation and identification of endophytic bacteria *Bacillus* sp. ME9 that exhibits biocontrol activity against *Xanthomonas phaseoli* pv. *manihotis*. *Biology*, *12*(9), 1231. https://doi.org/10.3390/biology12091231

Ferrarini, E., Špacapan, M., Lam, V. B., McCann, A., Cesa-Luna, C., Marahatta, B. P., De Pauw, E., De Mot, R., Venturi, V., & Höfte, M. (2022). Versatile role of *Pseudomonas fuscovaginae* cyclic lipopeptides in plant and microbial interactions. *Frontiers in Plant Science*, *13*, 1008980. https://doi.org/10.3389/fpls.2022.1008980

Fessia, A., Barra, P., Barros, G., & Nesci, A. (2022). Could *Bacillus* biofilms enhance the effectivity of biocontrol strategies in the phyllosphere? *Journal of Applied Microbiology*, *133*(4), 2148–2166. https://doi.org/10.1111/jam.15596

Fessia, A., Ponzio, R., Arcibia, L., Barros, G., & Nesci, A. (2024). Effects of different light wavelengths on *Bacillus subtilis* and *Bacillus velezensis*, two biocontrol agents isolated from the maize phyllosphere. *Archives of Microbiology*, *206*, 104. https://doi.org/10.1007/s00203-024-03836-5

Fiaboe, K. R., Agboka, K., Agboyi, L. K., Koffi, D., Ofoe, R., Kpadonou, G. E., Agnamba, A. O., Assogba, K., Adjevi, M. K. A., Zanou, K. T., & Fening, O. K. (2021). First report and distribution of the South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Togo. *Phytoparasitica*, *49*(2), 167–177. https://doi.org/10.1007/s12600-020-00841-4

Figueredo, E. F., Cruz, T. A. da, Almeida, J. R. de, Batista, B. D., Marcon, J., Andrade, P. A. M. de, Hayashibara, C. A. de A., Rosa, M. S., Azevedo, J. L., & Quecine, M. C. (2023). The key role of indole-3-acetic acid biosynthesis by *Bacillus thuringiensis* RZ2MS9 in promoting maize growth revealed by the ipdC gene knockout mediated by the CRISPR-Cas9 system. *Microbiological Research*, *266*, 127218. https://doi.org/10.1016/j.micres.2022.127218

Fira, D., Dimki, I., Beri, T., & Lozo, J. (2018). Biological control of plant pathogens by *Bacillus* species. *Journal of Biotechnology*, *285*, 44–55. https://doi.org/10.1016/j.jbiotec.2018.07.044

Flühe, L., Burghaus, O., Wieckowski, B. M., Giessen, T. W., Linne, U., & Marahiel, M. A. (2013). Two [4Fe-4S] clusters containing radical SAM enzyme SkfB catalyze thioether bond formation during the maturation of the sporulation killing factor. *Journal of the American Chemical Society*, *135*(3), 959–962. https://doi.org/10.1021/ja310542g

FPS Health, F. C. S. and E. (2023). *Phytoweb | Plant protection and Fertilising Products*. https://fytoweb.be/en

Freitas, M. A., Medeiros, F. H. V., Melo, I. S., Pereira, P. F., Peñaflor, M. F. G. V., Bento, J. M. S., & Paré, P. W. (2019). Stem inoculation with bacterial strains *Bacillus amyloliquefaciens* (GB03) and *Microbacterium imperiale* (MAIIF2a) mitigates *Fusarium* root rot in cassava. *Phytoparasitica*, *47*, 135–142. https://doi.org/10.1007/s12600-018-0706-2

Freyre-González, J. A., Manjarrez-Casas, A. M., Merino, E., Martinez-Nuñez, M., Perez-Rueda, E., & Gutiérrez-Ríos, R.-M. (2013). Lessons from the modular organization of the transcriptional regulatory network of *Bacillus subtilis*. *BMC Systems Biology*, *7*, 127. http://www.biomedcentral.com/1752-0509/7/127

Fu, R., & Feng, H. (2024). Deciphering bacterial chemorepulsion: The complex response of microbes to environmental stimuli. *Microorganisms*, *12*(8), 1706. https://doi.org/10.3390/microorganisms12081706

Fu, Y., Zhou, L., & Kuipers, O. P. (2023). Discovery, biosynthesis, and characterization of a lanthipeptide from *Bacillus subtilis* EH11 with a unique lanthionine ring pattern. *Cell Reports Physical Science*, *4*(8), 101524. https://doi.org/10.1016/j.xcrp.2023.101524

Fuentes, B., Choque, A., Gómez, F., Alarcón, J., Castro-Nallar, E., Arenas, F., Contreras, D., Mörchen, R., Amelung, W., Knief, C., Moradi, G., Klumpp, E., Saavedra, C. P., Prietzel, J., Klysubun, W., Remonsellez, F., & Bol, R. (2022). Influence of physical-chemical soil parameters on microbiota composition and diversity in a deep hyperarid core of the Atacama desert. *Frontiers in Microbiology*, *12*, 794743. https://doi.org/10.3389/fmicb.2021.794743

Gaberell, L., & Viret, G. (2020). *Les géants de l'agrochimie gagnent des milliards grâce à des pesticides cancérogènes ou néfastes pour les abeilles*. https://www.publiceye.ch/fr/thematiques/pesticides/analyse-ventes-pesticides-2018

Gaidashova, S., Karemera, F., & Karamura, E. (2010). Agronomic performance of introduced banana varieties in lowlands of Rwanda. *African Crop Science Journal*, *16*(1), 9–16. https://doi.org/10.4314/acsj.v16i1.54321

Galiano-Carneiro, A. L., & Miedaner, T. (2017). Genetics of resistance and pathogenicity in the maize *Setosphaeria turcica* pathosystem and implications for breeding. *Frontiers in Plant Science*, *8*, 1490. https://doi.org/10.3389/fpls.2017.01490

Galli, M., Feldmann, F., Vogler, U. K., & Kogel, K. H. (2024). Can biocontrol be the game-changer in integrated pest management? A review of definitions, methods and strategies. *Journal of Plant Diseases and Protection*, *131*(2), 265–291. https://doi.org/10.1007/s41348-024-00878-1

Garrido-Sanz, D., Čaušević, S., Vacheron, J., Heiman, C. M., Sentchilo, V., van der Meer, J. R., & Keel, C. (2023). Changes in structure and assembly of a species-rich soil natural community with contrasting nutrient availability upon establishment of a plant-beneficial *Pseudomonas* in the wheat rhizosphere. *Microbiome*, *11*(1), 214. https://doi.org/10.1186/s40168-023-01660-5

Garrido-Sanz, D., Manzano, J., Martín, M., Redondo-Nieto, M., & Rivilla, R. (2018). Metagenomic analysis of a biphenyl-degrading soil bacterial consortium reveals the

metabolic roles of specific populations. *Frontiers in Microbiology*, *9*, 232. https://doi.org/10.3389/fmicb.2018.00232

Gastélum, G., Ángeles-Morales, A., Arellano-Wattenbarger, G., Coronado, Y., Guevara-Hernandez, E., & Rocha, J. (2024). Biofilm formation and maize root-colonization of seed-endophytic bacilli isolated from native maize landraces. *Applied Soil Ecology*, *199*, 105390. https://doi.org/10.1016/j.apsoil.2024.105390

Gerst, M. M., Somogyi, Á., Yang, X., & Yousef, A. E. (2022). Detection and characterization of a rare two-component lantibiotic, amyloliquecidin GF610 produced by *Bacillus velezensis*, using a combination of culture, molecular and bioinformatic analyses. *Journal of Applied Microbiology*, *132*(2), 994–1007. https://doi.org/10.1111/jam.15290

Ghadge, V., Kumar, P., Singh, S., Mathew, D. E., Bhattacharya, S., Nimse, S. B., & Shinde, P. B. (2020). Natural melanin produced by the endophytic *Bacillus subtilis* 4NP-BL associated with the halophyte *Salicornia brachiata*. *Journal of Agricultural and Food Chemistry*, *68*(25), 6854–6863. https://doi.org/10.1021/ACS.JAFC.0C01997

Giorgio, A., Lo Cantore, P., Shanmugaiah, V., & Lamorte, D. (2016). Rhizobacteria isolated from common bean in southern Italy as potential biocontrol agents against common bacterial blight. *Eur J Plant Pathol*, *144*, 297–309. https://doi.org/10.1007/s10658-015-0767-8

Gohil, R. B., Raval, V. H., Panchal, R. R., & Rajput, K. N. (2022). Plant growth-promoting activity of *Bacillus* sp. PG-8 isolated from fermented Panchagavya and its effect on the growth of *Arachis hypogea*. *Frontiers in Agronomy*, *4*, 805454. https://doi.org/10.3389/fagro.2022.805454

Gong, W., Wang, J., Chen, Z., Xia, B., & Lu, G. (2011). Solution structure of LCI, a novel antimicrobial peptide from *Bacillus subtilis*. *Biochemistry*, *50*(18), 3621–3627. https://doi.org/10.1021/bi200123w

González-Pastor, J. E., Hobbs, E. C., & Losick, R. (2003). Cannibalism by sporulating bacteria. *Science*, *301*(5632), 510–513. https://doi.org/10.1126/science.1086462

Gordon, M. I., Thomas, W. J., & Putnam, M. L. (2024). Transmission and management of pathogenic *Agrobacterium tumefaciens* and *Rhodococcus fascians* in select ornamentals. *Plant Disease*, *108*(1), 50–61. https://doi.org/10.1094/PDIS-11-22-2557-RE

Goswami, G., Panda, D., Samanta, R., Boro, R. C., Modi, M. K., Bujarbaruah, K. M., & Barooah, M. (2018a). *Bacillus megaterium* adapts to acid stress condition through a network of genes: Insight from a genome-wide transcriptome analysis. *Scientific Reports*, *8*(1), 16105. https://doi.org/10.1038/s41598-018-34221-0

Goswami, G., Panda, D., Samanta, R., Boro, R. C., Modi, M. K., Bujarbaruah, K. M., & Barooah, M. (2018b). Bacillus megaterium adapts to acid stress condition through a network of genes: Insight from a genome-wide transcriptome analysis. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-34221-0

Gotor-Vila, A., Usall, J., Torres, R., Abadias, M., & Teixidó, N. (2017). Formulation of the biocontrol agent *Bacillus amyloliquefaciens* CPA-8 using different approaches:

Liquid, freeze-drying and fluid-bed spray-drying. *BioControl*, *62*(4), 545–555. https://doi.org/10.1007/s10526-017-9802-3

Govender, V., Korsten, L., & Sivakumar, D. (2005). Semi-commercial evaluation of *Bacillus licheniformis* to control mango postharvest diseases in South Africa. *Postharvest Biology and Technology*, *38*(1), 57–65. https://doi.org/10.1016/j.postharvbio.2005.04.005

Grandclément, C., Tannières, M., Moréra, S., Dessaux, Y., & Faure, D. (2015). Quorum quenching: Role in nature and applied developments. *FEMS Microbiology Reviews*, *40*(1), 86–116. https://doi.org/10.1093/femsre/fuv038

Grant, J. R., Enns, E., Marinier, E., Mandal, A., Herman, E. K., Chen, C. Y., Graham, M., Van Domselaar, G., & Stothard, P. (2023). Proksee: In-depth characterization and visualization of bacterial genomes. *Nucleic Acids Research*, *51*(W1), W484–W492. https://doi.org/10.1093/nar/gkad326

Grigoreva, A., Andreeva, J., Bikmetov, D., Rusanova, A., Serebryakova, M., Garcia, A. H., Slonova, D., Nair, S. K., Lippens, G., Severinov, K., & Dubiley, S. (2021). Identification and characterization of andalusicin: N-terminally dimethylated class III lantibiotic from *Bacillus thuringiensis* sv. *andalousiensis*. *IScience*, *24*(5), 102480. https://doi.org/10.1016/j.isci.2021.102480

Grinter, R., Milner, J., & Walker, D. (2012). Ferredoxin containing bacteriocins suggest a novel mechanism of iron uptake in *Pectobacterium* spp. *PLoS ONE*, *7*(3), e33033. https://doi.org/10.1371/journal.pone.0033033

Groupe de la Banque mondiale. (2023). *Les données ouvertes de la Banque mondiale*. https://donnees.banquemondiale.org/

Grubbs, K. J., Bleich, R. M., Santa Maria, K. C., Allen, S. E., Farag, S., Shank, E. A., & Bowers, A. A. (2017). Large-scale bioinformatics analysis of *Bacillus* genomes uncovers conserved roles of natural products in bacterial physiology. *MSystems*, *2*(6), e00040-17. https://doi.org/10.1128/mSystems.00040-17

Gu, S., Shao, J., He, R., Xiong, G., Qu, Z., Shao, Y., Yu, L., Zhang, D., Wang, F., Xu, R., Guo, P., Xi, N., Li, Y., Wu, Y., Wei, Z., & Li, Z. (2025). Forging the iron-net: Towards a quantitative understanding of microbial communities via siderophore-mediated interactions. *Quantitative Biology*, *13*(2), e84. https://doi.org/10.1002/qub2.84

GUCE. (2022). *Quantités des pesticides en RDC*. https://invoice.segucerdc.cd/invoice/Account/Login

Guimarães Pacifico, M., Eckstein, B., & Bettiol, W. (2021). Screening of *Bacillus* for the development of bioprotectants for the control of *Fusarium oxysporum* f. sp. *vasinfectum* and *Meloidogyne incognita*. *Biological Control*, *164*, 104764. https://doi.org/10.1016/j.biocontrol.2021.104764

Guo, J., Gao, W., Zhang, X., Pan, W., Zhang, X., Man, Z., & Cai, Z. (2024). Enhancing the thermostability and catalytic activity of *Bacillus subtilis* chitosanase by saturation mutagenesis of Lys242. *Biotechnology Journal*, *19*(1), 2300010. https://doi.org/10.1002/biot.202300010

Gupta, A., Chug, A., & Singh, A. P. (2024). Meteorological factor-based tomato early blight prediction using hyperparameter tuning of intelligent classifiers. *Agricultural Research*, *13*(2), 232–242. https://doi.org/10.1007/s40003-023-00691-6

Gurmessa, B. (2021). Soil acidity challenges and the significance of liming and organic amendments in tropical agricultural lands with reference to Ethiopia. *Environment, Development and Sustainability*, *23*(1), 77–99. https://doi.org/10.1007/s10668-020-00615-2

Haghverdi, M., Taghavi, S. M., Zarei, S., Mafakheri, H., Abachi, H., Briand, M., Taghouti, G., Portier, P., Jacques, M.-A., & Osdaghi, E. (2025). Pink-pigmented variant of *Clavibacter michiganensis* expands phenotypic range of tomato bacterial canker pathogen. *Phytopathology*®, *115*(4), 343–353. https://doi.org/10.1094/PHYTO-07-24-0236-R

Halami, P. M. (2019). Sublichenin, a new subtilin-like lantibiotics of probiotic bacterium *Bacillus licheniformis* MCC 2512 T with antibacterial activity. *Microbial Pathogenesis*, *128*, 139–146. https://doi.org/10.1016/j.micpath.2018.12.044

Hamrouni, R., Regus, F., Farnet Da Silva, A. M., Orsière, T., Boudenne, J.-L., Laffont-Schwob, I., Christen, P., & Dupuy, N. (2025). Current status and future trends of microbial and nematode-based biopesticides for biocontrol of crop pathogens. *Critical Reviews in Biotechnology*, *45*(2), 333–352. https://doi.org/10.1080/07388551.2024.2370370ï

Han, Q., Wu, F., Wang, X., Qi, H., Shi, L., Ren, A., Liu, Q., Zhao, M., & Tang, C. (2015). The bacterial lipopeptide iturins induce *Verticillium dahliae* cell death by affecting fungal signalling pathways and mediate plant defence responses involved in pathogen-associated molecular pattern-triggered immunity. *Environmental Microbiology*, *17*(4), 1166–1188. https://doi.org/10.1111/1462-2920.12538

Han, S., Chen, J., Zhao, Y., Cai, H., & Guo, C. (2021). *Bacillus subtilis* HSY21 can reduce soybean root rot and inhibit the expression of genes related to the pathogenicity of *Fusarium oxysporum*. *Pesticide Biochemistry and Physiology*, *178*, 104916. https://doi.org/10.1016/j.pestbp.2021.104916

Han, X., Shen, D., Xiong, Q., Bao, B., Zhang, W., Dai, T., Zhao, Y., Borriss, R., & Fan, B. (2021). The plant-beneficial rhizobacterium *Bacillus velezensis* FZB42 controls the soybean pathogen *phytophthora sojae* due to bacilysin production. *Applied and Environmental Microbiology*, *87*(23), e01601-21. https://doi.org/10.1128/AEM.01601-21

Han, Z., Lin, Q., Zhang, S., Zhou, X., Li, S., Sun, F., Shen, C., & Su, X. (2023). High PCBs mineralization capability of a resuscitated strain *Bacillus* sp. LS1 and its survival in PCB-contaminated soil. *Science of the Total Environment*, *856*, 159224. https://doi.org/10.1016/j.scitotenv.2022.159224

Hao, K., Ullah, H., Qin, X., Li, H., Li, F., & Guo, P. (2019). Effectiveness of *Bacillus pumilus* PDSLzg-1, an innovative hydrocarbon-degrading bacterium conferring antifungal and plant growth-promoting function. *3 Biotech*, *9*(8), 305. https://doi.org/10.1007/s13205-019-1842-1

Haque, F., Fan, C., & Lee, Y. Y. (2023). From waste to value: Addressing the relevance of waste recovery to agricultural sector in line with circular economy. *Journal of Cleaner Production*, *415*, 137873. https://doi.org/10.1016/j.jclepro.2023.137873

Haque, M., Mosharaf, K., Khatun, M., Haque, A., Biswas, S., Islam, S., Islam, M., Shozib, B. H., Miah, M. U., Molla, A. H., & Siddiquee, M. A. (2020). Biofilm producing rhizobacteria with multiple plant growth-promoting traits promote growth of tomato under water-deficit stress. *Frontiers in Microbiology*, *11*, 542053. https://doi.org/10.3389/fmicb.2020.542053

Harahagazwe, D., Ndayiragije, P., & Ntimpirangeza, M. (2007). *Les maladies et ravageurs de quelques cultures à racines et tubercules*. ISABU. file:///C:/Users/32467/Downloads/MaladiesetravageursdesracinesettuberculesauBDI%20(1).pdf

Harwood, C. R., & Kikuchi, Y. (2022). The ins and outs of *Bacillus* proteases: Activities, functions and commercial significance. *FEMS Microbiology Reviews*, *46*(1), fuab046. https://doi.org/10.1093/femsre/fuab046

Hasan, A., Tabassum, B., Hashim, M., & Khan, N. (2024). Role of plant growth promoting rhizobacteria (PGPR) as a plant growth enhancer for sustainable agriculture: A Review. *Bacteria*, *3*(2), 59–75. https://doi.org/10.3390/bacteria3020005

Hasan, N., Khan, I. U., Farzand, A., Heng, Z., Moosa, A., Saleem, M., & Canming, T. (2022). *Bacillus altitudinis* HNH7 and *Bacillus velezensis* HNH9 promote plant growth through upregulation of growth-promoting genes in upland cotton. *Journal of Applied Microbiology*, *132*(5), 3812–3824. https://doi.org/10.1111/jam.15511

Hashem, A., Tabassum, B., & Fathi Abd_Allah, E. (2019). *Bacillus subtilis*: A plant-growth promoting rhizobacterium that also impacts biotic stress. *Saudi Journal of Biological Sciences*, *26*(6), 1291–1297. https://doi.org/10.1016/j.sjbs.2019.05.004

Hassanisaadi, M. (2024). Surfactin as a multifaceted biometabolite for sustainable plant defense: A review. *Journal of Plant Pathology*, *107*, 149–165. https://doi.org/10.1007/s42161-024-01645-9

Hazarika, D. J., Bora, S. S., Naorem, R. S., Sharma, D., Boro, R. C., & Barooah, M. (2023). Genomic insights into *Bacillus subtilis* MBB3B9 mediated aluminium stress mitigation for enhanced rice growth. *Scientific Reports*, *13*, 16467. https://doi.org/10.1038/s41598-023-42804-9

He, A. L., Zhao, L. Y., Ren, W., Li, H. R., Paré, P. W., Zhao, Q., & Zhang, J. L. (2023). A volatile producing *Bacillus subtilis* strain from the rhizosphere of *Haloxylon ammodendron* promotes plant root development. *Plant and Soil*, *486*(1–2), 661–680. https://doi.org/10.1007/s11104-023-05901-2

He, P., Cui, W., Munir, S., He, P., Huang, R., Li, X., Wu, Y., Wang, Y., Yang, J., Tang, P., He, Y., & He, P. (2023). Fengycin produced by *Bacillus subtilis* XF-1 plays a major role in the biocontrol of chinese cabbage clubroot via direct effect and defense stimulation. *Journal of Cellular Physiology*, *239*(10), 1–12. https://doi.org/10.1002/jcp.30991

Hegemann, J. D., & Süssmuth, R. D. (2020). Matters of class: Coming of age of class III and IV lanthipeptides. *RSC Chemical Biology*, *1*(3), 110–127. https://doi.org/10.1039/d0cb00073f

Heinzmann, S., Entian, K. D., & Stein, T. (2006). Engineering *Bacillus subtilis* ATCC 6633 for improved production of the lantibiotic subtilin. *Applied Microbiology and Biotechnology*, *69*(5), 532–536. https://doi.org/10.1007/s00253-005-0023-9

Helepciuc, F. E., & Todor, A. (2023). Making the best of research investment in pathogens control through biocontrol. How is research correlated with agricultural microbial biological control product availability? *PLoS Pathogens*, *19*(1), e1011071. https://doi.org/10.1371/journal.ppat.1011071

Helmy, N. M., & Parang, K. (2023). Cyclic peptides with antifungal properties derived from bacteria, fungi, plants, and synthetic sources. *Pharmaceuticals*, *16*(6), 892. https://doi.org/10.3390/ph16060892

Henz, S. R., Huson, D. H., Auch, A. F., Nieselt-Struwe, K., & Schuster, S. C. (2005). Whole-genome prokaryotic phylogeny. *Bioinformatics*, *21*(10), 2329–2335. https://doi.org/10.1093/bioinformatics/bth324

Hernandez Garcia, A., & Nair, S. K. (2023). Structure and function of a class III metal-independent lanthipeptide synthetase. *ACS Central Science*, *9*(10), 1944–1956. https://doi.org/10.1021/acscentsci.3c00484

Herzner, A. M., Dischinger, J., Szekat, C., Josten, M., Schmitz, S., Yakéléba, A., Reinartz, R., Jansen, A., Sahl, H. G., Piel, J., & Bierbaum, G. (2011). Expression of the lantibiotic mersacidin in *Bacillus amyloliquefaciens* FZB42. *PLoS ONE*, *6*(7), e22389. https://doi.org/10.1371/journal.pone.0022389

Hidalgo-Castellanos, J., Marín-Peña, A. J., Herrera-Cervera, J. A., & López-Gómez, M. (2022). Polyamines: Key elements in the rhizobia-legume symbiosis? *Phytochemistry Reviews*, *21*(1), 127–140. https://doi.org/10.1007/s11101-021-09751-7

Hoff, G., Arias, A. A., Boubsi, F., Pršic, J., Meyer, T., Ibrahim, H. M. M., Steels, S., Luzuriaga, P., Legras, A., Franzil, L., Lequart-Pillon, M., Rayon, C., Osorio, V., de Pauw, E., Lara, Y., Deboever, E., de Coninck, B., Jacques, P., Deleu, M., … Ongena, M. (2021). Surfactin stimulated by pectin molecular patterns and root exudates acts as a key driver of the *Bacillus*-plant mutualistic interaction. *MBio*, *12*(6), e01774-21. https://doi.org/10.1128/mBio.01774-21

Hossain, M. A., Hossain, M. S., & Akter, M. (2023). Challenges faced by plant growth-promoting bacteria in field-level applications and suggestions to overcome the barriers. *Physiological and Molecular Plant Pathology*, *126*, 102029. https://doi.org/10.1016/j.pmpp.2023.102029

Hu, J., Yang, T., Friman, V. P., Kowalchuk, G. A., Hautier, Y., Li, M., Wei, Z., Xu, Y., Shen, Q., & Jousset, A. (2021). Introduction of probiotic bacterial consortia promotes plant growth via impacts on the resident rhizosphere microbiome. *Proceedings of the Royal Society B*, *288*, 20211396. https://doi.org/10.1098/rspb.2021.1396

Hu, Q., Xiao, Y., Liu, Z., Huang, X., Dong, B., & Wang, Q. (2024). *Bacillus subtilis* QM3, a plant growth-promoting rhizobacteria, can promote wheat seed germination by gibberellin pathway. *Journal of Plant Growth Regulation*, *43*(8), 2682–2695. https://doi.org/10.1007/s00344-024-11298-8

Huang, C. J., Pauwelyn, E., Ongena, M., Bleyaert, P., & Höfte, M. (2024). Both GacS-regulated lipopeptides and the type three secretion system contribute to *Pseudomonas cichorii* induced necrosis in lettuce and chicory. *Research in Microbiology*, *176*, 104249. https://doi.org/10.1016/j.resmic.2024.104249

Huang, X., Cui, C., Hou, E., Li, F., Liu, W., Jiang, L., Luo, Y., & Xu, X. (2022). Acidification of soil due to forestation at the global scale. *Forest Ecology and Management*, *505*, 119951. https://doi.org/10.1016/j.foreco.2021.119951

Huang, Y., Roseboom, W., Brul, S., & Kramer, G. (2023). Multi-omics analysis of *Bacillus subtilis* spores formed at different environmental temperatures reveal differences at the morphological and molecular level. *BioRxiv*. https://doi.org/10.1101/2023.06.22.546136

Hudson, G. A., & Mitchell, D. A. (2018). RiPP antibiotics: Biosynthesis and engineering potential. *Current Opinion in Microbiology*, *45*, 61–69. https://doi.org/10.1016/j.mib.2018.02.010

Idris, A. L., Li, W., Huang, F., Lin, F., Guan, X., & Huang, T. (2024). Impacts of UV radiation on *Bacillus* biocontrol agents and their resistance mechanisms. *World Journal of Microbiology and Biotechnology*, *40*, 58. https://doi.org/10.1007/s11274-023-03856-1

Igiehon, B. C., Babalola, O. O., & Hassen, A. I. (2024). Rhizosphere competence and applications of plant growth-promoting rhizobacteria in food production - A review. *Scientific African*, *23*, e02081. https://doi.org/10.1016/j.sciaf.2024.e02081

Ikram, M., Naeem, M., Zahoor, M., Hanafiah, M. M., Oyekanmi, A. A., Islam, N. U., Ullah, M., Mahnashi, M. H., Ali, A. Al, Jalal, N. A., Bantun, F., Momenah, A. M., & Sadiq, A. (2022). *Bacillus subtilis* : As an efficient bacterial strain for the reclamation of water loaded with textile Azo dye, Orange II. *International Journal of Molecular Sciences*, *23*(18), 10637. https://doi.org/10.3390/ijms231810637

Im, S. M., Yu, N. H., Joen, H. W., Kim, S. O., Park, H. W., Park, A. R., & Kim, J.-C. (2020). Biological control of tomato bacterial wilt by oxydifficidin and difficidin-producing *Bacillus methylotrophicus* DR-08. *Pesticide Biochemistry and Physiology*, *163*, 130–137. https://doi.org/10.1016/j.pestbp.2019.11.007

Imran, M., Abo-Elyousr, K. A. M., Mousa, M. A. A., & Saad, M. M. (2022). A study on the synergetic effect of *Bacillus amyloliquefaciens* and dipotassium phosphate on *Alternaria solani* causing early blight disease of tomato. *European Journal of Plant Pathology*, *162*(1), 63–77. https://doi.org/10.1007/s10658-021-02384-8

Iqbal, M., Naveed, M., Sanaullah, M., Brtnicky, M., Hussain, M. I., Kucerik, J., Holatko, J., & Mustafa, A. (2023). Plant microbe mediated enhancement in growth and yield of canola (*Brassica napus* L.) plant through auxin production and increased nutrient acquisition. *Journal of Soils and Sediments*, *23*(3), 1233–1249. https://doi.org/10.1007/s11368-022-03386-7

Iqbal, S., Begum, F., Rabaan, A. A., Aljeldah, M., Al Shammari, B. R., Alawfi, A., Alshengeti, A., Sulaiman, T., & Khan, A. (2023). Classification and multifaceted potential of secondary metabolites produced by *Bacillus subtilis* group: A Comprehensive review. *Molecules*, *28*(3), 927. https://doi.org/10.3390/molecules28030927

Iqbal, S., Ullah, N., & Janjua, H. A. (2021). *In Vitro* evaluation and genome mining of *Bacillus subtilis* strain RS10 reveals its biocontrol and plant growth-promoting potential. *Agriculture*, *11*(12), 1273. https://doi.org/10.3390/agriculture11121273

Iqbal, Z., Ahmad, M., Raza, M. A., Hilger, T., & Rasche, F. (2024). Phosphate-solubilizing *Bacillus* sp. modulate soil exoenzyme activities and improve wheat growth. *Microbial Ecology*, *87*(1), 31. https://doi.org/10.1007/s00248-023-02340-5

Irfan, M., Asghar, U., Nadeem, M., Nelofer, R., & Syed, Q. (2016). Optimization of process parameters for xylanase production by *Bacillus* sp. in submerged fermentation. *Journal of Radiation Research and Applied Sciences*, *9*(2), 139–147. https://doi.org/10.1016/j.jrras.2015.10.008

Isabirye, B. E., & Rwomushana, I. (2016). Current and future potential distribution of maize chlorotic mottle virus and risk of maize lethal necrosis disease in Africa. *J. Crop Prot*, *2016*(2), 215–228. https://doi.org/10.18869/modares.jcp.5.2.215

Ishida, K., Nakamura, A., & Kojima, S. (2022). Crystal structure of the AlbEF complex involved in subtilosin A biosynthesis. *Structure*, *30*(12), 1637-1646.e3. https://doi.org/10.1016/j.str.2022.10.002

Islam, T., Rabbee, M. F., Choi, J., & Baek, K. H. (2022). Biosynthesis, molecular regulation, and application of bacilysin produced by *Bacillus* species. *Metabolites*, *12*(5), 397. https://doi.org/10.3390/metabo12050397

Ivanova, L. A., Egorov, V. V., Zabrodskaya, Y. A., Shaldzhyan, A. A., Baranchikov, A. Ye., Tsvigun, N. V., Lykholay, A. N., Yapryntsev, A. D., Lebedev, D. V., & Kulminskaya, A. A. (2023). Matrix is everywhere: Extracellular DNA is a link between biofilm and mineralization in *Bacillus cereus* planktonic lifestyle. *Npj Biofilms and Microbiomes*, *9*, 9. https://doi.org/10.1038/s41522-023-00377-5

Jacob, S. M., & Paranthaman, S. (2023). Biofertilizers: an advent for eco-friendly and sustainable agriculture development. *Vegetos*, *36*(4), 1141–1153. https://doi.org/10.1007/s42535-022-00550-9

Jain, C., Rodriguez-R, L. M., Phillippy, A. M., Konstantinidis, K. T., & Aluru, S. (2018). High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nature Communications*, *9*(1), 5114. https://doi.org/10.1038/s41467-018-07641-9

Jalal, A., Filho, M. C. M. T., da Silva, E. C., da Silva Oliveira, C. E., Freitas, L. A., & do Nascimento, V. (2022). Plant growth-promoting bacteria and nitrogen fixing bacteria: Sustainability of non-legume crops. In K. D. Maheshwari, R. Dobhal, & S. Dheeman (Eds.), *Nitrogen Fixing Bacteria: Sustainable Growth of Non-legumes. Microorganisms for Sustainability* (Vol. 36, pp. 233–275). Springer. https://doi.org/10.1007/978-981-19-4906-7_11

Jang, S., Choi, S.-K., Zhang, H., Zhang, S., Ryu, C.-M., & Kloepper, J. W. (2023). History of a model plant growth-promoting rhizobacterium, *Bacillus velezensis* GB03: From isolation to commercialization. *Frontiers in Plant Science*, *14*, 1279896. https://doi.org/10.3389/fpls.2023.1279896

Jayakumar, A., Nair, I. C., & Radhakrishnan, E. K. (2021). Environmental adaptations of an extremely plant beneficial *Bacillus subtilis* Dcl1 identified through the genomic and metabolomic analysis. *Microbial Ecology*, *81*(3), 687–702. https://doi.org/10.1007/s00248-020-01605-7

Jensen, C. N. G., Pang, J. K. Y., Hahn, C. M., Gottardi, M., Husted, S., Moelbak, L., Kovács, Á. T., Fimognari, L., & Schulz, A. (2024). Differential influence of *Bacillus subtilis* strains on *Arabidopsis* root architecture through common and distinct plant hormonal pathways. *Plant Science*, *339*, 111936. https://doi.org/10.1016/j.plantsci.2023.111936

Jha, Y., Yadav, K. A., & Mohamed, H. I. (2024). Plant growth-promoting bacteria and exogenous phytohormones alleviate the adverse effects of drought stress in pigeon pea plants. *Plant and Soil*, *505*(1–2), 163–183. https://doi.org/10.1007/s11104-023-06155-8

Ji, S. H., Gururani, M. A., & Chun, S. C. (2014). Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. *Microbiological Research*, *169*(1), 83–98. https://doi.org/10.1016/j.micres.2013.06.003

Jiang, B., Wu, C., Liang, Y., Li, X., Li, J., & Song, G. (2023). *Bacillus* co-culture inhibits quorum sensing in *Pseudomonas aeruginosa*. *Current Microbiology*, *80*(4), 123. https://doi.org/10.1007/s00284-023-03218-y

Jimbere. (2019). Le cancer au Burundi : « Le diagnostic et le traitement coûtent les yeux de la tête ». *Jimbere Magazine*.

Kai, M. (2020). Diversity and distribution of volatile secondary metabolites throughout *Bacillus subtilis* isolates. *Frontiers in Microbiology*, *11*, 559. https://doi.org/10.3389/fmicb.2020.00559

Kakar, K. U., Duan, Y.-P., Nawaz, Z., Sun, G., Almoneafy, A. A., Hassan, M. A., Elshakh, A., Li, B., & Xie, G.-L. (2014). A novel rhizobacterium Bk7 for biological control of brown sheath rot of rice caused by *Pseudomonas fuscovaginae* and its mode of action. *European Journal of Plant Pathology*, *138*(4), 819–834. https://doi.org/10.1007/s10658-013-0356-7

Kalamara, M., Abbott, J., Sukhodub, T., Macphee, C., & Stanley-Wall, N. R. (2023). The putative role of the epipeptide EpeX in *Bacillus subtilis* intraspecies competition. *Microbiology*, *169*(6), 001344. https://doi.org/10.1099/mic.0.001344

Kalamara, M., Spacapan, M., Mandic-Mulec, I., & Stanley-Wall, N. R. (2018). Social behaviours by *Bacillus subtilis*: Quorum sensing, kin discrimination and beyond. *Molecular Microbiology*, *110*(6), 863–878. https://doi.org/10.1111/mmi.14127

Kalyon, B., Helaly, S. E., Scholz, R., Nachtigall, J., Vater, J., Borriss, R., & Süssmuth, R. D. (2011). Plantazolicin A and B: Structure elucidation of ribosomally synthesized

thiazole/oxazole peptides from *Bacillus amyloliquefaciens* FZB42. *Organic Letters*, *13*(12), 2996–2999. https://doi.org/10.1021/ol200809m

Kang, E., Li, Y., Zhang, X., Yan, Z., Wu, H., Li, M., Yan, L., Zhang, K., Wang, J., & Kang, X. (2021). Soil pH and nutrients shape the vertical distribution of microbial communities in an alpine wetland. *Science of the Total Environment*, *774*, 145780. https://doi.org/10.1016/j.scitotenv.2021.145780

Kang, X.-M., Luo, W. L., Guo, J., & Sun, Q. (2023). Screening and preliminary application of raw amylase-producing bacteria in fermented grains by natural fermentation of solid-state vinegar. *China Condiment*, *48*(10), 78–84.

Kanyange, L., Kamau, J., Ombori, O., Ndayiragije, A., & Muthini, M. (2019). Genotyping for blast (*Pyricularia oryzae*) resistance genes in F2 population of Supa aromatic rice (*Oryza sativa* L.). *International Journal of Genomics*, *2019*(1), 1–10. https://doi.org/10.1155/2019/5246820

Karačić, V., Miljaković, D., Marinković, J., Ignjatov, M., Milošević, D., Tamindžić, G., & Ivanović, M. (2024). *Bacillus* species: Excellent biocontrol agents against tomato diseases. *Microorganisms*, *12*(3), 457. https://doi.org/10.3390/microorganisms12030457

Karlowski, W. M., Varshney, D., & Zielezinski, A. (2023). Taxonomically restricted genes in *Bacillus* may form clusters of homologs and can be traced to a large reservoir of noncoding sequences. *Genome Biology and Evolution*, *15*(3), evad023. https://doi.org/10.1093/gbe/evad023

Karthika, S., Varghese, S., & Jisha, M. S. (2020). Exploring the efficacy of antagonistic rhizobacteria as native biocontrol agents against tomato plant diseases. *3 Biotech*, *10*(7), 320. https://doi.org/10.1007/s13205-020-02306-1

Kaspar, F., Neubauer, P., & Gimpel, M. (2019). Bioactive secondary metabolites from *Bacillus subtilis* : A Comprehensive review. *Journal of Natural Products*, *82*(7), 2038–2053. https://doi.org/10.1021/acs.jnatprod.9b00110

Katsuyama, Y., & Ohnishi, Y. (2012). Type III polyketide synthases in microorganisms. In A. D. Hopwood (Ed.), *Methods in Enzymology* (Vol. 515, pp. 359–377). Academic Press, Elsevier. https://doi.org/10.1016/B978-0-12-394290-6.00017-3

Kaur, Y., & Das, N. (2023). Roles of polyamines in growth and development of the Solanaceous crops under normal and stressful conditions. *Journal of Plant Growth Regulation*, *42*(8), 4989–5010. https://doi.org/10.1007/s00344-022-10841-9

Kavatsurwa, S. M., Kiremire, B., Wasswa, J., & Mpiana, P. T. (2014). Dithiocarbamates residues level in selected vegetables from Bukavu, Democratic Republic of Congo. *Journal of Physical and Chemical Sciences*, *1*(3), 1–7. https://zenodo.org/records/999907

Kawulka, K. E., Sprules, T., Diaper, C. M., Whittal, R. M., McKay, R. T., Mercier, P., Zuber, P., & Vederas, J. C. (2004). Structure of subtilosin A, a cyclic antimicrobial peptide from *Bacillus subtilis* with unusual sulfur to α-carbon cross-links: Formation and reduction of α-thio-α-amino acid derivatives ˈ. *Biochemistry*, *43*(12), 3385–3395. https://doi.org/10.1021/bi0359527

Kaya, C., Ashraf, M., Alyemeni, M. N., Rinklebe, J., & Ahmad, P. (2023). Alleviation of arsenic toxicity in pepper plants by aminolevulinic acid and heme through modulating its sequestration and distribution within cell organelles. *Environmental Pollution*, *330*, 121747. https://doi.org/10.1016/j.envpol.2023.121747

Kenfaoui, J., Dutilloy, E., Benchlih, S., Lahlali, R., Ait-Barka, E., & Esmaeel, Q. (2024). *Bacillus velezensis*: A versatile ally in the battle against phytopathogens-insights and prospects. *Applied Microbiology and Biotechnology*, *108*(1), 439. https://doi.org/10.1007/s00253-024-13255-7

Kenig, M., & Abraham, E. P. (1976). Antimicrobial activities and antagonists of bacilysin and anticapsin. *Journal of General Microbiology*, *94*(1), 37–45. https://doi.org/10.1099/00221287-94-1-37

Keren-Paz, A., Maan, H., Karunker, I., Olender, T., Kapishnikov, S., Dersch, S., Kartvelishvily, E., Wolf, S. G., Gal, A., Graumann, P. L., & Kolodkin-Gal, I. (2022). The roles of intracellular and extracellular calcium in *Bacillus subtilis* biofilms. *IScience*, *25*(6), 104308. https://doi.org/10.1016/j.isci.2022.104308

Keshmirshekan, A., de Souza Mesquita, L. M., & Ventura, S. P. M. (2024). Biocontrol manufacturing and agricultural applications of *Bacillus velezensis*. *Trends in Biotechnology*, *42*(8), 986–1001. https://doi.org/10.1016/j.tibtech.2024.02.003

Keswani, C., Singh, S. P., García-Estrada, C., Mezaache-Aichour, S., Glare, T. R., Borriss, R., Rajput, V. D., Minkina, T. M., Ortiz, A., & Sansinenea, E. (2022). Biosynthesis and beneficial effects of microbial gibberellins on crops for sustainable agriculture. *Journal of Applied Microbiology*, *132*(3), 1597–1615. https://doi.org/10.1111/jam.15348

Khade, O., & Sruthi, K. (2024). The rhizosphere microbiome: A key modulator of plant health and their role in secondary metabolites production. In H. Sarma & J. S. Joshi (Eds.), *Biotechnology of emerging microbes: Prospects for agriculture and environment* (pp. 327–349). Elsevier. https://doi.org/10.1016/B978-0-443-15397-6.00016-4

Khan, A., Doshi, H. V., & Thakur, M. C. (2016). *Bacillus* spp.: A prolific siderophore producer. In M. T. Islam, M. Rahman, P. Pandey, C. K. Jha, & A. Aeron (Eds.), *Bacilli and agrobiotechnology* (pp. 309–323). Springer. https://doi.org/10.1007/978-3-319-44409-3_13

Khan, A. R., Mustafa, A., Hyder, S., Valipour, M., Rizvi, Z. F., Gondal, A. S., Yousuf, Z., Iqbal, R., & Daraz, U. (2022). *Bacillus* spp. as bioagents: Uses and application for sustainable agriculture. *Biology*, *11*(12), 1763. https://doi.org/10.3390/biology11121763

Kharshandi, F., & Kayang, H. (2023). Antagonistic potential of rhizobacterial isolates against fungal pathogens causing rhizome rot in turmeric. *Archives of Microbiology*, *205*, 221. https://doi.org/10.1007/s00203-023-03565-1

Kibiriti, C., Ndayiragiye, S., Gourdin, J., & Hollebosch, P. (1986). *Analyse des végétaux et des aliments. Modes opératoires.* (Vol. 1). Laboratoire de chimie agricole-Institut des sciences agronomiques du Burundi.

Kieber, J. J., & Schaller, G. E. (2018). Cytokinin signaling in plant development. *Development*, *145*(4), dev149344. https://doi.org/10.1242/dev.149344

Kierul, K., Voigt, B., Albrecht, D., Chen, X. H., Carvalhais, L. C., & Borriss, R. (2015). Influence of root exudates on the extracellular proteome of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. *Microbiology*, *161*(1), 131–147. https://doi.org/10.1099/mic.0.083576-0

Kijana, R., Abang, M., Edema, R., Mukankusi, C., & Buruchara, R. (2017). Prevalence of angular leaf spot disease and sources of resistance in common bean in eastern Democratic Republic of Congo. *African Crop Science Journal*, *25*(1), 109. https://doi.org/10.4314/acsj.v25i1.8

Kim, M., Jung, D.-H., Seo, D.-H., Chung, W.-H., & Seo, M.-J. (2020). Genome analysis of *Lactobacillus plantarum* subsp. *plantarum* KCCP11226 reveals a well-conserved C30 carotenoid biosynthetic pathway. *3 Biotech*, *10*(4), 150. https://doi.org/10.1007/s13205-020-2149-y

Kim, M., Oh, H.-S., Park, S.-C., & Chun, J. (2014). Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *International Journal of Systematic and Evolutionary Microbiology*, *64*(Pt_2), 346–351. https://doi.org/10.1099/ijs.0.059774-0

Kim, M.-J., Radhakrishnan, R., Kang, S.-M., You, Y.-H., Jeong, E.-J., Kim, J.-G., & Lee, I.-J. (2017). Plant growth promoting effect of *Bacillus amyloliquefaciens* H-2-5 on crop plants and influence on physiological changes in soybean under soil salinity. *Physiology and Molecular Biology of Plants*, *23*(3), 571–580. https://doi.org/10.1007/s12298-017-0449-4

Kleerebezem, M. (2004). Quorum sensing control of lantibiotic production; nisin and subtilin autoregulate their own biosynthesis. *Peptides*, *25*(9), 1405–1414. https://doi.org/10.1016/j.peptides.2003.10.021

Kleerebezem, M., Bongers, R., Rutten, G., Vos, W. M. de, & Kuipers, O. P. (2004). Autoregulation of subtilin biosynthesis in *Bacillus subtilis*: The role of the *spa*-box in subtilin-responsive promoters. *Peptides*, *25*(9), 1415–1424. https://doi.org/10.1016/j.peptides.2003.11.025

Korangi Alleluya, V., Argüelles Arias, A., Ribeiro, B., De Coninck, B., Helmus, C., Delaplace, P., & Ongena, M. (2023). *Bacillus* lipopeptide-mediated biocontrol of peanut stem rot caused by *Athelia rolfsii*. *Frontiers in Plant Science*, *14*, 1069971. https://doi.org/10.3389/fpls.2023.1069971

Korangi Alleluya, V., Kubindana, G., Fingu-mabola, J. C., Sulu, A., Kasereka, G., Matamba, A., & Ndindir, J. (2021). Utilisation des biopesticides pour une agriculture durable en République Démocratique du Congo (Synthèse bibliographique). *Revue Africaine d'Environnement et d'Agriculture*, *2*, 53–67. www.rafea-congo.com

Kosakivska, I. V., Vedenicheva, N. P., Babenko, L. M., Voytenko, L. V., Romanenko, K. O., & Vasyuk, V. A. (2022). Exogenous phytohormones in the regulation of growth

and development of cereals under abiotic stresses. *Molecular Biology Reports*, *49*(1), 617–628. https://doi.org/10.1007/s11033-021-06802-2

Kulimushi, P. Z., Arias, A. A., Franzil, L., Steels, S., & Ongena, M. (2017). Stimulation of fengycin-type antifungal lipopeptides in Bacillus amyloliquefaciens in the presence of the maize fungal pathogen Rhizomucor variabilis. *Frontiers in Microbiology*, *8*(MAY), 1–12. https://doi.org/10.3389/fmicb.2017.00850

Kulimushi, P. Z., Basime, G. C., Nachigera, G. M., Thonart, P., & Ongena, M. (2018). Efficacy of *Bacillus amyloliquefaciens* as biocontrol agent to fight fungal diseases of maize under tropical climates: From lab to field assays in South Kivu. *Environmental Science and Pollution Research*, *25*(30), 29808–29821. https://doi.org/10.1007/s11356-017-9314-9

Kumar, J., Ramlal, A., Mallick, D., & Mishra, V. (2021). An overview of some biopesticides and their importance in plant protection for commercial acceptance. *Plants*, *10*(6), 1–15. https://doi.org/10.3390/plants10061185

Kumar, R., Singh, A., Shukla, E., Singh, P., Khan, A., Singh, N. K., & Srivastava, A. (2024). Siderophore of plant growth promoting rhizobacterium origin reduces reactive oxygen species mediated injury in *Solanum* spp. caused by fungal pathogens. *Journal of Applied Microbiology*, *135*(2), lxae036. https://doi.org/10.1093/jambio/lxae036

Kumar, S., Anjali, Arutselvan, R., Masurkar, P., Singh, U. B., Tripathi, R., Bhupenchandra, I., Minkina, T., & Keswani, C. (2024). *Bacillus subtilis*-mediated induction of disease resistance and promotion of plant growth of vegetable crops. In V. Mageshwaran, B. U. Singh, K. A. Saxena, & B. H. Singh (Eds.), *Applications of Bacillus and Bacillus-derived genera in agriculture, biotechnology and beyond. Microorganisms for sustainability* (Vol. 51, pp. 165–211). Springer. https://doi.org/10.1007/978-981-99-8195-3_9

Kurepa, J., & Smalle, J. A. (2022). Auxin/cytokinin antagonistic control of the shoot/root growth ratio and its relevance for adaptation to drought and nutrient deficiency stresses. *International Journal of Molecular Sciences*, *23*(4), 1933. https://doi.org/10.3390/ijms23041933

Kutnu, M., Işlerel, E. T., Tunçbağ, N., & Özcengiz, G. (2022). Comparative biological network analysis for differentially expressed proteins as a function of bacilysin biosynthesis in *Bacillus subtilis*. *Integrative Biology* , *14*(5), 99–110. https://doi.org/10.1093/intbio/zyac010

Lahlali, R., Ezrari, S., Radouane, N., Kenfaoui, J., Esmaeel, Q., El Hamss, H., Belabess, Z., & Barka, E. A. (2022). Biological control of plant pathogens: A global perspective. *Microorganisms*, *10*(3), 596. https://doi.org/10.3390/MICROORGANISMS10030596

Laller, R., Khosla, P. K., Negi, N., Avinash, H., Kusum, Thakur, N., Kashyap, S., Shukla, S. K., & Hussain, I. (2023). Bacterial volatiles as PGPRs: Inducing plant defense mechanisms during stress periods. *South African Journal of Botany*, *159*, 131–139. https://doi.org/10.1016/j.sajb.2023.05.041

Lam, V. B., Meyer, T., Arias, A. A., Ongena, M., Oni, F. E., & Höfte, M. (2021). *Bacillus* cyclic lipopeptides iturin and fengycin control rice blast caused by *Pyricularia oryzae* in potting and acid sulfate soils by direct antagonism and induced systemic resistance. *Microorganisms*, *9*(7), 1441. https://doi.org/10.3390/microorganisms9071441

Lammel, D. R., Barth, G., Ovaskainen, O., Cruz, L. M., Zanatta, J. A., Ryo, M., de Souza, E. M., & Pedrosa, F. O. (2018). Direct and indirect effects of a pH gradient bring insights into the mechanisms driving prokaryotic community structures. *Microbiome*, *6*(1), 106. https://doi.org/10.1186/s40168-018-0482-8

Landa, B. B., Navas-Cortés, J. A., & Jiménez-Díaz, R. M. (2004). Influence of temperature on plant–rhizobacteria interactions related to biocontrol potential for suppression of *Fusarium* wilt of chickpea. *Plant Pathology*, *53*(3), 341–352. https://doi.org/10.1111/j.0032-0862.2004.01008.x

Langa-Lomba, N., González-García, V., Venturini-Crespo, M. E., Casanova-Gascón, J., Barriuso-Vargas, J. J., & Martín-Ramos, P. (2023). Comparison of the efficacy of *Trichoderma* and *Bacillus* strains and commercial biocontrol products against grapevine *Botryosphaeria dieback* pathogens. *Agronomy*, *13*(2), 533. https://doi.org/10.3390/agronomy13020533

Lawton, E. M., Cotter, P. D., Hill, C., & Ross, R. P. (2007). Identification of a novel two-peptide lantibiotic, haloduracin, produced by the alkaliphile *Bacillus halodurans* C-125. *FEMS Microbiology Letters*, *267*(1), 64–71. https://doi.org/10.1111/j.1574-6968.2006.00539.x

Leathers, T. D., Saunders, L. P., Bowman, M. J., Price, N. P. J., Bischoff, K. M., Rich, J. O., Skory, C. D., & Nunnally, M. S. (2020). Inhibition of *Erwinia amylovora* by *Bacillus nakamurai*. *Current Microbiology*, *77*(5), 875–881. https://doi.org/10.1007/s00284-019-01845-y

Lee, J., Kim, S., Jung, H., Koo, B.-K., Han, J. A., & Lee, H.-S. (2023). Exploiting bacterial genera as biocontrol agents: Mechanisms, interactions and applications in sustainable agriculture. *Journal of Plant Biology*, *66*(6), 485–498. https://doi.org/10.1007/s12374-023-09404-6

Lee, S. I., Kim, D.-R., & Kwak, Y.-S. (2024). Genome analysis of *Streptomyces recifensis* SN1E1 to investigate mechanisms for inhibiting fire blight disease. *Journal of Applied Microbiology*, *135*(10), lxae253. https://doi.org/10.1093/jambio/lxae253

Legg, J. P., Owor, B., Sseruwagi, P., & Ndunguru, J. (2006). Cassava mosaic virus disease in East and Central Africa: Epidemiology and management of a regional pandemic. *Advances in Virus Research*, *67*, 355–418. https://doi.org/10.1016/S0065-3527(06)67010-3

Leistikow, K. R., May, D. S., Suh, W. S., Vargas Asensio, G., Schaenzer, A. J., Currie, C. R., & Hristova, K. R. (2024). *Bacillus subtilis*-derived peptides disrupt quorum sensing and biofilm assembly in multidrug-resistant *Staphylococcus aureus*. *MSystems*, *9*(8), 1–27. https://doi.org/10.1128/msystems.00712-24

Lewis, K. A., Tzilivakis, J., Warner, D. J., & Green, A. (2016). An international database for pesticide risk assessments and management. *Human and Ecological Risk Assessment*, *22*(4), 1050–1064. https://doi.org/10.1080/10807039.2015.1133242

Li, B., Yan, Z. Y., Liu, X. N., Zhou, J., Wu, X. Y., Wei, P., Jia, H. H., & Yong, X. Y. (2019). Increased fermentative adenosine production by gene-targeted Bacillus subtilis mutation. *Journal of Biotechnology*, *298*, 1–4. https://doi.org/10.1016/j.jbiotec.2019.04.007

Li, C., Alam, K., Zhao, Y., Hao, J., Yang, Q., Zhang, Y., Li, R., & Li, A. (2021). Mining and biosynthesis of bioactive lanthipeptides from microorganisms. *Frontiers in Bioengineering and Biotechnology*, *9*, 692466. https://doi.org/10.3389/fbioe.2021.692466

Li, H. P., Han, Q. Q., Liu, Q. M., Gan, Y. N., Rensing, C., Rivera, W. L., Zhao, Q., & Zhang, J. L. (2023). Roles of phosphate-solubilizing bacteria in mediating soil legacy phosphorus availability. *Microbiological Research*, *272*, 127375. https://doi.org/10.1016/j.micres.2023.127375

Li, J., Peng, C., Mao, A., Zhong, M., & Hu, Z. (2024). An overview of microbial enzymatic approaches for pectin degradation. *International Journal of Biological Macromolecules*, *254*, 127804. https://doi.org/10.1016/j.ijbiomac.2023.127804

Li, S., Liu, Y., Wang, J., Yang, L., Zhang, S., Xu, C., & Ding, W. (2017). Soil acidification aggravates the occurrence of bacterial wilt in South China. *Frontiers in Microbiology*, *8*(APR), 703. https://doi.org/10.3389/fmicb.2017.00703

Li, S. M. M., Piccoli, A. D., McDowell, T., MacDonald, J., Renaud, J., & Yuan, Z. C. (2021). Evaluating the biocontrol potential of canadian strain *Bacillus velezensis* 1B-23 via its surfactin production at various pHs and temperatures. *BMC Biotechnology*, *21*, 31. https://doi.org/10.1186/s12896-021-00690-x

Li, W., Sun, L., Wu, H., Gu, W., Lu, Y., Liu, C., Zhang, J., Li, W., Zhou, C., Geng, H., Li, Y., Peng, H., Shi, C., Wang, D., & Peng, G. (2024). *Bacillus velezensis* YXDHD1-7 prevents early blight disease by promoting growth and enhancing defense enzyme activities in tomato plants. *Microorganisms*, *12*(5), 921. https://doi.org/10.3390/microorganisms12050921

Li, X., Zhang, Y. L., Li, J., Gao, J., Jiang, Y., & Chen, C. Q. (2022). Genomic and transcriptomic analyses of *Bacillus methylotrophicus* NJ13 reveal a molecular response strategy combating *Ilyonectria robusta* causing ginseng rusty root rot. *Biological Control*, *172*, 104972. https://doi.org/10.1016/j.biocontrol.2022.104972

Liboga, O. B., Litucha, B. J., Ngama, B. F., Balimo, I. F., & Kayawa, L. J. (2020). Comportement de cinq variétés de riz pluvial (*Oryza sativa* L.) à la pyriculariose et la verse dans les conditions naturelles à Kisangani, République Démocratique du Congo. *Journal of Applied Biosciences*, *145*, 14965–14973. https://doi.org/10.35759/JABs.v145.11

Lin, H., Lai, C., Yu, G., Sunahara, I. G., Liu, L., Ullah, H., & Liu, J. (2024). Root exudate-driven rhizospheric recruitment of plant growth-promoting rhizobacteria. *Pedosphere*, *35*(1), 216–228. https://doi.org/10.1016/j.pedsph.2024.03.005

Liu, G., Zhang, K., Gong, H., Yang, K., Wang, X., Zhou, G., Cui, W., Chen, Y., & Yang, Y. (2023). Whole genome sequencing and the lignocellulose degradation potential of *Bacillus subtilis* RLI2019 isolated from the intestine of termites. *Biotechnology for Biofuels and Bioproducts*, *16*(1), 30. https://doi.org/10.1186/s13068-023-02375-3

Liu, N., Sun, H., Tang, Z., Zheng, Y., Qi, G., & Zhao, X. (2023). Transcription factor Spo0A regulates the biosynthesis of difficidin in *Bacillus amyloliquefaciens*. *Microbiology Spectrum*, *11*(4), 1–13. https://doi.org/10.1128/spectrum.01044-23

Liu, N., Xu, S., Yao, X., Zhang, G., Mao, W., Hu, Q., Feng, Z., & Gong, Y. (2016). Studies on the control of *Ascochyta* blight in field peas (*Pisum sativum* L.) caused by *Ascochyta pinodes* in Zhejiang Province, China. *Frontiers in Microbiology*, *7*, 481. https://doi.org/10.3389/fmicb.2016.00481

Liu, S., Huang, J., Zhang, C., Wang, L., Fan, C., & Zhong, C. (2022). Probing the growth and mechanical properties of *Bacillus subtilis* biofilms through genetic mutation strategies. *Synthetic and Systems Biotechnology*, *7*(3), 965–971. https://doi.org/10.1016/j.synbio.2022.05.005

Liu, Y., Dai, C., Zuo, Y., Qiao, J., Shen, J., Yin, X., Liu, Y., & Yongfeng Liu, O. (2024). Characterization of siderophore produced by *Bacillus velezensis* YL2021 and its application in controlling rice sheath blight and rice blast. *Phytopathology®*, *114*(12), 1–58. https://doi.org/10.1094/PHYTO-04-24-0148-R PDF

Liu, Y., Lai, Q., Du, J., & Shao, Z. (2016). *Bacillus zhangzhouensis* sp. nov. and *Bacillus australimaris* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, *66*(3), 1193–1199. https://doi.org/10.1099/ijsem.0.000856

Liu, Y., Teng, K., Wang, T., Dong, E., Zhang, M., Tao, Y., & Zhong, J. (2020). Antimicrobial *Bacillus velezensis* HC6: Production of three kinds of lipopeptides and biocontrol potential in maize. *Journal of Applied Microbiology*, *128*(1), 242–254. https://doi.org/10.1111/jam.14459

Liu, Y., Xu, Z., Chen, L., Xun, W., Shu, X., Chen, Y., Sun, X., Wang, Z., Ren, Y., Shen, Q., & Zhang, R. (2024a). Root colonization by beneficial rhizobacteria. *FEMS Microbiology Reviews*, *48*(1), fuad066. https://doi.org/10.1093/femsre/fuad066

Liu, Y., Xu, Z., Chen, L., Xun, W., Shu, X., Chen, Y., Sun, X., Wang, Z., Ren, Y., Shen, Q., & Zhang, R. (2024b). Root colonization by beneficial rhizobacteria. *FEMS Microbiology Reviews*, *48*(1), 1–20. https://doi.org/10.1093/femsre/fuad066

Liu, Y., Yue, Z., Sun, Z., & Li, C. (2023). Harnessing native *Bacillus* spp. for sustainable wheat production. *Applied and Environmental Microbiology*, *89*(2), 01247–22. https://doi.org/10.1128/aem.01247-22

Liu, Z., Budiharjo, A., Wang, P., Shi, H., Fang, J., Borriss, R., Zhang, K., & Huang, X. (2013). The highly modified microcin peptide plantazolicin is associated with nematicidal activity of *Bacillus amyloliquefaciens* FZB42. *Applied Microbiology and Biotechnology*, *97*(23), 10081–10090. https://doi.org/10.1007/s00253-013-5247-5

Lobato, M. C., Feldman, M. L., Machinandiarena, M. F., & Olivieri, F. P. (2024). First insights into the role of polyamines in biotic stress resistance induced by potassium

phosphite in potato. *Potato Research*, *67*(1), 255–270. https://doi.org/10.1007/s11540-023-09633-9

Lokuruka, N. I. M. (2021). Food and nutrition security in East Africa (Rwanda, Burundi and South Sudan): Status, challenges and prospects. In B. Mahmoud (Ed.), *Food Security in Africa* (pp. 75–90). IntechOpen. https://doi.org/10.5772/intechopen.95037

Lopes, M. J. dos S., Dias-Filho, M. B., & Gurgel, E. S. C. (2021). Successful plant growth-promoting microbes: Inoculation methods and abiotic factors. *Frontiers in Sustainable Food Systems*, *5*, 606454. https://doi.org/10.3389/fsufs.2021.606454

Lourenzi, C. R., Loss, A., Souza, M., Comin, J. J., Lovato, P. E., & Sousa Soares, C. R. F. (2022). The role of PGPR secondary metabolites in alleviating allelopathic effects (biotic Stress) and induced tolerance in plants. In R. Z. Sayyed & V. G. Uarrota (Eds.), *Secondary metabolites and volatiles of PGPR in plant-growth promotion* (pp. 133–152). Springer. https://doi.org/10.1007/978-3-031-07559-9_8

Luiz, C. B., Villalun, M., Eyre, M., Bushe, C. B., Brill, E., & Keith, M. L. (2024). First report of bacterial feaf spot caused by *Pseudomonas cichorii* on *Monstera adansonii* in Hawaii, U.S.A. *Plant Disease*, *108*(1), 201. https://doi.org/https://doi.org/10.1094/PDIS-06-23-1224-PDN

Lukanda, M., Owati, A., Ogunsanya, P., Valimunzigha, K., Katsongo, K., Ndemere, H., & Kumar, P. L. (2014). First report of maize chlorotic mottle virus Infecting maize in the Democratic Republic of the Congo. *Plant Disease*, *98*(10), 1448–1448. https://doi.org/10.1094/PDIS-05-14-0484-PDN

Lund, P. A., De Biase, D., Liran, O., Scheler, O., Mira, N. P., Cetecioglu, Z., Fernández, E. N., Bover-Cid, S., Hall, R., Sauer, M., & O'Byrne, C. (2020). Understanding how microorganisms respond to acid pH is central to their control and successful exploitation. *Frontiers in Microbiology*, *11*, 556140. https://doi.org/10.3389/fmicb.2020.556140

Luo, L., Zhao, C., Wang, E., Raza, A., & Yin, C. (2022). *Bacillus amyloliquefaciens* as an excellent agent for biofertilizer and biocontrol in agriculture: An overview for its mechanisms. *Microbiological Research*, *259*, 127016. https://doi.org/10.1016/j.micres.2022.127016

Lyng, M., Jørgensen, J. P. B., Schostag, M. D., Jarmusch, S. A., Aguilar, D. K. C., Lozano-Andrade, C. N., & Kovács, Á. T. (2024). Competition for iron shapes metabolic antagonism between *Bacillus subtilis* and *Pseudomonas marginalis*. *The ISME Journal*, *18*(1), 1–13. https://doi.org/10.1093/ismejo/wrad001

Ma, X., Shen, S., Li, W., & Wang, J. (2023). Bioherbicidal potential of *Bacillus altitudinis* D30202 on *Avena fatua* L.: A whole-genome sequencing analysis. *Journal of Applied Genetics*, *64*(4), 809–817. https://doi.org/10.1007/s13353-023-00788-2

Magan, N. (2020). Importance of ecological windows for efficacy of biocontrol agents. In A. , De Cal, P. , Melgarejo, & N. Magan (Eds.), *How research can stimulate the development of commercial biological control against plant diseases. Progress in*

biological control (Vol. 21, pp. 1–14). Springer. https://doi.org/10.1007/978-3-030-53238-3_1

Mahapatra, S., Yadav, R., & Ramakrishna, W. (2022). *Bacillus subtilis* impact on plant growth, soil health and environment: Dr. Jekyll and Mr. Hyde. *Journal of Applied Microbiology*, *132*(5), 3543–3562. https://doi.org/10.1111/jam.15480

Mahmood, I., Imadi, S. R., Shazadi, K., Gul, A., & Hakeem, K. R. (2016). Effects of pesticides on environment. In R. K. Hakeem, S. M. Akhtar, & A. N. S. Abdullah (Eds.), *Plant, soil and microbes* (pp. 253–269). Springer. https://doi.org/10.1007/978-3-319-27455-3_13

Mahmood, I., Rizvi, R., Sumbul, A., & Ansari, R. A. (2019). Potential role of plant growth promoting rhizobacteria in alleviation of biotic stress. In A. R. Ansari & I. Mahmood (Eds.), *Plant health under biotic stress. Microbial interactions* (Vol. 2, pp. 177–188). Springer Singapore. https://doi.org/10.1007/978-981-13-6040-4_9

Mahmood, T. (2022). Lipopeptides, powerful antifungal weapons produced by *Bacillus* species. *Plant Bulletin*, *1*(1), 55–68. https://doi.org/10.55627/pbulletin.001.01.0143

Mahuku, G., Lockhart, B. E., Wanjala, B., Jones, M. W., Kimunye, J. N., Stewart, L. R., Cassone, B. J., Sevgan, S., Nyasani, J. O., Kusia, E., Kumar, P. L., Niblett, C. L., Kiggundu, A., Asea, G., Pappu, H. R., Wangai, A., Prasanna, B. M., & Redinbaugh, M. G. (2015). Maize lethal necrosis (MLN), an emerging threat to maize-based food security in sub-Saharan Africa. *Phytopathology®*, *105*(7), 956–965. https://doi.org/10.1094/PHYTO-12-14-0367-FI

Malit, J. J. L., Wu, C., Liu, L. L., & Qian, P. Y. (2021). Global genome mining reveals the distribution of diverse thioamidated RiPP biosynthesis gene clusters. *Frontiers in Microbiology*, *12*, 635389. https://doi.org/10.3389/fmicb.2021.635389

Malliarakis, D., Pagoulatou, M. G., Mpalantinaki, E., Trantas, E., Ververidis, F., & Goumas, D. E. (2023). Phylogenetic diversity of *Clavibacter michiganensis* subsp. *michiganensis* isolates causing bacterial canker of tomato in Greece. *Journal of Plant Pathology*, *105*(4), 1403–1419. https://doi.org/10.1007/s42161-023-01375-4

Maloney, E. M., Sykes, H., Morrissey, C., Peru, K. M., Headley, J. V, & Liber, K. (2020). Environmental toxicology comparing the acute toxicity of imidacloprid with alternative systemic insecticides in the aquatic insect *Chironomus dilutus*. *Environmental Toxicology and Chemistry*, *39*(3), 587–594. https://doi.org/10.1002/etc.4639

Manirakiza, N., Bisore, S., Ndihokubwayo, N., Niyuhire, E., Bigumandondera, P., & Nimbona, G. (2023). Caractérisation des déchets solides municipaux générés dans la municipalité de Kinama, Bujumbura-Burundi. *Afrique Science*, *22*(5), 10–21. http://www.afriquescience.net

Manirakiza, P., Covaci, A., Nizigiymana, L., Ntakimazi, G., & Schepens, P. (2002). Persistent chlorinated pesticides and polychlorinated biphenyls in selected fish species from Lake Tanganyika, Burundi, Africa. *Environmental Pollution*, *117*(3), 447–455. https://doi.org/10.1016/S0269-7491(01)00188-9

Marcano, I.-E., Díaz-Alcántara, C.-A., Seco, V., Urbano, B., & González-Andrés, F. (2016). Induced systemic resistance could explain the reduction in the incidence of

black Sigatoka (*Mycosphaerella fijiensis*) in banana plants inoculated with bacteria isolated from banana tree roots in the Dominican Republic. In F. González-Andrés & E. James (Eds.), *Biological nitrogen fixation and beneficial plant-microbe interaction* (pp. 155–170). Springer. https://doi.org/10.1007/978-3-319-32528-6_14

Marian, M., & Shimizu, M. (2019). Improving performance of microbial biocontrol agents against plant diseases. *Journal of General Plant Pathology*, *85*(5), 329–336. https://doi.org/10.1007/s10327-019-00866-6

Marrone, P. G. (2024). Status of the biopesticide market and prospects for new bioherbicides. *Pest Management Science*, *80*(1), 81–86. https://doi.org/10.1002/ps.7403

Marschner, P., & Rengel, Z. (2023). Nutrient availability in soils. In Z. Rengel, I. Cakmak, & P. J. White (Eds.), *Marschner's mineral nutrition of plants* (4th ed., pp. 499–522). Academic Press, Elsevier. https://doi.org/10.1016/B978-0-12-819773-8.00003-4

Maruthi, M. N., Jeremiah, S. C., Mohammed, I. U., & Legg, J. P. (2017). The role of the whitefly, *Bemisia tabaci* (Gennadius), and farmer practices in the spread of cassava brown streak ipomoviruses. *Journal of Phytopathology*, *165*(11–12), 707–717. https://doi.org/10.1111/jph.12609

Masoudi, A., Joseph, R. A., & Keyhani, N. O. (2024). Viral- and fungal-mediated behavioral manipulation of hosts: Summit disease. *Applied Microbiology and Biotechnology*, *108*(1), 492. https://doi.org/10.1007/s00253-024-13332-x

Matavacas, J., & von Wachenfeldt, C. (2022). Update on the protein homeostasis network in *Bacillus subtilis*. *Frontiers in Microbiology*, *13*, 865141. https://doi.org/10.3389/fmicb.2022.865141

Maurya, A. K., Agarwal, R., & Gupta, R. (2025). Unraveling the crosstalk among ethylene, nitric oxide, and polyamines in tailoring the abiotic stress resilience in plants. *Stress Biology*, *5*(1), 20. https://doi.org/10.1007/s44154-024-00198-2

Mazumdar, D., Saha, S. P., & Ghosh, S. (2020). Isolation, screening and application of a potent PGPR for enhancing growth of chickpea as affected by nitrogen level. *International Journal of Vegetable Science*, *26*(4), 333–350. https://doi.org/10.1080/19315260.2019.1632401

Mbabou Mbianzoue, E. (2023). *Evaluation de l'efficacité de Bacillus nakamurai BDI-IS1 dans le biocontrôle de la maladie des taches anguleuses du haricot, causée par Pseudocercospora griseola*. Université Catholique de Louvain.

Mehta, P., Walia, A., Kulshrestha, S., Chauhan, A., & Shirkot, C. K. (2015). Efficiency of plant growth-promoting P-solubilizing *Bacillus circulans* CB7 for enhancement of tomato growth under net house conditions. *Journal of Basic Microbiology*, *55*(1), 33–44. https://doi.org/10.1002/jobm.201300562

Meier-Kolthoff, J. P., Auch, A. F., Klenk, H.-P., & Öker, M. G. (2013). Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics*, *14*, 60. https://doi.org/10.1186/1471-2105-14-60

Meier-Kolthoff, J. P., & Göker, M. (2019). TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nature Communications*, *10*(1), 2182. https://doi.org/10.1038/s41467-019-10210-3

Meier-Kolthoff, J. P., Göker, M., Spröer, C., & Klenk, H. P. (2013). When should a DDH experiment be mandatory in microbial taxonomy? *Archives of Microbiology*, *195*(6), 413–418. https://doi.org/10.1007/s00203-013-0888-4

Meier-Kolthoff, J. P., Klenk, H.-P., & Göker, M. (2014). Taxonomic use of DNA G+C content and DNA–DNA hybridization in the genomic age. *International Journal of Systematic and Evolutionary Microbiology*, *64*(Pt_2), 352–356. https://doi.org/10.1099/ijs.0.056994-0

Mekonnen, E., Kebede, A., Nigussie, A., Kebede, G., & Tafesse, M. (2021). Isolation and characterization of urease-producing soil bacteria. *International Journal of Microbiology*, *2021*, 8888641. https://doi.org/10.1155/2021/8888641

Mercado, V., & Olmos, J. (2022). Bacteriocin production by *Bacillus* species: Isolation, characterization, and application. *Probiotics and Antimicrobial Proteins*, *14*(6), 1151–1169. https://doi.org/10.1007/s12602-022-09966-w

Mhatre, P. H., Thorat, Y. E., Manimaran, B., Divya, K. L., Bairwa, A., Chavan, S. N., Pokhare, S. S., Dukare, A. S., & Karthik, C. (2024). Nematicidal activity of secondary metabolites from soil microbes. In K. K. Chaudhary, K. M. Meghvansi, & S. Siddiqui (Eds.), *Sustainable management of nematodes in agriculture, Vol.2: Role of microbes-assisted strategies, sustainability in plant and crop protection 19. Sustainability in plant and crop protection* (Vol. 19, pp. 297–324). Springer Nature. https://doi.org/10.1007/978-3-031-52557-5_12

Miao, S., Liang, J., Xu, Y., Yu, G., & Shao, M. (2023). Bacillaene, sharp objects consist in the arsenal of antibiotics produced by *Bacillus*. *Journal of Cellular Physiology*, *239*(8), e31228. https://doi.org/10.1002/jcp.30974

Miethke, M., Klotz, O., Linne, U., May, J. J., Beckering, C. L., & Marahiel, M. A. (2006). Ferri-bacillibactin uptake and hydrolysis in *Bacillus subtilis*. *Molecular Microbiology*, *61*(6), 1413–1427. https://doi.org/10.1111/j.1365-2958.2006.05321.x

Miethke, M., Monteferrante, C. G., Marahiel, M. A., & van Dijl, J. M. (2013). The *Bacillus subtilis* EfeUOB transporter is essential for high-affinity acquisition of ferrous and ferric iron. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1833*(10), 2267–2278. https://doi.org/10.1016/j.bbamcr.2013.05.027

Miljaković, D., Marinković, J., & Balešević-Tubić, S. (2020). The significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegetable crops. *Microorganisms*, *8*(7), 1037. https://doi.org/10.3390/microorganisms8071037

Miller, R. A., Beno, S. M., Kent, D. J., Carroll, L. M., Martin, N. H., Boor, K. J., & Kovac, J. (2016). *Bacillus wiedmannii* sp. nov., a psychrotolerant and cytotoxic *Bacillus cereus* group species isolated from dairy foods and dairy environments. *International Journal of Systematic and Evolutionary Microbiology*, *66*(11), 4744–4753. https://doi.org/10.1099/ijsem.0.001421

Milton, M. E., & Cavanagh, J. (2023). The biofilm regulatory network from *Bacillus subtilis*: A structure-function analysis. *Journal of Molecular Biology*, *435*(3), 167923. https://doi.org/10.1016/j.jmb.2022.167923

MINAGRI-Burundi. (2016). *Plan de gestion des pestes*. http://documents1.worldbank.org/

MINAGRI-Burundi. (2018). *Composante burundaise plan de gestion des pestes (PGP)*. https://bi.chm-cbd.net/sites/bi/files/2020-09/plan-gest-pestes-bi.pdf

Minengu, J. D. D., Mwengi, I., & Maleke, M. (2018). Agriculture familiale dans les zones péri-urbaines de Kinshasa : analyse , enjeux et perspectives (synthèse bibliographique). *Revue Africaine d'Environnement et d'Agriculture*, *1*(1), 60–69.

Mirsam, H., Hary Kalqutny, S., Aqil, M., Azrai, M., Pakki, S., Muis, A., Djaenuddin, N., & Wahid Rauf, A. (2021). Indigenous fungi from corn as a potential plant growth promoter and its role in *Fusarium verticillioides* suppression on corn. *Heliyon*, *7*, e07926. https://doi.org/10.1016/j.heliyon.2021.e07926

Mithöfer, A., & Boland, W. (2016). Do you speak chemistry? *EMBO Reports*, *17*(5), 626–629. https://doi.org/10.15252/embr.201642301

Mizumoto, S., Hirai, M., & Shoda, M. (2006). Production of lipopeptide antibiotic iturin A using soybean curd residue cultivated with *Bacillus subtilis* in solid-state fermentation. *Applied Microbiology and Biotechnology*, *72*(5), 869–875. https://doi.org/10.1007/s00253-006-0389-3

Mnif, I., & Ghribi, D. (2015). Lipopeptide surfactants: Production, recovery and pore forming capacity. *Peptides*, *71*, 100–112. https://doi.org/10.1016/j.peptides.2015.07.006

Mofid, M. R., Finking, R., Essen, L. O., & Marahiel, M. A. (2004). Structure-based mutational analysis of the 4′-phosphopantetheinyl transferases Sfp from *Bacillus subtilis*: Carrier protein recognition and reaction mechanism. *Biochemistry*, *43*(14), 4128–4136. https://doi.org/10.1021/bi036013h

Mohan, M. S., Salim, S. A., Pakhira, P., & Busi, S. (2024). Microbial production of polyketides and non-ribosomal peptides and their applications. In V. Kothari, S. Ray, & P. Kumar (Eds.), *Microbial products for health and nutrition* (pp. 365–390). Springer Nature Singapore. https://doi.org/10.1007/978-981-97-4235-6_15

Molohon, K. J., Melby, J. O., Lee, J., Evans, B. S., Dunbar, K. L., Bumpus, S. B., Kelleher, N. L., & Mitchell, D. A. (2011). Structure determination and interception of biosynthetic intermediates for the plantazolicin class of highly discriminating antibiotics. *ACS Chem. Biol*, *6*(12), 1307–1313. https://doi.org/10.1021/cb200339d

Molohon, K. J., Saint-Vincent, P. M. B., Park, S., Doroghazi, J. R., Maxson, T., Hershfield, J. R., Flatt, K. M., Schroeder, N. E., Ha, T., & Mitchell, D. A. (2016). Plantazolicin is an ultranarrow-spectrum antibiotic that targets the *Bacillus anthracis* membrane. *ACS Infectious Diseases*, *2*(3), 207–220. https://doi.org/10.1021/acsinfecdis.5b00115

Montalbán-López, M., Scott, T. A., Ramesh, S., Rahman, I. R., Van Heel, A. J., Viel, J. H., Bandarian, V., Dittmann, E., Genilloud, O., Goto, Y., Grande Burgos, M. J., Hill, C., Kim, S., Koehnke, J., Latham, J. A., Link, A. J., Martínez, B., Nair, S. K.,

Nicolet, Y., … Van Der Donk, W. A. (2021). New developments in RiPP discovery, enzymology and engineering. *Natural Product Reports*, *38*(1), 130–239. https://doi.org/10.1039/d0np00027b

Morales, A. E., Soto, N., Delgado, C., Hernández, Y., Carrillo, L., Ferrero, C., & Enríquez, G. A. (2023). Expression of Mn-sod, PAL1, aos1 and HPL genes in soybean plants overexpressing the NmDef02 defensin. *Transgenic Research*, *32*(3), 223–233. https://doi.org/10.1007/s11248-023-00350-0

Mordor Intelligence. (2025). *Africa biopesticides market size & share analysis - Growth trends trends & forecasts up to 2030*.

Moreno-Velandia, C. A., Ongena, M., Kloepper, J. W., & Cotes, A. M. (2021). Biosynthesis of cyclic lipopeptides by *Bacillus velezensis* Bs006 and its antagonistic activity are modulated by the temperature and culture media conditions. *Current Microbiology*, *78*(9), 3505–3515. https://doi.org/10.1007/s00284-021-02612-8

Morrissey, J., & Guerinot, M. Lou. (2009). Iron uptake and transport in plants: The good, the bad, and the ionome. *Chemical Reviews*, *109*(10), 4553–4567. https://doi.org/10.1021/cr900112r

Mosela, M., Andrade, G., Massucato, L. R., de Araújo Almeida, S. R., Nogueira, A. F., de Lima Filho, R. B., Zeffa, D. M., Mian, S., Higashi, A. Y., Shimizu, G. D., Teixeira, G. M., Branco, K. S., Faria, M. V., Giacomin, R. M., Scapim, C. A., & Gonçalves, L. S. A. (2022). *Bacillus velezensis* strain Ag75 as a new multifunctional agent for biocontrol, phosphate solubilization and growth promotion in maize and soybean crops. *Scientific Reports*, *12*(1), 15284. https://doi.org/10.1038/s41598-022-19515-8

Mosley, L. M., Rengasamy, P., & Fitzpatrick, R. (2024). Soil pH: Techniques, challenges and insights from a global dataset. *European Journal of Soil Science*, *75*(6), e70021. https://doi.org/10.1111/ejss.70021

Motasim, A. M., Samsuri, Abd. W., Nabayi, A., Akter, A., Haque, M. A., Abdul Sukor, A. S., & Adibah, A. Mohd. (2024). Urea application in soil: Processes, losses, and alternatives-a review. *Discover Agriculture*, *2*, 42. https://doi.org/10.1007/s44279-024-00060-z

MsangoSoko, K., Gandotra, S., Bhattacharya, R., Ramakrishnan, B., Sharma, K., & Subramanian, S. (2022). Screening and characterization of lipase producing bacteria isolated from the gut of a lepidopteran larvae, *Samia ricini*. *Journal of Asia-Pacific Entomology*, *25*(1), 101856. https://doi.org/10.1016/J.ASPEN.2021.101856

Msimbira, L. A., & Smith, D. L. (2020). The roles of plant growth promoting microbes in enhancing plant tolerance to acidity and alkalinity stresses. *Frontiers in Sustainable Food Systems*, *4*, 106. https://doi.org/10.3389/fsufs.2020.00106

Mu, F., Chen, X., Fu, Z., Wang, X., Guo, J., Zhao, X., & Zhang, B. (2023). Genome and transcriptome analysis to elucidate the biocontrol mechanism of *Bacillus amyloliquefaciens* XJ5 against *Alternaria solani*. *Microorganisms*, *11*(8), 2055. https://doi.org/10.3390/microorganisms11082055

Muhindo, H., Yasenge, S., Casinga, C., Songbo, M., Dhed'a, B., Alicai, T., Pita, J., & Monde, G. (2020). Incidence, severity and distribution of cassava brown streak

disease in northeastern Democratic Republic of Congo. *Cogent Food & Agriculture*, *6*(1), 1789422. https://doi.org/10.1080/23311932.2020.1789422

Mukherjee, S., Pandey, V., Parvez, A., Qi, X., & Hussain, T. (2022). *Bacillus* as a versatile tool for crop improvement and agro-industry. In M. T. Islam, M. Rahman, P. Pandey, M. H. Boehme, & G. Haesaer (Eds.), *Bacilli in agrobiotechnology, bacilli in climate resilient agriculture and bioprospecting* (pp. 429–452). Sringer International . https://doi.org/10.1007/978-3-030-85465-2_19

Mukwa, L. F. T., Muengula, M., Zinga, I., Kalonji, A., Iskra-Caruana, M. L., & Bragard, C. (2014). Occurrence and distribution of banana bunchy top virus related agro-ecosystem in South Western, Democratic Republic of Congo. *American Journal of Plant Sciences*, *05*(05), 647–658. https://doi.org/10.4236/ajps.2014.55079

Muleta, D., Assefa, F., & Granhall, U. (2007). *In vitro* antagonism of rhizobacteria isolated from *Coffea arabica* L. against emerging fungal coffee pathogens. *Engineering in Life Sciences*, *7*(6), 577–586. https://doi.org/10.1002/elsc.200700004

Muliele, T. M., Manzenza, C. M., Ekuke, L. W., Diaka, C. P., Ndikubwayo, D. M., Kapalay, O. M., & Mundele, A. N. (2018). Utilisation et gestion des pesticides en cultures maraîchères : Cas de la zone de Nkolo dans la province du Kongo Central, République démocratique du Congo. *Journal of Applied Biosciences*, *119*(1), 11954. https://doi.org/10.4314/jab.v119i1.11

Müller, S., Strack, S. N., Hoefler, B. C., Straight, P. D., Kearns, D. B., & Kirby, J. R. (2014). Bacillaene and sporulation protect *Bacillus subtilis* from predation by *Myxococcus xanthus*. *Applied and Environmental Microbiology*, *80*(18), 5603–5610. https://doi.org/10.1128/AEM.01621-14

Müller, S., Strack, S. N., Ryan, S. E., Kearns, D. B., & Kirby, J. R. (2015). Predation by *Myxococcus xanthus* induces *Bacillus subtilis* to form spore-filled megastructures. *Applied and Environmental Microbiology*, *81*(1), 203–210. https://doi.org/10.1128/AEM.02448-14

Munganyinka, E., Ateka, E. M., Kihurani, A. W., Kanyange, M. C., Tairo, F., Sseruwagi, P., & Ndunguru, J. (2018). Cassava brown streak disease in Rwanda, the associated viruses and disease phenotypes. *Plant Pathology*, *67*(2), 377–387. https://doi.org/10.1111/PPA.12789

Muñoz-Torres, P., Cárdenas-Ninasivincha, S., & Aguilar, Y. (2024). Exploring the agricultural applications of microbial melanin. *Microorganisms*, *12*(7), 1352. https://doi.org/10.3390/microorganisms12071352

Munyuli, T., Cihire, K., Rubabura, D., Mitima, K., Kalimba, Y., Tchombe, N., Mulangane, E. K., Birhashwira, O., Umoja, M., Cinyabuguma, E., Mukadi, T. T., Ilunga, M. T., & Mukendi, R. T. (2017). Farmers' perceptions, believes, knowledge and management practices of potato pests in South-Kivu Province, Eastern of Democratic Republic of Congo. *Open Agriculture*, *2*(1), 362–385. https://doi.org/10.1515/opag-2017-0040

Murphy, R. O., Cotton, J. S., Owens, I. M., Carroll, J. D., Martin, K. M., Held, D., Lawrence, K., & Beckmann, J. F. (2025). Fast screening libraries of plant growth

promoting rhizobacteria (PGPRs) for insecticidal activity. *Journal of Applied Microbiology*, *136*(3), Ixaf054. https://doi.org/10.1093/jambio/lxaf054

Musonerimana, S., Bez, C., Licastro, D., Habarugira, G., Bigirimana, J., & Venturi, V. (2020). Pathobiomes revealed that *Pseudomonas fuscovaginae* and *Sarocladium oryzae* are independently associated with rice sheath rot. *Microbial Ecology*, *80*(3), 627–642. https://doi.org/10.1007/s00248-020-01529-2

Nabahungu, N. L., & Visser, S. M. (2013). Farmers' knowledge and perception of agricultural wetland management in Rwanda. *Land Degradation and Development*, *24*(4), 363–374. https://doi.org/10.1002/ldr.1133

Nadeem, H., Khan, A., Gupta, R., Hashem, M., Alamri, S., Siddiqui, M. A., & Ahmad, F. (2023). Stress combination: When two negatives may become antagonistic, synergistic or additive for plants? *Pedosphere*, *33*(2), 287–300. https://doi.org/10.1016/j.pedsph.2022.06.031

Naguib, M. D., Nabil, A., & Ahmed, M. (2020). Onion dry scales extract induce resistance against bacterial wilt in eggplant through improving polyamines and antioxidant metabolism. *Biocatalysis and Agricultural Biotechnology*, *28*, 101743. https://doi.org/10.1016/j.bcab.2020.101743

Nair, I. M., & Kochupurackal, J. (2023). Squalene hopene cyclases and oxido squalene cyclases: Potential targets for regulating cyclisation reactions. *Biotechnology Letters*, *45*(5–6), 573–588. https://doi.org/10.1007/s10529-023-03366-y

Nakamura, H., Hisano, T., Rahman, M. M., Tosha, T., Shirouzu, M., & Shiro, Y. (2022). Structural basis for heme detoxification by an ATP-binding cassette-type efflux pump in gram-positive pathogenic bacteria. *PNAS*, *119*(27), e2123385119. https://doi.org/10.1073/pnas

Nakamura, L. K. (1989). Taxonomic relationship of black-pigmented *Bacillus subtilis* strains and a proposal for *Bacillus atrophaeus* sp. nov. *International Journal of Systematic Bacteriology*, *39*(3), 295–300. https://doi.org/10.1099/00207713-39-3-295/CITE/REFWORKS

Nakato, V., Mahuku, G., & Coutinho, T. (2018). Xanthomonas campestris pv. musacearum: a major constraint to banana, plantain and enset production in central and east Africa over the past decade. *Molecular Plant Pathology*, *19*(3), 525–536. https://doi.org/10.1111/mpp.12578

Nandy, S., Mandal, S., Gupta, S. K., Anand, U., Ghorai, M., Mundhra, A., Rahman, M. H., Ray, P., Mitra, S., Ray, D., Lal, M. K., Tiwari, R. K., Nongdam, P., Pandey, D. K., Shekhawat, M. S., Jha, N. K., Jha, S. K., Kumar, M., Radha, … Dey, A. (2023). Role of polyamines in molecular regulation and cross-talks against drought tolerance in plants. *Journal of Plant Growth Regulation*, *42*(8), 4901–4917. https://doi.org/10.1007/s00344-022-10802-2

Nannan, C., Vu, H. Q., Gillis, A., Caulier, S., Nguyen, T. T. T., & Mahillon, J. (2021). Bacilysin within the *Bacillus subtilis* group: Gene prevalence versus antagonistic activity against Gram-negative foodborne pathogens. *Journal of Biotechnology*, *327*, 28–35. https://doi.org/10.1016/j.jbiotec.2020.12.017

Navarro-Cartagena, S., & Micol, J. L. (2023). Is auxin enough? Cytokinins and margin patterning in simple leaves. *Trends in Plant Science*, *28*(1), 54–73. https://doi.org/10.1016/j.tplants.2022.08.019

Ndayambaje, B., Amuguni, H., Coffin-Schmitt, J., Sibo, N., Ntawubizi, M., & VanWormer, E. (2019). Pesticide application practices and knowledge among small-scale local rice growers and communities in Rwanda: A cross-sectional study. *International Journal of Environmental Research and Public Health*, *16*(23), 4770. https://doi.org/10.3390/ijerph16234770

Ndayihanzamaso, P., Mostert, D., Matthews, M. C., Mahuku, G., Jomanga, K., Mpanda, H. J., Mduma, H., Brown, A., Uwimana, B., Swennen, R., Tumuhimbise, R., & Viljoen, A. (2020). Evaluation of mchare and matooke bananas for resistance to fusarium oxysporum f. Sp. cubense race 1. *Plants*, *9*(9), 1–15. https://doi.org/10.3390/plants9091082

Ndayihanzamaso, P., Niko, N., Niyongere, C., Bizimana, S., Nibasumba, A., Lepoint, P., Tinzaara, W., Kaboneka, S., Sakayoya, E., Jogo, W., Mugiraneza, T., & Karamura, E. (2016). Distribution, incidence and farmers knowledge of banana *Xanthomonas* wilt in Burundi. *African Journal of Agricultural Research*, *11*(38), 3615–3621. https://doi.org/10.5897/ajar2016.11210

Ndikumana, T., Manirakiza, N., Girukwishaka, S., Mboninyibuka D., Irakoze Pacifique, Bigumandondera Patrice, & Jung, G. C. (2024). Quantification of biogas emitted from the Mubone landfill. *J. Mater. Environ. Sci*, *15*(1), 151–161. http://www.jmaterenvironsci.com

Ndisanze, A. M., Kamana, E., Nirere, C., & Ilkay, K. (2022). Assessment of the pesticides utilization and the pesticide residues presence in fresh and tomato products for the tomato supply chain in Rwanda. *Food and Nutrition Sciences*, *13*(12), 963–972. https://doi.org/10.4236/FNS.2022.1312067

Ndungo, V., Fiaboe, K. K. M., & Mwangi, M. (2008). Banana *Xanthomonas* wilt in the DR Congo : Impact, spread and management. *Journal of Applied Biosciences*, *1*(1), 1–7. www.biosciences.elewa.org

Negash, A. W., & Tsehai, B. A. (2020). Current Applications of Bacteriocin. *International Journal of Microbiology*, *2020*, 4374891. https://doi.org/10.1155/2020/4374891

Negi, R., Sharma, B., Kaur, S., Kaur, T., Khan, S. S., Kumar, S., Ramniwas, S., Rustagi, S., Singh, S., Rai, A. K., Kour, D., Thakur, N., & Yadav, A. N. (2023). Microbial antagonists: Diversity, formulation and applications for management of pest-pathogens. *Egyptian Journal of Biological Pest Control*, *33*(1), 105. https://doi.org/10.1186/s41938-023-00748-2

Netzker, T., Shepherdson, E. M. F., Zambri, M. P., & Elliot, M. A. (2020). Bacterial volatile compounds: Functions in communication, cooperation, and competition. *Annual Review of Microbiology*, *74*(1), 409–430. https://doi.org/10.1146/annurev-micro-011320-015542

Neuenschwander, P. (2001). Biological control of the cassava mealybug in Africa: A review. *Biological Control*, *21*(3), 214–229. https://doi.org/10.1006/bcon.2001.0937

Newman, J., & Lewis, R. J. (2013). Exploring the role of SlrR and SlrA in the SinR epigenetic switch. *Communicative & Integrative Biology*, *6*(6), e25658. https://doi.org/10.4161/cib.25658

NG, C. W. W., YAN, W. H., TSIM, K. W. K., SO, P. S., XIA, Y. T., & TO, C. T. (2022). Effects of Bacillus subtilis and Pseudomonas fluorescens as the soil amendment. *Heliyon*, *8*(11). https://doi.org/10.1016/j.heliyon.2022.e11674

Ngalimat, M. S., Yahaya, R. S. R., Baharudin, M. M. A., Yaminudin, S. Mohd., Karim, M., Ahmad, S. A., & Sabri, S. (2021). A review on the biotechnological applications of the operational group *Bacillus amyloliquefaciens*. *Microorganisms*, *9*(3), 614. https://doi.org/10.3390/microorganisms9030614

Ngoubeyou, P. S. K., Wolkersdorfer, C., Ndibewu, P. P., & Augustyn, W. (2022). Toxicity of polychlorinated biphenyls in aquatic environments – A review. *Aquatic Toxicology*, *251*, 106284. https://doi.org/10.1016/j.aquatox.2022.106284

Ngweme, G. N., Al Salah, D. M. M., Laffite, A., Sivalingam, P., Grandjean, D., Konde, J. N., Mulaji, C. K., Breider, F., & Poté, J. (2021). Occurrence of organic micropollutants and human health risk assessment based on consumption of *Amaranthus viridis*, Kinshasa in the Democratic Republic of the Congo. *Science of The Total Environment*, *754*, 142175. https://doi.org/10.1016/j.scitotenv.2020.142175

Ngweme, G. N., Mbela, G. K., Sikulisimwa, C. P., Kyela, C. M., & Komanda, J. A. (2019). Analyse des connaissances, attitudes et pratiques des maraîchers de la ville de Kinshasa en rapport avec l'utilisation des pesticides et l'impact sur la santé humaine et sur l'environnement. *Afrique SCIENCE*, *15*(4), 122–133. http://www.afriquescience.net

Ni, J., Yu, L., Li, F., Li, Y., Zhang, M., Deng, Y., & Liu, X. (2023). Macrolactin R from *Bacillus siamensis* and its antifungal activity against *Botrytis cinerea*. *World Journal of Microbiology and Biotechnology*, *39*(5), 117. https://doi.org/10.1007/s11274-023-03563-x

Ni, Y., Yang, T., Ma, Y., Zhang, K., Soltis, P. S., Soltis, D. E., Gilbert, J. A., Zhao, Y., Fu, C., & Chu, H. (2021). Soil pH determines bacterial distribution and assembly processes in natural mountain forests of eastern China. *Global Ecology and Biogeography*, *30*(11), 2164–2177. https://doi.org/10.1111/geb.13373

Niassy, S., Agbodzavu, M. K., Kimathi, E., Mutune, B., Abdel-Rahman, E. F. M., Salifu, D., Hailu, G., Belayneh, Y. T., Felege, E., Tonnang, H. E. Z., Ekesi, S., & Subramanian, S. (2021). Bioecology of fall armyworm *Spodoptera frugiperda* (J. E. Smith), its management and potential patterns of seasonal spread in Africa. *PLOS ONE*, *16*(6), e0249042. https://doi.org/10.1371/journal.pone.0249042

Nihorimbere, G. (2025). *Biocontrol activity of Bacillus nakamurai BDI-IS1 and investigation of its mechanisms against Northern corn leaf blight*. Université Catholique de Louvain.

Nihorimbere, G., Korangi Alleluya, V., Nimbeshaho, F., Nihorimbere, V., Legrève, A., & Ongena, M. (2024). *Bacillus*-based biocontrol beyond chemical control in central

Africa: The challenge of turning myth into reality. *Frontiers in Plant Science*, *15*, 1349357. https://doi.org/10.3389/fpls.2024.1349357

Nihorimbere, V., Cawoy, H., Seyer, A., Brunelle, A., Thonart, P., & Ongena, M. (2012). Impact of rhizosphere factors on cyclic lipopeptide signature from the plant beneficial strain *Bacillus amyloliquefaciens* S499. *FEMS Microbiology Ecology*, *79*(1), 176–191.

Nihorimbere, V., Ongena, M., Cawoy, H., Brostaux, Y., Kakana, P., Jourdan, E., & Thonart, P. (2010). Beneficial effects of *Bacillus subtilis* on field-grown tomato in Burundi: Reduction of local *Fusarium* disease and growth promotion. *African Journal of Microbiology Research*, *4*(11), 1135–1142. http://www.academicjournals.org/ajmr

Nikolaidis, M., Hesketh, A., Mossialos, D., Iliopoulos, I., Oliver, S. G., & Amoutzias, G. D. (2022). A comparative analysis of the core proteomes within and among the *Bacillus subtilis* and *Bacillus cereus* evolutionary groups reveals the patterns of lineage- and species-specific adaptations. *Microorganisms*, *10*(9), 1720. https://doi.org/10.3390/microorganisms10091720

Nimbeshaho, F., Nihorimbere, G., Arias, A. A., Liénard, C., Steels, S., Nibasumba, A., Nihorimbere, V., Legrève, A., & Ongena, M. (2024). Unravelling the secondary metabolome and biocontrol potential of the recently described species *Bacillus nakamurai*. *Microbiological Research*, *288*, 127841. https://doi.org/10.1016/j.micres.2024.127841

Nishisaka, C. S., Ventura, J. P., Bais, H. P., & Mendes, R. (2024). Role of *Bacillus subtilis* exopolymeric genes in modulating rhizosphere microbiome assembly. *Environmental Microbiome*, *19*(1), 33. https://doi.org/10.1186/s40793-024-00567-4

Niyibizi, G. P., Gakuru, S. J. B., Rizinde, H. J. C., & Munenwa, S. Armand, Lwanzo, L. (2019). État de lieux des caféières face à la menace d'*Antestiopsis orbitalis* dans le territoire de Kalehe, à l'Est de la RD Congo. *Annales de l'UNIGOM*, *IX*(1), 87–99.

Niyongabo, J. M. V. (2023). Les cas de cancer inquiètent. *BurundiEco*. https://burundi-eco.com/les-cas-de-cancer-inquietent/

Niyongere, C., Losenge, T., Ateka, E. M., Ntukamazina, N., Ndayiragije, P., Simbare, A., Cimpaye, P., Nintije, P., Lepoint, P., & Blomme, G. (2013). Understanding banana bunchy top disease epidemiology in Burundi for an enhanced and integrated management approach. *Plant Pathology*, *62*(3), 562–570. https://doi.org/10.1111/j.1365-3059.2012.02676.x

Niyongere, C., Mbonihankuye, C., Mutshail, G., & Yamuremye, A. (2015). Utilization of pesticides in smallholder horticulture production pinpoint the need for cropping system changes in Burundi. *Acta Horticulturae*, *1105*, 213–220. https://doi.org/10.17660/ActaHortic.2015.1105.30

Nkuba, J., Tinzaara, W., Night, G., Niko, N., Jogo, W., Ndyetabula, I., Mukandala, L., Ndayihazamaso, P., Niyongere, C., Gaidashova, S., Rwomushana, I., Opio, F., & Karamura, E. (2015). Adverse impact of Banana *Xanthomonas* Wilt on farmers livelihoods in Eastern and Central Africa. *African Journal of Plant Science*, *9*(7), 279–286. https://doi.org/10.5897/AJPS2015.1292

Nkurunziza, G., Ndayisenga, M., Ndayihanzamaso, P., Ndayiragije, P., & Niyongabo, D. (2012). *Techniques de culture , de protection et de conservation du maïs – Cas des variétés à pollinisation libre, Manuel.* ISABU. https://isabu.bi/wp-content/uploads/2021/09/Manuel-de-formation_-Mais.pdf

Noor, A. O., Almasri, D. M., Basyony, A. F., Albohy, A., Almutairi, L. S., Alhammadi, S. S., Alkhamisi, M. A., Alsharif, S. A., & Elfaky, M. A. (2022). Biodiversity of N-acyl homoserine lactonase (aiiA) gene from *Bacillus subtilis*. *Microbial Pathogenesis*, *166*, 105543. https://doi.org/10.1016/j.micpath.2022.105543

Nordgaard, M., Blake, C., Maróti, G., Hu, G., Wang, Y., Strube, M. L., & Kovács, Á. T. (2022). Experimental evolution of *Bacillus subtilis* on *Arabidopsis thaliana* roots reveals fast adaptation and improved root colonization. *IScience*, *25*(6), 104406. https://doi.org/10.1016/j.isci.2022.104406

Northen, T. R., Kleiner, M., Torres, M., Kovács, Á. T., Nicolaisen, M. H., Krzyżanowska, D. M., Sharma, S., Lund, G., Jelsbak, L., Baars, O., Kindtler, N. L., Wippel, K., Dinesen, C., Ferrarezi, J. A., Marian, M., Pioppi, A., Xu, X., Andersen, T., Geldner, N., … Garrido-Oter, R. (2024). Community standards and future opportunities for synthetic communities in plant–microbiota research. *Nature Microbiology*, *9*(11), 2774–2784. https://doi.org/10.1038/s41564-024-01833-4

Novello, G., Bona, E., Toumatia, O., Vuolo, F., Bouras, N., Titouah, H., Zitouni, A., Gorrasi, S., Massa, N., Cesaro, P., Todeschini, V., Lingua, G., & Gamalero, E. (2023). Rhizosphere bacterial isolation from indigenous plants in arid and semi-arid algerian Soils: Implications for plant growth enhancement. *Processes*, *11*(10), 2907. https://doi.org/10.3390/pr11102907

Ntagisanimana, G., Yu, Z., & Ma, H. (2021). Current situation of solid waste management in East African countries and the proposal for sustainable management. *African Journal of Environmental Science and Technology*, *15*(1), 1–15. https://doi.org/10.5897/ajest2020.2911

Nyabyenda, P. (2005). *Les plantes cultivées en régions tropicales d'altitude d'Afrique : Généralités, légumineuses alimentaires, plantes à tubercules et racines, céréales* (Vol. 1). Presses Agronomiques de Gembloux.

Nyabyenda, P. (2006). *Les plantes cultivées en régions tropicales d'altitude d'Afrique : Cultures industrielles et d'exportation, cultures fruitières, cultures maraîchères* (Vol. 2). Presses Agronomiques de Gembloux.

Nzeyimana, F., Onwonga, R. N., Ayuke, F. O., Chemining'wa, G. N., Nabahungu, N. L., Bigirimana, J., & Noella Josiane, U. K. (2024). Determination of abundance and symbiotic effectiveness of native rhizobia nodulating soybean and other legumes in Rwanda. *Plant-Environment Interactions*, *5*(2), e10138. https://doi.org/10.1002/pei3.10138

O'Callaghan, M., Ballard, R. A., & Wright, D. (2022). Soil microbial inoculants for sustainable agriculture: Limitations and opportunities. *Soil Use and Management*, *38*(3), 1340–1369. https://doi.org/10.1111/sum.12811

Oiza, N., Moral-Vico, J., Sánchez, A., Oviedo, E. R., & Gea, T. (2022). Solid-state fermentation from organic wastes: A new generation of bioproducts. *Processes*, *10*(12), 2675. https://doi.org/10.3390/pr10122675

Okonya, J., Ocimati, W., Nduwayezu, A., Kantungeko, D., Niko, N., Blomme, G., Legg, J. P., & Kroschel, J. (2019). Farmer reported pest and disease impacts on root, tuber, and banana crops and livelihoods in Rwanda and Burundi. *Sustainability (Switzerland)*, *11*(6), 1–20. https://doi.org/10.3390/su11061592

Okonya, J., Petsakos, A., Suarez, V., Nduwayezu, A., Kantungeko, D., Blomme, G., Legg, J., & Kroschel, J. (2019). Pesticide use practices in root, tuber, and banana crops by smallholder farmers in Rwanda and Burundi. *International Journal of Environmental Research and Public Health*, *16*(3), 400. https://doi.org/10.3390/ijerph16030400

Okumura, K., Mikami, B., Oiki, S., Ogura, K., & Hashimoto, W. (2024). Expression, purification and preliminary crystallographic analysis of bacterial transmembrane protein EfeU for iron import. *Protein Expression and Purification*, *219*, 106487. https://doi.org/10.1016/j.pep.2024.106487

Olishevska, S., Nickzad, A., & Déziel, E. (2019). *Bacillus* and *Paenibacillus* secreted polyketides and peptides involved in controlling human and plant pathogens. *Applied Microbiology and Biotechnology*, *103*(3), 1189–1215. https://doi.org/10.1007/s00253-018-9541-0

Ongpipattanakul, C., Desormeaux, E. K., Dicaprio, A., Van Der Donk, W. A., Mitchell, D. A., & Nair, S. K. (2022). Mechanism of Action of Ribosomally Synthesized and Post-Translationally Modified Peptides. In *Chemical Reviews* (Vol. 122, Issue 18, pp. 14722–14814). American Chemical Society. https://doi.org/10.1021/acs.chemrev.2c00210

Ongpipattanakul, C., Desormeaux, K. E., DiCaprio, A., van der Donk, A. W., Mitchell, A. D., & Nair, K. S. (2022). Mechanism of action of ribosomally synthesized and post-translationally modified peptides. *Chemical Reviews*, *122*(18), 14722–14814. https://doi.org/10.1021/acs.chemrev.2c00210

Orozco-Mosqueda, Ma. del C., Fadiji, A. E., Babalola, O. O., & Santoyo, G. (2023). Bacterial elicitors of the plant immune system: An overview and the way forward. *Plant Stress*, *7*, 100138. https://doi.org/10.1016/j.stress.2023.100138

Orozco-Mosqueda, Ma. del C., Santoyo, G., & Glick, B. R. (2023). Recent advances in the bacterial phytohormone modulation of plant growth. *Plants*, *12*(3), 606. https://doi.org/10.3390/plants12030606

Osdaghi, E. (2023). Pectobacterium carotovorum *(bacterial soft rot)*.

Overbeek, R., Olson, R., Pusch, G. D., Olsen, G. J., Davis, J. J., Disz, T., Edwards, R. A., Gerdes, S., Parrello, B., Shukla, M., Vonstein, V., Wattam, A. R., Xia, F., & Stevens, R. (2014). The SEED and the rapid annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Research*, *42*(D1), D206–D214. https://doi.org/10.1093/nar/gkt1226

Özcengiz, G., & Alaeddinoglu, N. G. (1991). Bacilysin production by *Bacillus subtilis*: Effects of bacilysin, pH and temperature. *Folia Microbiologica*, *36*(6), 522–526. https://doi.org/10.1007/BF02884030

Özcengiz, G., & Öğülür, I. (2015). Biochemistry, genetics and regulation of bacilysin biosynthesis and its significance more than an antibiotic. *New Biotechnology*, *32*(6), 612–619. https://doi.org/10.1016/J.NBT.2015.01.006

Paik, S. H., Chakicherla, A., & Norman Hansen, J. (1998). Identification and characterization of the structural and transporter genes for, and the chemical and biological properties of, sublancin 168, a novel lantibiotic produced by *Bacillus subtilis* 168. *Journal of Biological Chemistry*, *273*(36), 23134–23142. https://doi.org/10.1074/jbc.273.36.23134

Paka, G. V., Mobambo, P. K., Omondi, B. A., & Staver, C. (2021). Evaluation de l'efficacité de la macro-propagation des cultivars de bananiers les plus préférés au Kongo Central, en RD Congo. *Afrique SCIENCE*, *19*(6), 76–88.

Pálfi, P., Bakacsy, L., Kovács, H., & Szepesi, Á. (2021). Hypusination, a metabolic posttranslational modification of eif5a in plants during development and environmental stress responses. In *Plants* (Vol. 10, Issue 7). MDPI AG. https://doi.org/10.3390/plants10071261

Pandey, C., Prabha, D., Negi, Y. K., Maheshwari, D. K., Dheeman, S., & Gupta, M. (2023). Macrolactin A mediated biocontrol of Fusarium oxysporum and Rhizoctonia solani infestation on Amaranthus hypochondriacus by Bacillus subtilis</i> BS-58. *Frontiers in Microbiology*, *14*, 11058449. https://doi.org/10.3389/fmicb.2023.1105849

Pandey, P., Patil, M., Priya, P., & Senthil-Kumar, M. (2024). When two negatives make a positive: The favorable impact of the combination of abiotic stress and pathogen infection on plants. *Journal of Experimental Botany*, *75*(3), 674–688. https://doi.org/10.1093/jxb/erad413

Pandit, M. A., Kumar, J., Gulati, S., Bhandari, N., Mehta, P., Katyal, R., Rawat, C. D., Mishra, V., & Kaur, J. (2022). Major biological control strategies for plant pathogens. *Pathogens*, *11*(2), 273. https://doi.org/10.3390/pathogens11020273

Pandith, S. A., Ramazan, S., Khan, M. I., Reshi, Z. A., & Shah, M. A. (2020). Chalcone synthases (CHSs): The symbolic type III polyketide synthases. *Planta*, *251*(1), 15. https://doi.org/10.1007/s00425-019-03307-y

Pang, Y., Yang, J., Chen, X., Jia, Y., Li, T., Jin, J., Liu, H., Jiang, L., Hao, Y., Zhang, H., & Xie, Y. (2021). An antifungal chitosanase from <Bacillus subtilis</i> SH21. *Molecules*, *26*, 1863. https://doi.org/10.3390/molecules26071863

Panicker, S., & Sayyed, R. Z. (2022). Hydrolytic enzymes from PGPR against plant fungal pathogens. In R. Z. Sayyed, A. Singh, & N. Ilyas (Eds.), *Antifungal metabolites of rhizobacteria for sustainable agriculture, Fungal Biology* (Vol. 29, Issue 3, pp. 211–238). https://doi.org/10.1007/978-3-031-04805-0_10

Panlilio, H., & Rice, C. V. (2021). The role of extracellular DNA in the formation, architecture, stability, and treatment of bacterial biofilms. *Biotechnology and Bioengineering*, *118*(6), 2129–2141. https://doi.org/10.1002/bit.27760

Pappalettere, L., Bartolini, S., & Toffanin, A. (2024). Enhancement of tomato seed germination and growth parameters through seed priming with auxin-producing plant growth promoting bacteria strains. *Seeds*, *3*(3), 479–492. https://doi.org/10.3390/seeds3030032

Park, J. S., Ryu, G. R., & Kang, B. R. (2022). Target mechanism of iturinic lipopeptide on differential expression patterns of defense-related genes against *Colletotrichum acutatum* in pepper. *Plants*, *11*(9), 1267. https://doi.org/10.3390/plants11091267

Park, S., Kim, D., Jang, I., Oh, H. Bin, & Choe, J. (2014). Structural and biochemical study of *Bacillus subtilis* HmoB in complex with heme. *Biochemical and Biophysical Research Communications*, *446*(1), 286–291. https://doi.org/10.1016/j.bbrc.2014.02.092

Parker, J. B., & Walsh, C. T. (2013). Action and timing of BacC and BacD in the late stages of biosynthesis of the dipeptide antibiotic bacilysin. *Biochemistry*, *52*(5), 889–901. https://doi.org/10.1021/bi3016229

Parte, A. C., Carbasse, J. S., Meier-Kolthoff, J. P., Reimer, L. C., & Göker, M. (2020). List of prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ. *International Journal of Systematic and Evolutionary Microbiology*, *70*(11), 5607–5612. https://doi.org/10.1099/ijsem.0.004332

Patani, A., Patel, M., Islam, S., Yadav, V. K., Prajapati, D., Yadav, A. N., Sahoo, D. K., & Patel, A. (2024). Recent advances in *Bacillus*-mediated plant growth enhancement: A paradigm shift in redefining crop resilience. *World Journal of Microbiology and Biotechnology*, *40*(2), 77. https://doi.org/10.1007/s11274-024-03903-5

Patel, K., Goswami, D., Dhandhukia, P., & Thakker, J. (2015). Techniques to study microbial phytohormones. In K. D. Maheshwari (Ed.), *Bacterial metabolites in sustainable agroecosystem, Sustainable development and biodiversity* (Vol. 12, pp. 1–27). Springer. https://doi.org/10.1007/978-3-319-24654-3_1

Patra, D., & Mandal, S. (2022). Non-rhizobia are the alternative sustainable solution for growth and development of the nonlegume plants. *Biotechnology and Genetic Engineering Reviews*, *39*(2), 435–464. https://doi.org/10.1080/02648725.2022.2152623

Pavlović, M., Šokarda Slavić, M., Kojić, M., Margetić, A., Ristović, M., Drulović, N., & Vujčić, Z. (2024). Unveiling novel insights into *Bacillus velezensis* 16B pectin lyase for improved fruit juice processing. *Food Chemistry*, *456*, 140030. https://doi.org/10.1016/j.foodchem.2024.140030

Pedersen, T. B., Nielsen, M. R., Kristensen, S. B., Spedtsberg, E. M. L., Sørensen, T., Petersen, C., Muff, J., Sondergaard, T. E., Nielsen, K. L., Wimmer, R., Gardiner, D. M., & Sørensen, J. L. (2022). Speed dating for enzymes! Finding the perfect phosphopantetheinyl transferase partner for your polyketide synthase. *Microbial Cell Factories*, *21*(1), 9. https://doi.org/10.1186/s12934-021-01734-9

Pedreira, T., Elfmann, C., & Stülke, J. (2022). The current state of SubtiWiki, the database for the model organism *Bacillus subtilis*. *Nucleic Acids Research*, *50*(D1), D875–D882. https://doi.org/10.1093/nar/gkab943

Penha, R. O., Vandenberghe, L. P. S., Faulds, C., Soccol, V. T., & Soccol, C. R. (2020). *Bacillus* lipopeptides as powerful pest control agents for a more sustainable and healthy agriculture: Recent studies and innovations. *Planta*, *251*(3), 70. https://doi.org/10.1007/s00425-020-03357-7

Perumal, V., Yao, Z., Kim, J. A., Kim, H. J., & Kim, J. H. (2019). Purification and characterization of a bacteriocin, bacBS2, produced by *Bacillus velezensis* BS2 isolated from meongge jeotgal. *Journal of Microbiology and Biotechnology*, *29*(7), 1033–1042. https://doi.org/10.4014/jmb.1903.03065

Pettinari, M. J., Pavan, M. E., & López, N. I. (2023). Melanin synthesis in bacteria: Who, how and why. In G. Gosset (Ed.), *Melanins: Functions, biotechnological production, and applications* (pp. 1–25). Springer. https://doi.org/10.1007/978-3-031-27799-3_1

Pfanzagl, V., Holcik, L., Maresch, D., Gorgone, G., Michlits, H., Furtmüller, P. G., & Hofbauer, S. (2018). Coproheme decarboxylases - Phylogenetic prediction versus biochemical experiments. *Archives of Biochemistry and Biophysics*, *640*, 27–36. https://doi.org/10.1016/j.abb.2018.01.005

Pi, H., & Helmann, J. D. (2017). Sequential induction of Fur-regulated genes in response to iron limitation in *Bacillus subtilis*. *Proceedings of the National Academy of Sciences*, *114*(48), 12785–12790. https://doi.org/10.1073/pnas.1713008114

Pleban, S., Ingel, F., & Chet, I. (1995). Control of *Rhizoctonia solani* and *Sclerotium rolfsii* in the greenhouse using endophytic *Bacillus* spp. *European Journal of Plant Pathology*, *101*(6), 665–672. https://doi.org/10.1007/BF01874870

PND. (2018). *Plan National de Développement du Burundi 2018-2027*. https://www.presidence.gov.bi/wp-content/uploads/2018/08/PND-Burundi-2018-2027-Version-Finale.pdf

Põllumaa, L., Alamäe, T., & Mäe, A. (2012). Quorum sensing and expression of virulence in Pectobacteria. *Sensors*, *12*(3), 3327–3349. https://doi.org/10.3390/s120303327

Polonca, S. (2020). Environment shapes the intra-species diversity of *Bacillus subtilis* isolates. *Microbial Ecology*, *79*(4), 853–864. https://doi.org/10.1007/s00248-019-01455-y

Pomerleau, M., Charron-Lamoureux, V., Léonard, L., Grenier, F., Rodrigue, S., & Beauregard, P. B. (2024). Adaptive laboratory evolution reveals regulators involved in repressing biofilm development as key players in *Bacillus subtilis* root colonization. *MSystems*, *9*(2), 1–16. https://doi.org/10.1128/msystems.00843-23

Potter, C., Klooster, S., & Krauter, C. (2003). Regional modeling of ammonia emissions from native soil sources in California. *Earth Interactions*, *7*(11), 1–28. https://doi.org/10.1175/1087-3562(2003)007<0001:RMOAEF>2.0.CO;2

Poulaki, E. G., & Tjamos, S. E. (2023). *Bacillus* species: Factories of plant protective volatile organic compounds. *Journal of Applied Microbiology*, *134*(3), lxad037. https://doi.org/10.1093/jambio/lxad037

Poveda, J., & González-Andrés, F. (2021). *Bacillus* as a source of phytohormones for use in agriculture. *Applied Microbiology and Biotechnology*, *105*(23), 8629–8645. https://doi.org/10.1007/s00253-021-11492-8

Prasad, B., Sharma, D., Kumar, P., & Chandra Dubey, R. (2023). Biocontrol potential of *Bacillus* spp. for resilient and sustainable agricultural systems. *Physiological and Molecular Plant Pathology*, *128*, 102173. https://doi.org/10.1016/j.pmpp.2023.102173

Prazdnova, E. V., Gorovtsov, A. V., Vasilchenko, N. G., Kulikov, M. P., Statsenko, V. N., Bogdanova, A. A., Refeld, A. G., Brislavskiy, Y. A., Chistyakov, V. A., & Chikindas, M. L. (2022). Quorum-sensing inhibition by Gram-positive bacteria. *Microorganisms*, *10*(2), 350. https://doi.org/10.3390/microorganisms10020350

Pršić, J., & Ongena, M. (2020). Elicitors of plant immunity triggered by beneficial bacteria. *Frontiers in Plant Science*, *11*, 594530. https://doi.org/10.3389/fpls.2020.594530

Puan, S. L., Erriah, P., Baharudin, M. M. A., Yahaya, N. M., Kamil, W. N. I. W. A., Ali, M. S. M., Ahmad, S. A., Oslan, S. N., Lim, S., & Sabri, S. (2023). Antimicrobial peptides from *Bacillus* spp. and strategies to enhance their yield. *Applied Microbiology and Biotechnology*, *107*(18), 5569–5593. https://doi.org/10.1007/s00253-023-12651-9

Qiao, J., Zhang, R., Liu, Y., & Liu, Y. (2023). Evaluation of the biocontrol efficiency of *Bacillus subtilis* wettable powder on pepper root rot caused by *Fusarium solani*. *Pathogens*, *12*(2), 225. https://doi.org/10.3390/pathogens12020225

Qin, Y., Wang, Y., He, Y., Zhang, Y., She, Q., Chai, Y., Li, P., & Shang, Q. (2019). Characterization of subtilin L-Q11, a novel class I bacteriocin synthesized by *Bacillus subtilis* L-Q11 isolated from orchard soil. *Frontiers in Microbiology*, *10*, 484. https://doi.org/10.3389/fmicb.2019.00484

Radhakrishnan, R., Hashem, A., & Abd_Allah, E. F. (2017). *Bacillus*: A biological tool for crop improvement through bio-molecular changes in adverse environments. *Frontiers in Physiology*, *8*, 667. https://doi.org/10.3389/fphys.2017.00667

Rahman, F. Bin, Sarkar, B., Moni, R., & Rahman, M. S. (2021). Molecular genetics of surfactin and its effects on different sub-populations of *Bacillus subtilis*. *Biotechnology Reports*, *32*, e00686. https://doi.org/10.1016/j.btre.2021.e00686

Rajer, F. U., Wu, H., Xie, Y., Xie, S., Raza, W., Tahir, H. A. S., & Gao, X. (2017). Volatile organic compounds produced by a soil-isolate, *Bacillus subtilis* FA26 induce adverse ultra-structural changes to the cells of *Clavibacter michiganensis* ssp. *sepedonicus*, the causal agent of bacterial ring rot of potato. *Microbiology*, *163*(4), 523–530. https://doi.org/10.1099/mic.0.000451

Rajesh, R., & Gummadi, S. N. (2022). α-Amylase and cellulase production by novel halotolerant *Bacillus* sp. PM06 isolated from sugarcane pressmud. *Biotechnology and Applied Biochemistry*, *69*(1), 149–159. https://doi.org/10.1002/bab.2091

Raju, D. V., Nagarajan, A., Pandit, S., Nag, M., Lahiri, D., & Upadhye, V. (2022). Effect of bacterial quorum sensing and mechanism of antimicrobial resistance. *Biocatalysis and Agricultural Biotechnology*, *43*, 102409. https://doi.org/10.1016/j.bcab.2022.102409

Ramalakshmi, V. D. L. P. D. K. A. K. (2020). Pest control by macroorganisms. In S. V. S. S. R. K. Raju (Ed.), *Recent trends in insect pest management* (Vol. 3, pp. 135–156). AkiNik Publications. https://doi.org/10.22271/ed.book.819

Ramírez-Pool, J. A., Calderón-Pérez, B., Ruiz-Medrano, R., Ortiz-Castro, R., & Xoconostle-Cazares, B. (2024). *Bacillus* strains as effective biocontrol agents against phytopathogenic bacteria and promoters of plant growth. *Microbial Ecology*, *87*(1), 76. https://doi.org/10.1007/s00248-024-02384-1

Ramyabharathi, S., & Raguchander, T. (2014). Efficacy of secondary metabolites produced by *Bacillus subtilis* EPCO16 against tomato wilt pathogen *Fusarium oxysporum* f.sp. *lycopersici*. *J Mycol Plant Pathol*, *44*(2), 148–153. https://www.cabidigitallibrary.org/doi/full/10.5555/20143262465

Rana, A., Sudakov, K., Carmeli, S., Miyara, S. B., Bucki, P., & Minz, D. (2024). Volatile organic compounds of the soil bacterium *Bacillus halotolerans* suppress pathogens and elicit defense-responsive genes in plants. *Microbiological Research*, *281*, 127611. https://doi.org/10.1016/j.micres.2024.127611

Raphel, S., & Halami, P. M. (2024). Genome mining of *Bacillus licheniformis* MCC2514 for the identification of lasso peptide biosynthetic gene cluster and its characterization. *Archives of Microbiology*, *206*(4), 143. https://doi.org/10.1007/s00203-024-03877-w

Rashid, G. M. M., & Bugg, T. D. H. (2021). Enhanced biocatalytic degradation of lignin using combinations of lignin-degrading enzymes and accessory enzymes. *Catalysis Science and Technology*, *11*(10), 3568–3577. https://doi.org/10.1039/d1cy00431j

Rasouli, S., Asl, M., Marandi, R., Emtyazjoo, M., & Zaeimdar, M. (2024). Investigation of total petroleum hydrocarbons and indigenous bacteria under aerobic conditions to increase the efficiency of conventional treatment systems for oil-polluted soils. *International Journal of Industrial Chemistry*, *15*(2), 152412. https://doi.org/10.57647/j.ijic.2024.1502.12

Rath, H., Sappa, P. K., Hoffmann, T., Gesell Salazar, M., Reder, A., Steil, L., Hecker, M., Bremer, E., Mäder, U., & Völker, U. (2020). Impact of high salinity and the compatible solute glycine betaine on gene expression of *Bacillus subtilis*. *Environmental Microbiology*, *22*(8), 3266–3286. https://doi.org/10.1111/1462-2920.15087

Raut, S. P., & Ranade, S. (2004). Diseases of banana and their management: Diagnosis and management. In S. A. M. H. Naqvi (Ed.), *Diseases of fruits and vegetables* (Vol. 2, pp. 37–52). Kluwer Academic. https://doi.org/10.1007/1-4020-2607-2_2

Raveau, R., Fontaine, J., & Lounès-Hadj Sahraoui, A. (2020). Essential oils as potential alternative biocontrol products against plant pathogens and weeds: A review. *Foods*, *9*(3), 365. https://doi.org/10.3390/foods9030365

Redinbaugh, M. G., & Stewart, L. R. (2018). Maize lethal necrosis: An emerging, synergistic viral disease. *Annual Review of Virology*, *5*(1), 301–322. https://doi.org/10.1146/annurev-virology-092917-043413

REMA. (2011). *Lake Victoria environmental management project phase II: The national integrated pest management (IPM) framework for Rwanda* (Issue June 2009). https://www.rema.gov.rw/rema_doc/LVEMP/IPM_Latest%20Version-2.pdf

Ren, H., Dommaraju, S. R., Huang, C., Cui, H., Pan, Y., Nesic, M., Zhu, L., Sarlah, D., Mitchell, D. A., & Zhao, H. (2023). Genome mining unveils a class of ribosomal peptides with two amino termini. *Nature Communications*, *14*(1), 1624. https://doi.org/10.1038/s41467-023-37287-1

Repka, L. M., Chekan, J. R., Nair, S. K., & van der Donk, W. A. (2017). Mechanistic understanding of lanthipeptide biosynthetic enzymes. *Chemical Reviews*, *117*(8), 5457–5520. https://doi.org/10.1021/acs.chemrev.6b00591

Reva, O. N., Weinel, C., Weinel, M., Böhm, K., Stjepandic, D., Hoheisel, J. D., & Tümmler, B. (2006). Functional genomics of stress response in *Pseudomonas putida* KT2440. *Journal of Bacteriology*, *188*(11), 4079–4092. https://doi.org/10.1128/JB.00101-06

Reyes-Estebanez, M., Sanmartín, P., Camacho-Chab, J. C., De la Rosa-García, S. C., Chan-Bacab, M. J., Águila-Ramírez, R. N., Carrillo-Villanueva, F., De la Rosa-Escalante, E., Arteaga-Garma, J. L., Serrano, M., & Ortega-Morales, B. O. (2020). Characterization of a native *Bacillus velezensis*-like strain for the potential biocontrol of tropical fruit pathogens. *Biological Control*, *141*, 104127. https://doi.org/10.1016/j.biocontrol.2019.104127

Ricci, E., Schwinghamer, T., Fan, D., Smith, D. L., & Gravel, V. (2019). Growth promotion of greenhouse tomatoes with *Pseudomonas* sp. and *Bacillus* sp. biofilms and planktonic cells. *Applied Soil Ecology*, *138*, 61–68. https://doi.org/10.1016/j.apsoil.2019.02.009

Richter, A., Blei, F., Hu, G., Schwitalla, J. W., Lozano-Andrade, C. N., Xie, J., Jarmusch, S. A., Wibowo, M., Kjeldgaard, B., Surabhi, S., Xu, X., Jautzus, T., Phippen, C. B. W., Tyc, O., Arentshorst, M., Wang, Y., Garbeva, P., Larsen, T. O., Ram, A. F. J., … Kovács, Á. T. (2024). Enhanced surface colonisation and competition during bacterial adaptation to a fungus. *Nature Communications*, *15*(1), 4486. https://doi.org/10.1038/s41467-024-48812-1

Rietveld, A. M., Dusingizimana, P., Blomme, G., Gaidashova, S. V, Ocimati, W., & Ntamwira, J. (2020). *A superior technology to control banana* Xanthomonas *wilt (BXW) in Rwanda*. https://promusa.org/seeMore.php?id=19677&recent

Rigolet, A. (2023). *Featuring the bioactive secondary metabolites of Bacillus in interspecies interactions*. University of Liège.

Riseh, R. S., Fathi, F., Vazvani, M. G., & Tarkka, M. T. (2025). Plant colonization by biocontrol bacteria and improved plant health: A review. *Frontiers in Bioscience-Landmark*, *30*(1), 23223. https://doi.org/10.31083/FBL23223

Roca, A., Cabeo, M., Enguidanos, C., Martínez-Checa, F., Sampedro, I., & Llamas, I. (2024). Potential of the quorum-quenching and plant-growth promoting halotolerant *Bacillus toyonensis* AA1EC1 as biocontrol agent. *Microbial Biotechnology*, *17*(3), e14420. https://doi.org/10.1111/1751-7915.14420

Rodenburg, J., Demont, M., Zwart, S. J., & Bastiaans, L. (2016). Parasitic weed incidence and related economic losses in rice in Africa. *Agriculture, Ecosystems & Environment*, *235*, 306–317. https://doi.org/10.1016/j.agee.2016.10.020

Roets-Dlamini, Y., Moonsamy, G., Lalloo, R., & Ramchuran, S. (2022). Use of *Bacillus* spp. in the bioremediation of fats, oils and greases (FOG's), and other waste substrates in food processing effluents. *Biocatalysis and Agricultural Biotechnology*, *42*, 102351. https://doi.org/10.1016/j.bcab.2022.102351

Rojas-Solis, D., García Rodríguez, Y. M., Larsen, J., Santoyo, G., & Lindig-Cisneros, R. (2023). Growth promotion traits and emission of volatile organic compounds of two bacterial strains stimulate growth of maize exposed to heavy metals. *Rhizosphere*, *27*, 100739. https://doi.org/10.1016/j.rhisph.2023.100739

Romero-Severson, J., Moran, T. E., Shrader, D. G., Fields, F. R., Pandey-Joshi, S., Thomas, C. L., Palmer, E. C., Shrout, J. D., Pfrender, M. E., & Lee, S. W. (2021). A seed-endophytic *Bacillus safensis* strain with antimicrobial activity has genes for novel bacteriocin-like antimicrobial peptides. *Frontiers in Microbiology*, *12*, 734216. https://doi.org/10.3389/fmicb.2021.734216

Rosazza, T., Eigentler, L., Earl, C., Davidson, F. A., & Stanley-Wall, N. R. (2023). *Bacillus subtilis* extracellular protease production incurs a context-dependent cost. *Molecular Microbiology*, *120*(2), 105–121. https://doi.org/10.1111/mmi.15110

Roser, M. (2023). *Employment in Agriculture*. https://ourworldindata.org/employment-in-agriculture

Rousk, J., Brookes, P. C., & Bååth, E. (2009). Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Applied and Environmental Microbiology*, *75*(6), 1589–1596. https://doi.org/10.1128/AEM.02775-08

Roy, B., Maitra, D., Biswas, A., Chowdhury, N., Ganguly, S., Bera, M., Dutta, S., Golder, S., Roy, S., Ghosh, J., & Mitra, A. K. (2024). Efficacy of high-altitude biofilm-forming novel *Bacillus subtilis* species as plant growth-promoting rhizobacteria on *Zea mays* L. *Applied Biochemistry and Biotechnology*, *196*(2), 643–666. https://doi.org/10.1007/s12010-023-04563-1

Roy, M. E., & Griffith, L. K. (2017). Characterization of a novel iron acquisition activity that coordinates the iron response with population density under iron-replete conditions in *Bacillus subtilis*. *Journal of Bacteriology*, *199*(1), e00487-16. https://doi.org/10.1128/JB.00487-16

Roy, T., Pal, N., & Das, N. (2024). Regulation of the polyamine pool in plants: Metabolic implications and stress mitigation, with emphasis on microbial influence. *Physiological and Molecular Plant Pathology*, *132*, 102317. https://doi.org/10.1016/j.pmpp.2024.102317

Rudakova, N. L., Khilyas, I. V., Danilova, I. V., Pudova, D. S., & Sharipova, M. R. (2023). Evaluating of the potential of *Bacillus pumilus* 3-19 as a plant growth-promoting strain. *Russian Journal of Plant Physiology*, *70*(8), 197. https://doi.org/10.1134/S1021443723603282

Runo, S., & Kuria, E. K. (2018). Habits of a highly successful cereal killer, Striga. *PLoS Pathogens*, *14*(1), 2–7. https://doi.org/10.1371/journal.ppat.1006731

Ruraduma, C., Ntukamazina, N., Ntibashirwa, S., & Niko, N. (2012). *Conduite de la culture du haricot (*Phaseolus vulgaris *L.) au Burundi*. ISABU.

Rutikanga, A. (2015). *Pesticides use and regulations in Rwanda status and potential for promotion of biological control methods*. University of Neuchatel.

Ryu, C.-M., Nelson, L. M., & de-Bashan, L. (2024). Editorial: Highlights from the 12th plant growth-promoting rhizobacteria workshop. *Frontiers in Plant Science*, *15*, 1470576. https://doi.org/10.3389/fpls.2024.1470576

Saberi Riseh, R., Vatankhah, M., Hassanisaadi, M., & Barka, E. A. (2024). Unveiling the role of hydrolytic enzymes from soil biocontrol bacteria in sustainable phytopathogen management. *Frontiers in Bioscience-Landmark*, *29*(3), 105. https://doi.org/10.31083/j.fbl2903105

Saeed, Q., Xiukang, W., Haider, F. U., Kučerik, J., Mumtaz, M. Z., Holatko, J., Naseem, M., Kintl, A., Ejaz, M., Naveed, M., Brtnicky, M., & Mustafa, A. (2021). Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: A comprehensive review of effects and mechanisms. *International Journal of Molecular Sciences*, *22*(19), 10529. https://doi.org/10.3390/ijms221910529

Sagar, A., Yadav, S. S., Sayyed, R. Z., Sharma, S., & Ramteke, P. W. (2022). *Bacillus subtilis*: A multifarious plant growth promoter, biocontrol agent, and bioalleviator of abiotic stress. In M. T. Islam, M. Rahman, & P. Pandey (Eds.), *Bacilli in agrobiotechnology plant stress tolerance, bioremediation, and bioprospecting* (pp. 561–580). Springer. https://doi.org/10.1007/978-3-030-85465-2_24

Saikia, K., Belwal, V. K., Datta, D., & Chaudhary, N. (2019). Aromatic-rich C-terminal region of LCI is a potent antimicrobial peptide in itself. *Biochemical and Biophysical Research Communications*, *519*(2), 372–377. https://doi.org/10.1016/j.bbrc.2019.09.013

Saini, N., Sahgal, M., & Singh, A. V. (2024). Mechanisms of cell wall degrading enzymes from *Bacillus methylotrophicus* and *Bacillus subtilis* in suppressing foliar blight pathogens. *Journal of Advances in Biology & Biotechnology*, *27*(8), 239–249. https://doi.org/10.9734/jabb/2024/v27i81136

Saiyam, D., Dubey, A., Malla, M. A., & Kumar, A. (2024). Lipopeptides from *Bacillus*: unveiling biotechnological prospects-sources, properties, and diverse applications. *Brazilian Journal of Microbiology*, *55*(1), 281–295. https://doi.org/10.1007/s42770-023-01228-3

Sakai, M., Masai, E., Asami, H., Sugiyama, K., Kimbara, K., & Fukuda, M. (2002). Diversity of 2,3-dihydroxybiphenyl dioxygenase genes in a strong PCB degrader, *Rhodococcus* sp. strain RHA1. *Journal of Bioscience and Bioengineering*, *93*(4), 421–427. https://doi.org/10.1016/S1389-1723(02)80078-0

Salazar, B., Ortiz, A., Keswani, C., Minkina, T., Mandzhieva, S., Pratap Singh, S., Rekadwad, B., Borriss, R., Jain, A., Singh, H. B., & Sansinenea, E. (2023). *Bacillus* spp. as bio-factories for antifungal secondary metabolites: Innovation beyond whole

organism formulations. *Microbial Ecology*, *86*(1), 1–24. https://doi.org/10.1007/s00248-022-02044-2

Salazar-Cerezo, S., Martínez-Montiel, N., García-Sánchez, J., Pérez-y-Terrón, R., & Martínez-Contreras, R. D. (2018). Gibberellin biosynthesis and metabolism: A convergent route for plants, fungi and bacteria. *Microbiological Research*, *208*, 85–98. https://doi.org/10.1016/j.micres.2018.01.010

Saleem, B. (2021). Phyllosphere microbiome: Plant defense strategies. In A. S. Lone & A. Malik (Eds.), *Microbiomes and the global climate change* (pp. 173–201). Springer Singapore. https://doi.org/10.1007/978-981-33-4508-9_11

Salwan, R., Sharma, M., Sharma, A., & Sharma, V. (2023). Insights into plant beneficial microorganism-triggered induced systemic resistance. *Plant Stress*, *7*, 100140. https://doi.org/10.1016/j.stress.2023.100140

Sanghvi, G., Patel, H., Vaishnav, D., Oza, T., Dave, G., Kunjadia, P., & Sheth, N. (2016). A novel alkaline keratinase from *Bacillus subtilis* DP1 with potential utility in cosmetic formulation. *International Journal of Biological Macromolecules*, *87*, 256–262. https://doi.org/10.1016/j.ijbiomac.2016.02.067

Santos, L. F., & Olivares, F. L. (2021). Plant microbiome structure and benefits for sustainable agriculture. *Current Plant Biology*, *26*, 100198. https://doi.org/10.1016/j.cpb.2021.100198

Santoyo, G., Hernández-Pacheco, C., Hernández-Salmerón, J., & Hernández-León, R. (2017). The role of abiotic factors modulating the plant-microbe-soil interactions: Toward sustainable agriculture. A review. *Spanish Journal of Agricultural Research*, *15*(1), e03R01. https://doi.org/10.5424/sjar/2017151-9990

Sartori, M., Nesci, A., García, J., Passone, M. A., Montemarani, A., & Etcheverry, M. (2017). Efficacy of epiphytic bacteria to prevent northern leaf blight caused by *Exserohilum turcicum* in maize. *Revista Argentina de Microbiología*, *49*(1), 75–82. https://doi.org/10.1016/J.RAM.2016.09.008

Sauer, K., Stoodley, P., Goeres, D. M., Hall-Stoodley, L., Burmølle, M., Stewart, P. S., & Bjarnsholt, T. (2022). The biofilm life cycle: Expanding the conceptual model of biofilm formation. *Nature Reviews Microbiology*, *20*(10), 608–620. https://doi.org/10.1038/s41579-022-00767-0

Saxena, D., Ben-Dov, E., Manasherob, R., Barak, Z., Boussiba, S., & Zaritsky, A. (2002). A UV tolerant mutant of *Bacillus thuringiensis* subsp. *kurstaki* producing melanin. *Current Microbiology*, *44*(1), 25–30. https://doi.org/10.1007/S00284-001-0069-6

Saxena, R., & Singh, R. (2011). Amylase production by solid-state fermentation of agro-industrial wastes using *Bacillus* sp. *Brazilian Journal of Microbiology*, *42*(4), 1334–1342. https://doi.org/10.1590/S1517-83822011000400014

Schmid, R., Heuckeroth, S., Korf, A., Smirnov, A., Myers, O., Dyrlund, T. S., Bushuiev, R., Murray, K. J., Hoffmann, N., Lu, M., Sarvepalli, A., Zhang, Z., Fleischauer, M., Dührkop, K., Wesner, M., Hoogstra, S. J., Rudt, E., Mokshyna, O., Brungs, C., … Pluskal, T. (2023). Integrative analysis of multimodal mass spectrometry data in MZmine 3. *Nature Biotechnology*, *41*(4), 447–449. https://doi.org/10.1038/s41587-023-01690-2

Schmidt, W., Thomine, S., & Buckhout, T. J. (2020). Editorial: Iron nutrition and interactions in plants. *Frontiers in Plant Science*, *10*, 1670. https://doi.org/10.3389/fpls.2019.01670

Schneider, K., Chen, X. H., Vater, J., Franke, P., Nicholson, G., Borriss, R., & Süssmuth, R. D. (2007). Macrolactin is the polyketide biosynthesis product of the pks2 cluster of *Bacillus amyloliquefaciens* FZB42. *Journal of Natural Products*, *70*(9), 1417–1423. https://doi.org/10.1021/np070070k

Scholz, R., Molohon, K. J., Nachtigall, J., Vater, J., Markley, A. L., Süssmuth, R. D., Mitchell, D. A., & Borriss, R. (2011). Plantazolicin, a novel microcin B17/streptolysin S-like natural product from *Bacillus amyloliquefaciens* FZB42. *Journal of Bacteriology*, *193*(1), 215–224. https://doi.org/10.1128/JB.00784-10

Scholz, R., Vater, J., Budiharjo, A., Wang, Z., He, Y., Dietel, K., Schwecke, T., Herfort, S., Lasch, P., & Borriss, R. (2014). Amylocyclicin, a novel circular bacteriocin produced by *Bacillus amyloliquefaciens* FZB42. *Journal of Bacteriology*, *196*(10), 1842–1852. https://doi.org/10.1128/JB.01474-14

Schumacher, K., Brameyer, S., & Jung, K. (2023). Bacterial acid stress response: From cellular changes to antibiotic tolerance and phenotypic heterogeneity. *Current Opinion in Microbiology*, *75*, 102367. https://doi.org/10.1016/j.mib.2023.102367

Seemann, T. (2014). Prokka: Rapid prokaryotic genome annotation. *Bioinformatics*, *30*(14), 2068–2069. https://doi.org/10.1093/bioinformatics/btu153

Semerikova, D., Egorova, D., & Demakov, V. (2022). A new strain of the genus *Bacillus*, perspective for biodegradation of biphenyl and its derivatives. *AIP Conference Proceedings*, *2390*, 030077. https://doi.org/10.1063/5.0069062

Septiani, P., Pramesti, Y., Ghildan, M., Aprilia, K. Z., Awaludin, R., Medina, S., Subandiyah, S., & Meitha, K. (2025). RNAi-based biocontrol for crops: a revised expectation for a non-recent technology. *Planta*, *261*, 44. https://doi.org/10.1007/s00425-025-04625-0

Serrão, C. P., Ortega, J. C. G., Rodrigues, P. C., & de Souza, C. R. B. (2024). *Bacillus* species as tools for biocontrol of plant diseases: A meta-analysis of twenty-two years of research, 2000–2021. *World Journal of Microbiology and Biotechnology*, *40*(4), 110. https://doi.org/10.1007/s11274-024-03935-x

Shafi, S., Reda, F. M., & Ismail, M. (2017). Production of terpenoids, terpene alcohol, fatty acids and N2 compounds by *Bacillus amyloliquefaciens* S5i4 isolated from archaeological egyptian soil. *Adv Tech Clin Microbiol*, *1*(3), 18. http://www.imedpub.com/advanced-techniques-in-clinical-microbiology/

Shaikh, I. A., Turakani, B., Malpani, J., Goudar, S. V., Mahnashi, M. H., Hamed Al-Serwi, R., Ghoneim, M. M., El-Sherbiny, M., Abdulaziz Mannasaheb, B., Alsaikhan, F., Sindagimath, V., Khan, A. A., Muddapur, U. M., Azzouz, S., Mohammed, T., & Shakeel Iqubal, S. M. (2023). Extracellular protease production, optimization, and partial purification from *Bacillus nakamurai* PL4 and its applications. *Journal of King Saud University - Science*, *35*(1), 102429. https://doi.org/10.1016/j.jksus.2022.102429

Shailendra Singh, G. G. (2015). Plant Growth Promoting Rhizobacteria (PGPR): Current and Future Prospects for Development of Sustainable Agriculture. *Journal of Microbial & Biochemical Technology*, *07*(02), 96–102. https://doi.org/10.4172/1948-5948.1000188

Shao, D., He, Y., Zhai, Y., Yang, X., Guo, Z., Tan, J., & Wei, M. (2025). Mechanisms of tomato growth promotion in three soils after applying *Bacillus* combinations. *Soil and Tillage Research*, *249*, 106477. https://doi.org/10.1016/j.still.2025.106477

Shao, J., Li, S., Zhang, N., Cui, X., Zhou, X., Zhang, G., Shen, Q., & Zhang, R. (2015). Analysis and cloning of the synthetic pathway of the phytohormone indole-3-acetic acid in the plant-beneficial *Bacillus amyloliquefaciens* SQR9. *Microbial Cell Factories*, *14*(1), 130. https://doi.org/10.1186/s12934-015-0323-4

Sharifi, R., & Ryu, C.-M. (2016). Are bacterial volatile compounds poisonous odors to a fungal pathogen *Botrytis cinerea*, alarm signals to *Arabidopsis* seedlings for eliciting induced resistance, or both? *Frontiers in Microbiology*, *7*, 196. https://doi.org/10.3389/fmicb.2016.00196

Sharma, B. A., SarbjitKaur, & Lore, J. S. (2023). Prevalence of pathogens causing sheath rot of rice in North India and its management. *Indian Phytopathology*, *76*(3), 727–741. https://doi.org/10.1007/s42360-023-00634-9

Sharma, K., Kreuze, J., Abdurahman, A., Parker, M., Nduwayezu, A., & Rukundo, P. (2021). Molecular diversity and pathogenicity of *Ralstonia solanacearum* species complex associated with bacterial wilt of potato in Rwanda. *Plant Disease*, *105*(4), 770–779. https://doi.org/10.1094/PDIS-04-20-0851-RE

Sharma, S., & Araujo, A. S. F. (2024). Microbial crosstalk: Decoding interactions to generate efficient SynComs. *Trends in Plant Science*. https://doi.org/10.1016/j.tplants.2024.11.006

Shayanthan, A., Ordoñez, P. A. C., & Oresnik, I. J. (2022). The role of synthetic microbial communities (SynCom) in sustainable agriculture. *Frontiers in Agronomy*, *4*, 896307. https://doi.org/10.3389/fagro.2022.896307

Sheffield, F. M. L. (1957). Virus diseases of sweet potato in East Africa. I. Identification of the viruses and their insect vectors. *Phytopathology*, *47*(10), 582–590. https://www.cabidigitallibrary.org/doi/full/10.5555/19580500967

Shen, L., Zhang, S., & Chen, G. (2021). Regulated strategies of cold-adapted microorganisms in response to cold: A review. *Environmental Science and Pollution Research*, *28*(48), 68006–68024. https://doi.org/10.1007/s11356-021-16843-6

Shen, N., Li, S., Li, S., Zhang, H., & Jiang, M. (2022). The siderophore-producing bacterium, *Bacillus siamensis* Gxun-6, has an antifungal activity against *Fusarium oxysporum* and promotes the growth of banana. *Egyptian Journal of Biological Pest Control*, *32*(1), 34. https://doi.org/10.1186/s41938-022-00533-7

Shenkarev, Z. O., Finkina, E. I., Nurmukhamedova, E. K., Balandin, S. V., Mineev, K. S., Nadezhdin, K. D., Yakimenko, Z. A., Tagaev, A. A., Temirov, Y. V., Arseniev, A. S., & Ovchinnikova, T. V. (2010). Isolation, structure elucidation, and synergistic antibacterial activity of a novel two-component lantibiotic lichenicidin from *Bacillus*

licheniformis VK21. *Biochemistry*, *49*(30), 6462–6472. https://doi.org/10.1021/bi100871b

Shifa, H., Gopalakrishnan, C., & Velazhahan, R. (2018). Management of late leaf spot (*Phaeoisariopsis personata*) and root rot (*Macrophomina phaseolina*) diseases of groundnut (*Arachis hypogaea* L.) with plant growth-promoting rhizobacteria, systemic acquired resistance inducers and plant extracts. *Phytoparasitica*, *46*(1), 19–30. https://doi.org/10.1007/s12600-018-0644-z

Shree, P., Singh, C. K., Sodhi, K. K., Surya, J. N., & Singh, D. K. (2023). Biofilms: Understanding the structure and contribution towards bacterial resistance in antibiotics. *Medicine in Microecology*, *16*, 100084. https://doi.org/10.1016/j.medmic.2023.100084

Shyntum, D. Y., Nkomo, N. P., Shingange, N. L., Gricia, A. R., Bellieny-Rabelo, D., & Moleleki, L. N. (2019). The impact of type VI secretion system, bacteriocins and antibiotics on bacterial competition of *Pectobacterium carotovorum* subsp. *brasiliense* and the regulation of carbapenem biosynthesis by iron and the ferric-uptake regulator. *Frontiers in Microbiology*, *10*, 2379. https://doi.org/10.3389/fmicb.2019.02379

Siddika, A., Rashid, A. A., Khan, S. N., Khatun, A., Karim, M. M., Prasad, P. V. V., & Hasanuzzaman, M. (2024). Harnessing plant growth-promoting rhizobacteria, *Bacillus subtilis* and *B. aryabhattai* to combat salt stress in rice: A study on the regulation of antioxidant defense, ion homeostasis, and photosynthetic parameters. *Frontiers in Plant Science*, *15*, 1419764. https://doi.org/10.3389/fpls.2024.1419764

Sikdar, R., & Elias, M. (2020). Quorum quenching enzymes and their effects on virulence, biofilm, and microbiomes: A review of recent advances. *Expert Review of Anti-Infective Therapy*, *18*(12), 1221–1233. https://doi.org/10.1080/14787210.2020.1794815

Simon, Z., Mtei, K., Gessesse, A., & Ndakidemi, P. A. (2014). Isolation and Characterization of Nitrogen Fixing Rhizobia from Cultivated and Uncultivated Soils of Northern Tanzania. *American Journal of Plant Sciences*, *05*(26), 4050–4067. https://doi.org/10.4236/ajps.2014.526423

Singh, N., & Bhatla, S. C. (2022). Heme oxygenase-nitric oxide crosstalk-mediated iron homeostasis in plants under oxidative stress. *Free Radical Biology and Medicine*, *182*, 192–205. https://doi.org/10.1016/j.freeradbiomed.2022.02.034

Singh, R., Caseys, C., & Kliebenstein, D. J. (2024). Genetic and molecular landscapes of the generalist phytopathogen *Botrytis cinerea*. *Molecular Plant Pathology*, *25*(1), e13404. https://doi.org/10.1111/mpp.13404

Soltan, H. A. H., Soltan, H. A. H., Dakhly, O. F., Mahmoud, M. A., & Fikry, Y. F. M. (2022). Microbiological and genetical identification of some vermicompost beneficial associated bacteria. *SVU-International Journal of Agricultural Sciences*, *4*(1), 21–36. https://doi.org/10.21608/SVUIJAS.2021.106875.1154

Song, G. C., Jeon, J. S., Sim, H. J., Lee, S., Jung, J., Kim, S. G., Moon, S. Y., & Ryu, C. M. (2022). Dual functionality of natural mixtures of bacterial volatile compounds on

plant growth. *Journal of Experimental Botany*, *73*(2), 571–583. https://doi.org/10.1093/jxb/erab466

Soni, R., & Keharia, H. (2021). Phytostimulation and biocontrol potential of Gram-positive endospore-forming bacilli. *Planta*, *254*(3), 49. https://doi.org/10.1007/s00425-021-03695-0

Sorde, K. L., & Ananthanarayan, L. (2019). Isolation, screening, and optimization of bacterial strains for novel transglutaminase production. *Preparative Biochemistry and Biotechnology*, *49*, 64–73. https://doi.org/10.1080/10826068.2018.1536986

Sreedharan, S. M., Rishi, N., & Singh, R. (2023). Microbial lipopeptides: Properties, mechanics and engineering for novel lipopeptides. *Microbiological Research*, *271*, 127363. https://doi.org/10.1016/j.micres.2023.127363

Sreekala, A. G. V., Saraswathy, S. M., Nathan, V. K., & Uppuluri, K. B. (2025). Genomic and biochemical investigations in the biomineralizing potential of an isolated marine ureolytic *Bacillus* sp. N₉. *Science of The Total Environment*, *964*, 178591. https://doi.org/10.1016/j.scitotenv.2025.178591

Srivastava, V., Patra, K., Pai, H., Aguilar-Pontes, M. V., Berasategui, A., Kamble, A., Di Pietro, A., & Redkar, A. (2024). Molecular dialogue during host manipulation by the vascular wilt fungus *Fusarium oxysporum*. *Annual Review of Phytopathology*, *62*(1), 97–126. https://doi.org/10.1146/annurev-phyto-021722-034823

Stauff, D. L., Bagaley, D., Torres, V. J., Joyce, R., Anderson, K. L., Kuechenmeister, L., Dunman, P. M., & Skaar, E. P. (2008). *Staphylococcus aureus* HrtA is an ATPase required for protection against heme toxicity and prevention of a transcriptional heme stress response. *Journal of Bacteriology*, *190*(10), 3588–3596. https://doi.org/10.1128/JB.01921-07

Stein, T. (2020). Oxygen-limiting growth conditions and deletion of the transition state regulator protein AbrB in *Bacillus subtilis* 6633 result in an increase in subtilosin production and a decrease in subtilin production. *Probiotics and Antimicrobial Proteins*, *12*(2), 725–731. https://doi.org/10.1007/s12602-019-09547-4

Stein, T., Borchert, S., Conrad, B., Feesche, J., Hofemeister, B., Hofemeister, J., & Entian, K.-D. (2002). Two different lantibiotic-like peptides originate from the ericin gene cluster of *Bacillus subtilis* A1/3. *Journal of Bacteriology*, *184*(6), 1703–1711. https://doi.org/10.1128/JB.184.6.1703-1711.2002

Stein, T., Borchert, S., Kiesau, P., Heinzmann, S., Klöss, S., Klein, C., Helfrich, M., & Entian, K. (2002). Dual control of subtilin biosynthesis and immunity in *Bacillus subtilis*. *Molecular Microbiology*, *44*(2), 403–416. https://doi.org/10.1046/j.1365-2958.2002.02869.x

Stein, T., Heinzmann, S., Kiesau, P., Himmel, B., & Entian, K. (2003). The *spa* -box for transcriptional activation of subtilin biosynthesis and immunity in *Bacillus subtilis*. *Molecular Microbiology*, *47*(6), 1627–1636. https://doi.org/10.1046/j.1365-2958.2003.03374.x

Steinke, K., Mohite, O. S., Weber, T., & Kovács, Á. T. (2021). Phylogenetic distribution of secondary metabolites in the *Bacillus subtilis* species complex. *MSystems*, *6*(2), e00057-21. https://doi.org/10.1128/msystems.00057-21

Stenberg, J. A. (2017). A conceptual framework for integrated pest management. *Trends in Plant Science*, *22*(9), 759–769. https://doi.org/10.1016/j.tplants.2017.06.010

Striednig, B., & Hilbi, H. (2022). Bacterial quorum sensing and phenotypic heterogeneity: How the collective shapes the individual. *Trends in Microbiology*, *30*(4), 379–389. https://doi.org/10.1016/j.tim.2021.09.001

Stülke, J., Grüppen, A., Bramkamp, M., & Pelzer, S. (2023). *Bacillus subtilis*, a swiss army knife in science and biotechnology. *Journal of Bacteriology*, *205*(5), e00102-23. https://doi.org/10.1128/jb.00102-23

Stülke, J., & Hillen, W. (2000). Regulation of carbon catabolism in *Bacillus* species. *Annual Review of Microbiology*, *54*(1), 849–880. https://doi.org/10.1146/annurev.micro.54.1.849

Su, Y. ting, Liu, C., Long, Z., Ren, H., & Guo, X. hua. (2019). Improved production of spores and bioactive metabolites from *Bacillus amyloliquefaciens* in solid-state fermentation by a rapid optimization process. *Probiotics and Antimicrobial Proteins*, *11*(3), 921–930. https://doi.org/10.1007/s12602-018-9474-z

Subramaniyam, R., & Vimala, R. (2012). Solid state and submerged fermentation for the production of bioactive substances: A comparative study. *International Journal of Science and Nature*, *3*(3), 480–486. https://www.researchgate.net/publication/232041875

Sudharsan, M., Rajendra Prasad, N., & Saravanan, R. (2023). Bacterial redox response factors in the management of environmental oxidative stress. *World Journal of Microbiology and Biotechnology*, *39*(1), 11. https://doi.org/10.1007/s11274-022-03456-5

Sun, D., Liao, J., Sun, L., Wang, Y., Liu, Y., Deng, Q., Zhang, N., Xu, D., Fang, Z., Wang, W., & Gooneratne, R. (2019). Effect of media and fermentation conditions on surfactin and iturin homologues produced by *Bacillus* natto NT-6: LC-MS analysis. *AMB Express*, *9*(1), 120. https://doi.org/10.1186/s13568-019-0845-y

Sun, J., Liu, Y., Lin, F., Lu, Z., & Lu, Y. (2021). CodY, ComA, DegU and Spo0A controlling lipopeptides biosynthesis in *Bacillus amyloliquefaciens* fmbJ. *Journal of Applied Microbiology*, *131*(3), 1289–1304. https://doi.org/10.1111/jam.15007

Sun, J., Lu, F., Luo, Y., Bie, L., Xu, L., & Wang, Y. (2023). OrthoVenn3: An integrated platform for exploring and visualizing orthologous data across genomes. *Nucleic Acids Research*, *51*(W1), W397–W403. https://doi.org/10.1093/nar/gkad313

Sun, W., Li, S., Zhang, G., Fu, G., Qi, H., & Li, T. (2023). Effects of climate change and anthropogenic activities on soil pH in grassland regions on the Tibetan Plateau. *Global Ecology and Conservation*, *45*, e02532. https://doi.org/10.1016/j.gecco.2023.e02532

Sun, W., & Shahrajabian, M. H. (2025). Biostimulant and beyond: *Bacillus* spp., the Important plant growth-promoting rhizobacteria (PGPR)-based biostimulant for sustainable agriculture. *Earth Systems and Environment*. https://doi.org/10.1007/s41748-024-00552-4

Sun, X., Xu, Z., Xie, J., Hesselberg-Thomsen, V., Tan, T., Zheng, D., Strube, M. L., Dragoš, A., Shen, Q., Zhang, R., & Kovács, Á. T. (2022). *Bacillus velezensis*

stimulates resident rhizosphere *Pseudomonas stutzeri* for plant health through metabolic interactions. *ISME Journal*, *16*(3), 774–787. https://doi.org/10.1038/s41396-021-01125-3

Takigawa, H., Sugiyama, M., & Shibuya, Y. (2010). C35-terpenes from *Bacillus subtilis* KSM 6-10. *Journal of Natural Products*, *73*(2), 204–207. https://doi.org/10.1021/np900705q

Tang, J., Tang, D.-J., Dubrow, Z. E., Bogdanove, A., & An, S. (2021). *Xanthomonas campestris* pathovars. *Trends in Microbiology*, *29*(2), 182–183. https://doi.org/10.1016/j.tim.2020.06.003

Tasiaux, T. (2024). *Caractérisation de l'activité de biocontrôle de Bacillus nakamurai sur deux souches d'Exserohilum turcicum inoculées sur onze cultivars de maïs burundais*. Université Catholique de Louvain.

Tawfik, E., Hammad, I., & Bakry, A. (2022). Production of transgenic *Allium cepa* by nanoparticles to resist *Aspergillus niger* infection. *Molecular Biology Reports*, *49*(3), 1783–1790. https://doi.org/10.1007/s11033-021-06988-5

Tchatchambe, N. B. J., Losimba, K. J., Kirongozi, B. F., Adheka, G. J., & Onautshu, O. D. (2019). Macro-propagation and micro-propagation of BBTV-free plants in Kisangani, DR Congo. *Scholars Bulletin*, *5*(5), 178–183. https://doi.org/10.21276/sb.2019.5.5.1

Teixidó, N., Usall, J., & Torres, R. (2022). Insight into a successful development of biocontrol agents: Production, formulation, packaging, and shelf life as key aspects. *Horticulturae*, *8*(4), 305. https://doi.org/10.3390/horticulturae8040305

Thakur, N., Sood, R., & Parmar, S. (2022). Reviving back the ecological sustainability through microbial bioprospection. In A. Kumar (Ed.), *Microbial biocontrol: Sustainable agriculture and phytopathogen management* (1st ed., Vol. 1, pp. 279–299). Springer. https://doi.org/10.1007/978-3-030-87512-1_12

Thanh, K. Le, & Yen, T. T. (2023). Isolation and selection of antagonistic bacteria against *Cercospora arachidicola* causing brown spot on peanut. *HAYATI Journal of Biosciences*, *30*(5), 927–936. https://doi.org/10.4308/hjb.30.5.927-936

Théatre, A., Hoste, A. C. R., Rigolet, A., Benneceur, I., Bechet, M., Ongena, M., Deleu, M., & Jacques, P. (2022). *Bacillus* sp.: A remarkable source of bioactive lipopeptides. *Adv Biochem Eng Biotechnol*, *181*, 123–180. https://doi.org/10.1007/10_2021_182

Thresh, J. M., & Cooter, R. J. (2005). Strategies for controlling cassava mosaic virus disease in Africa. *Plant Pathology*, *54*(5), 587–614. https://doi.org/10.1111/J.1365-3059.2005.01282.X

Tian, D., Song, X., Li, C., Zhou, W., Qin, L., Wei, L., Di, W., Huang, S., Li, B., Huang, Q., Long, S., He, Z., & Wei, S. (2021). Antifungal mechanism of *Bacillus amyloliquefaciens* strain GKT04 against *Fusarium* wilt revealed using genomic and transcriptomic analyses. *MicrobiologyOpen*, *10*(3), e1192. https://doi.org/10.1002/mbo3.1192

Timofeeva, A. M., Galyamova, M. R., & Sedykh, S. E. (2022). Bacterial siderophores: Classification, biosynthesis, perspectives of use in agriculture. *Plants*, *11*(22), 3065. https://doi.org/10.3390/plants11223065

Tinivella, F., Hirata, L. M., Celan, M. A., Wright, S. A. I., Amein, T., Schmitt, A., Koch, E., Van Der Wolf, J. M., Groot, S. P. C., Stephan, D., Garibaldi, A., Gullino, M. L., Gullino, M. L., Celan, M. A., Wright, S. A. I., Amein, T., Schmitt, A., Koch, E., Stephan, : D, … Groot, S. P. C. (2009). Control of seed-borne pathogens on legumes by microbial and other alternative seed treatments. *Eur J Plant Pathol*, *123*, 139–151. https://doi.org/10.1007/s10658-008-9349-3

Tiwari, M., Kumar, R., Subramanian, S., Doherty, C. J., & Jagadish, S. V. K. (2023). Auxin-cytokinin interplay shapes root functionality under low-temperature stress. *Trends in Plant Science*, *28*(4), 447–459. https://doi.org/10.1016/j.tplants.2022.12.004

Tran, C., Cock, I. E., Chen, X., & Feng, Y. (2022). Antimicrobial *Bacillus*: Metabolites and their mode of action. *Antibiotics*, *11*(1), 88. https://doi.org/10.3390/antibiotics11010088

Tran, P., Lander, S. M., & Prindle, A. (2024). Active pH regulation facilitates *Bacillus subtilis* biofilm development in a minimally buffered environment. *MBio*, *15*(3), e03387-23. https://doi.org/10.1128/mbio.03387-23

Trejo, M., Douarche, C., Bailleux, V., Poulard, C., Mariot, S., Regeard, C., & Raspaud, E. (2013). Elasticity and wrinkled morphology of *Bacillus subtilis* pellicles. *Proceedings of the National Academy of Sciences*, *110*(6), 2011–2016. https://doi.org/10.1073/pnas.1217178110

Tut, G., Magan, N., Brain, P., & Xu, X. (2021). Critical evaluation of two commercial biocontrol agents for their efficacy against *B. cinerea* under *in vitro* and *in vivo* conditions in relation to different abiotic factors. *Agronomy*, *11*(9), 1868. https://doi.org/10.3390/agronomy11091868

Tyagi, A., Ali, S., Ramakrishna, G., Singh, A., Park, S., Mahmoudi, H., & Bae, H. (2023). Revisiting the role of polyamines in plant growth and abiotic stress resilience: Mechanisms, crosstalk, and future perspectives. *Journal of Plant Growth Regulation*, *42*(8), 5074–5098. https://doi.org/10.1007/s00344-022-10847-3

Usta, M. (2021). Determination of honey bee (*Apis mellifera*) bacterial flora, cry gene analysis and honey bee health. *Uludag Aricilik Dergisi*, *21*(2), 157–167. https://doi.org/10.31467/uluaricilik.954479

Uwamahoro, F., Berlin, A., Bucagu, C., Bylund, H., & Yuen, J. (2020). *Ralstonia solanacearum* causing potato bacterial wilt: Host range and cultivars' susceptibility in Rwanda. *Plant Pathology*, *69*(3), 559–568. https://doi.org/10.1111/ppa.13140

Vaishnavi, J., & Osborne, W. J. (2021). Microbial volatiles: Small molecules with an important role in intra- and interbacterial genus interactions-quorum sensing. In A. Kumar, J. Sing, & J. Samuel (Eds.), *Volatiles and metabolites of microbes* (pp. 35–50). Academic Press, Elsevier. https://doi.org/10.1016/B978-0-12-824523-1.00005-5

Valdés-Velasco, L. M., Favela-Torres, E., Théatre, A., Arguelles-Arias, A., Saucedo-Castañeda, J. G., & Jacques, P. (2022). Relationship between lipopeptide biosurfactant and primary metabolite production by *Bacillus* strains in solid-state and submerged fermentation. *Bioresource Technology*, *345*, 126556. https://doi.org/10.1016/j.biortech.2021.126556

Valencia-Marin, M. F., Chávez-Avila, S., Guzmán-Guzmán, P., Orozco-Mosqueda, M. del C., de los Santos-Villalobos, S., Glick, B. R., & Santoyo, G. (2024). Survival strategies of *Bacillus* spp. in saline soils: Key factors to promote plant growth and health. *Biotechnology Advances*, *70*, 108303. https://doi.org/10.1016/j.biotechadv.2023.108303

Valencia-Marin, M. F., Chávez-Avila, S., Sepúlveda, E., Delgado-Ramírez, C. S., Meza-Contreras, J. J., Orozco-Mosqueda, M. del C., De Los Santos-Villalobos, S., Babalola, O. O., Hernández-Martinez, R., & Santoyo, G. (2025). Stress-tolerant *Bacillus* strains for enhancing tomato growth and biocontrol of *Fusarium oxysporum* under saline conditions: Functional and genomic characterization. *World Journal of Microbiology and Biotechnology*, *41*(3), 96. https://doi.org/10.1007/s11274-025-04308-8

van der Donk, W. A., & Nair, S. K. (2014). Structure and mechanism of lanthipeptide biosynthetic enzymes. *Current Opinion in Structural Biology*, *29*, 58–66. https://doi.org/10.1016/j.sbi.2014.09.006

Van Gijsegem, F., Hugouvieux-Cotte-Pattat, N., Kraepiel, Y., Lojkowska, E., Moleleki, L. N., Gorshkov, V., & Yedidia, I. (2021). Molecular interactions of *Pectobacterium* and *Dickeya* with Plants. In F. Van Gijsegem, M. J. van der Wolf, & K. I. Toth (Eds.), *Plant diseases caused by Dickeya and Pectobacterium species* (pp. 85–147). Springer International Publishing. https://doi.org/10.1007/978-3-030-61459-1_4

Van Heel, A. J., De Jong, A., Song, C., Viel, J. H., Kok, J., & Kuipers, O. P. (2018). BAGEL4: A user-friendly web server to thoroughly mine RiPPs and bacteriocins. *Nucleic Acids Research*, *46*(1), 278–281. https://doi.org/10.1093/nar/gky383

van Heel, A. J., Montalban-Lopez, M., Oliveau, Q., & Kuipers, O. P. (2017). Genome-guided identification of novel head-to-tail cyclized antimicrobial peptides, exemplified by the discovery of pumilarin. *Microbial Genomics*, *3*(10), e000134. https://doi.org/10.1099/mgen.0.000134

Vargas-Bautista, C., Rahlwes, K., & Straight, P. (2014). Bacterial competition reveals differential regulation of the *pks* genes by *Bacillus subtilis*. *Journal of Bacteriology*, *196*(4), 717–728. https://doi.org/10.1128/JB.01022-13

Vasilchenko, A. S., Lukyanov, D. A., Dilbaryan, D. S., Usachev, K. S., Poshvina, D. V., Taldaev, A. Kh., Nikandrova, A. A., Imamutdinova, A. N., Garaeva, N. S., Bikmullin, A. G., Klochkova, E. A., Rusanov, A. L., Romashin, D. D., Luzgina, N. G., Osterman, I. A., Sergiev, P. V., & Teslya, A. V. (2025). Macrolactin A is an inhibitor of protein biosynthesis in bacteria. *Biochimie*, *232*, 25–34. https://doi.org/10.1016/j.biochi.2025.01.003

Velasco-Muñoz, J. F., Aznar-Sánchez, J. A., López-Felices, B., & Román-Sánchez, I. M. (2022). Circular economy in agriculture. An analysis of the state of research based

on the life cycle. *Sustainable Production and Consumption*, *34*, 257–270. https://doi.org/10.1016/j.spc.2022.09.017

Verma, P., Yadav, A. N., Khannam, K. S., Kumar, S., Saxena, A. K., & Suman, A. (2016). Molecular diversity and multifarious plant growth promoting attributes of Bacilli associated with wheat (*Triticum aestivum* L.) rhizosphere from six diverse agro-ecological zones of India. *Journal of Basic Microbiology*, *56*(1), 44–58. https://doi.org/10.1002/jobm.201500459

Veselova, M. A., Plyuta, V. A., & Khmel, I. A. (2019). Volatile compounds of bacterial origin: Structure, biosynthesis, and biological activity. *Microbiology*, *88*(3), 261–274. https://doi.org/10.1134/S0026261719030160

Waadt, R., Seller, C. A., Hsu, P.-K., Takahashi, Y., Munemasa, S., & Schroeder, J. I. (2022). Plant hormone regulation of abiotic stress responses. *Nature Reviews Molecular Cell Biology*, *23*(10), 680–694. https://doi.org/10.1038/s41580-022-00479-6

Waigi, M. G., Kang, F., Goikavi, C., Ling, W., & Gao, Y. (2015). Phenanthrene biodegradation by sphingomonads and its application in the contaminated soils and sediments: A review. *International Biodeterioration & Biodegradation*, *104*, 333–349. https://doi.org/10.1016/j.ibiod.2015.06.008

Wan, C., Fan, X., Lou, Z., Wang, H., Olatunde, A., & Rengasamy, K. R. R. (2022). Iturin: Cyclic lipopeptide with multifunction biological potential. *Critical Reviews in Food Science and Nutrition*, *62*(29), 7976–7988. https://doi.org/10.1080/10408398.2021.1922355

Wan, W., Tan, J., Wang, Y., Qin, Y., He, H., Wu, H., Zuo, W., & He, D. (2020). Responses of the rhizosphere bacterial community in acidic crop soil to pH: Changes in diversity, composition, interaction, and function. *Science of The Total Environment*, *700*, 134418. https://doi.org/10.1016/j.scitotenv.2019.134418

Wang, B., Yuan, J., Zhang, J., Shen, Z., Zhang, M., Li, R., Ruan, Y., & Shen, Q. (2013). Effects of novel bioorganic fertilizer produced by *Bacillus amyloliquefaciens* W19 on antagonism of *Fusarium* wilt of banana. *Biology and Fertility of Soils*, *49*(4), 435–446. https://doi.org/10.1007/s00374-012-0739-5

Wang, C., & Kuzyakov, Y. (2024a). Mechanisms and implications of bacterial-fungal competition for soil resources. *The ISME Journal*, *18*(1), wrae073. https://doi.org/10.1093/ismejo/wrae073

Wang, C., & Kuzyakov, Y. (2024b). Soil organic matter priming: The pH effects. *Global Change Biology*, *30*(6), e17349. https://doi.org/10.1111/gcb.17349

Wang, H., Liu, R., You, M. P., Barbetti, M. J., & Chen, Y. (2021). Pathogen Biocontrol Using Plant Growth-Promoting Bacteria (PGPR): Role of Bacterial Diversity. *Microorganisms 2021, Vol. 9, Page 1988*, *9*(9), 1988. https://doi.org/10.3390/MICROORGANISMS9091988

Wang, J., Ping, Y., Liu, W., He, X., & Du, C. (2024). Improvement of lipopeptide production in *Bacillus subtilis* HNDF2-3 by overexpression of the *sfp* and *comA* genes. *Preparative Biochemistry & Biotechnology*, *54*(2), 184–192. https://doi.org/10.1080/10826068.2023.2209890

Wang, J., Zhang, Y., Jin, J., Li, Q., Zhao, C., Nan, W., Wang, X., Ma, R., & Bi, Y. (2018). An intact cytokinin-signaling pathway is required for *Bacillus* sp. LZR216-promoted plant growth and root system architecture alteration in *Arabidopsis thaliana* seedlings. *Plant Growth Regulation*, *84*(3), 507–518. https://doi.org/10.1007/s10725-017-0357-1

Wang, M., Wang, Y., Wang, M., Liu, M., & Cheng, A. (2023). Heme acquisition and tolerance in Gram-positive model bacteria: An orchestrated balance. *Heliyon*, *9*(7), e18233. https://doi.org/10.1016/j.heliyon.2023.e18233

Wang, P., Liu, W., Han, C., Wang, S., Bai, M., & Song, C. (2024). Reactive oxygen species: Multidimensional regulators of plant adaptation to abiotic stress and development. *Journal of Integrative Plant Biology*, *66*(3), 330–367. https://doi.org/10.1111/jipb.13601

Wang, R., Liang, X., Long, Z., Wang, X., Yang, L., Lu, B., & Gao, J. (2021). An LCI-like protein APC2 protects ginseng root from *Fusarium solani* infection. *Journal of Applied Microbiology*, *130*(1), 165–178. https://doi.org/10.1111/jam.14771

Wang, S., Na, X., Yang, L., Liang, C., He, L., Jin, J., Liu, Z., Qin, J., Li, J., Wang, X., & Bi, Y. (2021). *Bacillus megaterium* strain WW1211 promotes plant growth and lateral root initiation via regulation of auxin biosynthesis and redistribution. *Plant and Soil*, *466*(1–2), 491–504. https://doi.org/10.1007/s11104-021-05055-z

Wang, S., Sun, L., Zhang, W., Chi, F., Hao, X., Bian, J., & Li, Y. (2020). *Bacillus velezensis* BM21, a potential and efficient biocontrol agent in control of corn stalk rot caused by *Fusarium graminearum*. *Egyptian Journal of Biological Pest Control*, *30*(1), 9. https://doi.org/10.1186/s41938-020-0209-6

Wang, S.-Y., Herrera-Balandrano, D. D., Wang, Y.-X., Shi, X.-C., Chen, X., Jin, Y., Liu, F.-Q., & Laborda, P. (2022). Biocontrol ability of the *Bacillus amyloliquefaciens* group, *B. amyloliquefaciens*, *B. velezensis*, *B. nakamurai*, and *B. siamensis*, for the management of fungal postharvest diseases: A review. *Journal of Agricultural Food Chemistry*, *70*, 6591–6616. https://doi.org/10.1021/acs.jafc.2c01745

Wang, W., Zhao, Z., Yang, J., Lian, X., Xie, X., Chen, H., Wang, M., & Zheng, H. (2024). Application of oil-degrading agents consisted of thermophilic *Bacillus subtilis* and *Bacillus glycinifermentans* in food waste. *Environmental Technology*, *45*(23), 4704–4714. https://doi.org/10.1080/09593330.2023.2283064

Wang, Y., Pei, Y., Wang, X., Dai, X., & Zhu, M. (2024). Antimicrobial metabolites produced by the plant growth-promoting rhizobacteria (PGPR): *Bacillus* and *Pseudomonas*. *Advanced Agrochem*, *3*(3), 206–221. https://doi.org/10.1016/j.aac.2024.07.007

Wang, Y., Zhang, C., Liang, J., Wu, L., Gao, W., & Jiang, J. (2020). Iturin A extracted from *Bacillus subtilis* WL-2 affects *Phytophthora infestans* via cell structure disruption, oxidative stress, and energy supply dysfunction. *Frontiers in Microbiology*, *11*, 536083. https://doi.org/10.3389/fmicb.2020.536083

Wang, Z., Liu, C., Shi, Y., Huang, M., Song, Z., Simal-Gandara, J., Li, N., & Shi, J. (2024). Classification, application, multifarious activities and production improvement of lipopeptides produced by *Bacillus*. *Critical Reviews in Food*

Science and Nutrition, *64*(21), 7451–7464. https://doi.org/10.1080/10408398.2023.2185588

Wei, Z., Huang, J., Yang, T., Jousset, A., Xu, Y., Shen, Q., & Friman, V. P. (2017). Seasonal variation in the biocontrol efficiency of bacterial wilt is driven by temperature-mediated changes in bacterial competitive interactions. *Journal of Applied Ecology*, *54*(5), 1440–1448. https://doi.org/10.1111/1365-2664.12873

Wei, Z., Shan, C., Zhang, L., Ge, D., Wang, Y., Xia, X., Liu, X., & Zhou, J. (2021). A novel subtilin-like lantibiotics subtilin JS-4 produced by *Bacillus subtilis* JS-4, and its antibacterial mechanism against *Listeria monocytogenes*. *LWT*, *142*, 110993. https://doi.org/10.1016/j.lwt.2021.110993

Weiland-Bräuer, N. (2021). Friends or foes-microbial interactions in nature. *Biology*, *10*, 496. https://doi.org/10.3390/biology10060496

Wendimu, Y. G., & Gurmu, K. A. (2024). Insect vectors of plant viruses: Host interactions, their effects, and future opportunities. *Advances in Agriculture*, *2024*(1), 6006985. https://doi.org/10.1155/aia/6006985

WFP. (2021). *Burundi Annual Country Report*. https://docs.wfp.org/api/documents/WFP-0000137887/download/

Wilks, J. C., Kitko, R. D., Cleeton, S. H., Lee, G. E., Ugwu, C. S., Jones, B. D., BonDurant, S. S., & Slonczewski, J. L. (2009). Acid and base stress and transcriptomic responses in *Bacillus subtilis*. *Applied and Environmental Microbiology*, *75*(4), 981–990. https://doi.org/10.1128/AEM.01652-08

Willocquet, L., Savary, S., & Singh, K. P. (2023). Revisiting the use of disease index and of disease scores in plant pathology. *Indian Phytopathology*, *76*(3), 909–914. https://doi.org/10.1007/s42360-023-00663-4

World Bank Group. (2021). *Climate change knowledge portal for development practitioners and policy makers*.

Wu, G., Zhou, J., Zheng, J., Abdalmegeed, D., Tian, J., Wang, M., Sun, S., Sedjoah, R. C. A. A., Shao, Y., Sun, S., & Xin, Z. (2023). Construction of lipopeptide mono-producing *Bacillus* strains and comparison of their antimicrobial activity. *Food Bioscience*, *53*, 102813. https://doi.org/10.1016/j.fbio.2023.102813

Wu, L., Wu, H., Chen, L., Xie, S., Zang, H., Borriss, R., & Gao, X. (2014). Bacilysin from *Bacillus amyloliquefaciens* FZB42 has specific bactericidal activity against harmful algal bloom species. *Applied and Environmental Microbiology*, *80*(24), 7512–7520. https://doi.org/10.1128/AEM.02605-14

Wu, L., Wu, H., Chen, L., Yu, X., Borriss, R., & Gao, X. (2015). Difficidin and bacilysin from *Bacillus amyloliquefaciens* FZB42 have antibacterial activity against *Xanthomonas oryzae* rice pathogens. *Scientific Reports*, *5*(1), 12975. https://doi.org/10.1038/srep12975

Wu, R., Kong, L., & Liu, F. (2024). Regulation of biofilm gene expression by DNA replication in *Bacillus subtilis*. *Journal of Cellular and Molecular Medicine*, *28*(12), e18481. https://doi.org/10.1111/jcmm.18481

Wu, T., Xiao, F., & Li, W. (2021). Macrolactins: biological activity and biosynthesis. *Marine Life Science and Technology*, *3*(1), 62–68. https://doi.org/10.1007/s42995-020-00068-6

Wu, Y., Guo, W., Zhao, J., Ding, L., & Chen, X. (2018). Isolation and identification of a novel LCI like antibacterial protein from *Bacillus* sp. MD-5 reveals its potential application in controlling *Staphylococcus aureus* in food industry. *Food Control*, *89*, 142–149. https://doi.org/10.1016/j.foodcont.2018.01.026

Xia, Y., Jiang, X., Guo, S., Wang, Y., Mu, Y., & Shen, J. (2024). Glycine betaine modulates extracellular polymeric substances to enhance microbial salinity tolerance. *Environmental Science and Ecotechnology*, *20*, 100406. https://doi.org/10.1016/j.ese.2024.100406

Xiang, D., Yang, X., Liu, B., Chu, Y., Liu, S., & Li, C. (2023). Bio-priming of banana tissue culture plantlets with endophytic *Bacillus velezensis* EB1 to improve *Fusarium* wilt resistance. *Frontiers in Microbiology*, *14*, 1146331. https://doi.org/10.3389/fmicb.2023.1146331

Xie, S., Jiang, L., Wu, Q., Wan, W., Gan, Y., Zhao, L., & Wen, J. (2022). Maize root exudates recruit *Bacillus amyloliquefaciens* OR2-30 to inhibit *Fusarium graminearum* infection. *Phytopathology*, *112*(9), 1886–1893. https://doi.org/10.1094/PHYTO-01-22-0028-R

Xie, S., Liu, J., Gu, S., Chen, X., Jiang, H., & Ding, T. (2020). Antifungal activity of volatile compounds produced by endophytic *Bacillus subtilis* DZSY21 against *Curvularia lunata*. *Annals of Microbiology*, *70*(1), 2. https://doi.org/10.1186/s13213-020-01553-0

Xie, S., Zang, H., Jun Wu, H., Uddin Rajer, F., & Gao, X. (2018). Antibacterial effects of volatiles produced by *Bacillus* strain D13 against *Xanthomonas oryzae* pv. *oryzae*. *Molecular Plant Pathology*, *19*(1), 49–58. https://doi.org/10.1111/mpp.12494

Xing, Y., Wang, X., & Mustafa, A. (2025). Exploring the link between soil health and crop productivity. *Ecotoxicology and Environmental Safety*, *289*, 117703. https://doi.org/10.1016/j.ecoenv.2025.117703

Xu, W., Yang, Q., Yang, F., Xie, X., Goodwin, P. H., Deng, X., Tian, B., & Yang, L. (2022). Evaluation and genome analysis of *Bacillus subtilis* YB-04 as a potential biocontrol agent against *Fusarium* wilt and growth promotion agent of cucumber. *Frontiers in Microbiology*, *13*, 885430. https://doi.org/10.3389/fmicb.2022.885430

Xu, X., & Kovács, Á. T. (2024). How to identify and quantify the members of the *Bacillus* genus? *Environmental Microbiology*, *26*(2), e16593. https://doi.org/10.1111/1462-2920.16593

Xu, Y., & Li, Z. (2020). CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. *Computational and Structural Biotechnology Journal*, *18*, 2401–2415. https://doi.org/10.1016/j.csbj.2020.08.031

Xu, Z., Liu, Y., Zhang, N., Xun, W., Feng, H., Miao, Y., Shao, J., Shen, Q., & Zhang, R. (2023). Chemical communication in plant-microbe beneficial interactions: a toolbox for precise management of beneficial microbes. *Current Opinion in Microbiology*, *72*, 102269. https://doi.org/10.1016/j.mib.2023.102269

Xu, Z., Zhang, R., Wang, D., Qiu, M., Feng, H., Zhang, N., & Shen, Q. (2014). Enhanced control of cucumber wilt disease by *Bacillus amyloliquefaciens* SQR9 by altering the regulation of its DegU phosphorylation. *Applied and Environmental Microbiology*, *80*(9), 2941–2950. https://doi.org/10.1128/AEM.03943-13

Xue, D., Older, E. A., Zhong, Z., Shang, Z., Chen, N., Dittenhauser, N., Hou, L., Cai, P., Walla, M. D., Dong, S. H., Tang, X., Chen, H., Nagarkatti, P., Nagarkatti, M., Li, Y. X., & Li, J. (2022). Correlational networking guides the discovery of unclustered lanthipeptide protease-encoding genes. *Nature Communications*, *13*(1), 1–14. https://doi.org/10.1038/s41467-022-29325-1

Xue, D., Shang, Z., Older, E. A., Zhong, Z., Pulliam, C., Peter, K., Nagarkatti, M., Nagarkatti, P., Li, Y.-X., & Li, J. (2023). Refactoring and heterologous expression of class III lanthipeptide biosynthetic gene clusters lead to the discovery of *N* , *N* -dimethylated lantibiotics from Firmicutes. *ACS Chemical Biology*, *18*(3), 508–517. https://doi.org/10.1021/acschembio.2c00849

Xue, Y., Yu, C., Ouyang, H., Huang, J., & Kang, X. (2024). Uncovering the molecular composition and architecture of the *Bacillus subtilis* biofilm via solid-state NMR Spectroscopy. *Journal of the American Chemical Society*, *146*(17), 11906–11923. https://doi.org/10.1021/jacs.4c00889

Xue, Y., Zhang, Y., Huang, K., Wang, X., Xing, M., Xu, Q., & Guo, Y. (2023). A novel biocontrol agent *Bacillus velezensis* K01 for management of gray mold caused by *Botrytis cinerea*. *AMB Express*, *13*(1), 91. https://doi.org/10.1186/s13568-023-01596-x

Xue, Z., Sun, H., Hong, H., Zhang, Z., Zhang, Y., Guo, Z., Le, S., & Chen, H. (2024). Comparative analysis of folding and unfolding dynamics and free-energy landscapes in homologous cold shock proteins with variable thermal stabilities. *Physical Review Research*, *6*(2), 023170. https://doi.org/10.1103/PhysRevResearch.6.023170

Yadav, R., Singh, S., & Singh, A. N. (2022). Biopesticides: Current status and future prospects. *Proceedings of the International Academy of Ecology and Environmental Sciences*, *12*(3), 211–233. www.iaees.org

Yafetto, L. (2022). Application of solid-state fermentation by microbial biotechnology for bioprocessing of agro-industrial wastes from 1970 to 2020: A review and bibliometric analysis. *Heliyon*, *8*(3), e09173. https://doi.org/10.1016/j.heliyon.2022.e09173

Yahia Mahfouz, A., & Daigham, G. (2020). Micro flora diversity of Luxor and Aswan temples "guardians of temples." *Sylwan*, *164*(3), 24–43. https://www.researchgate.net/publication/348937427

Yamamoto, S., Shiraishi, S., & Suzuki, S. (2015). Are cyclic lipopeptides produced by *Bacillus amyloliquefaciens* S13-3 responsible for the plant defence response in strawberry against *Colletotrichum gloeosporioides*? *Letters in Applied Microbiology*, *60*(4), 379–386. https://doi.org/10.1111/lam.12382

Yan, Y., Lu, J., Huang, J., Feng, P., Ling, Y., & Yi, Y. (2022). Recombinant expression and enzymatic properties of chitosanase from *Bacillus nakamurai* in *Escherichia*

coli. *Journal of Chinese Institute of Food Science & Technology*, *22*(9), 74–81. https://doi.org/10.16429/j.1009-7848.2022.09.008

Yang, L., Zhou, Y., Guo, L., Yang, L., Wang, J., Liang, C., & Huang, J. (2022). The effect of banana rhizosphere chemotaxis and chemoattractants on *Bacillus velezensis* LG14-3 root colonization and suppression of banana *Fusarium* wilt disease. *Sustainability*, *15*(1), 351. https://doi.org/10.3390/su15010351

Yang, Q., Zhang, H., You, J., Yang, J., Zhang, Q., Zhao, J., Aimaier, R., Zhang, J., Han, S., Zhao, H., & Zhao, H. (2023). Transcriptome and metabolome analyses reveal that *Bacillus subtilis* BS-Z15 lipopeptides mycosubtilin homologue mediates plant defense responses. *Frontiers in Plant Science*, *13*, 1088220. https://doi.org/10.3389/fpls.2022.1088220

Yang, R., Lei, S., Xu, X., Jin, H., Sun, H., Zhao, X., Pang, B., & Shi, J. (2020). Key elements and regulation strategies of NRPSs for biosynthesis of lipopeptides by *Bacillus*. *Applied Microbiology and Biotechnology*, *104*(19), 8077–8087. https://doi.org/10.1007/s00253-020-10801-x

Yang, T., Xin, Y., Liu, T., Li, Z., Liu, X., Wu, Y., Wang, M., & Xiang, M. (2022). Bacterial volatile-mediated suppression of root-knot nematode (*Meloidogyne incognita*). *Plant Disease*, *106*(5), 1358–1365. https://doi.org/10.1094/PDIS-06-21-1139-RE

Yannarell, S. M., Beaudoin, E. S., Talley, H. S., Schoenborn, A. A., Orr, G., Anderton, C. R., Chrisler, W. B., & Shank, E. A. (2023). Extensive cellular multi-tasking within *Bacillus subtilis* biofilms. *MSystems*, *8*(4), e00891-22. https://doi.org/10.1128/msystems.00891-22

Yaraguppi, D. A., Bagewadi, Z. K., Patil, N. R., & Mantri, N. (2023). Iturin: A promising cyclic lipopeptide with diverse applications. *Biomolecules*, *13*(10), 1515. https://doi.org/10.3390/biom13101515

Ye, L., Wang, J.-Y., Liu, X.-F., Guan, Q., Dou, N.-X., Li, J., Zhang, Q., Gao, Y.-M., Wang, M., Li, J.-S., & Zhou, B. (2022). Nematicidal activity of volatile organic compounds produced by *Bacillus altitudinis* AMCC 1040 against *Meloidogyne incognita*. *Archives of Microbiology*, *204*(8), 521. https://doi.org/10.1007/s00203-022-03024-3

Ye, T., Zhang, W., Feng, Z., Fan, X., Xu, X., Mishra, S., Zhang, L., & Chen, S. (2020). Characterization of a novel quorum-quenching bacterial strain, *Burkholderia anthina* HN-8, and its biocontrol potential against black rot disease caused by *Xanthomonas campestris* pv. *campestris*. *Microorganisms*, *8*(10), 1–17. https://doi.org/10.3390/microorganisms8101485

Yi, H. S., Ahn, Y. R., Song, G. C., Ghim, S. Y., Lee, S., Lee, G., & Ryu, C. M. (2016). Impact of a bacterial volatile 2,3-butanediol on Bacillus subtilis rhizosphere robustness. *Frontiers in Microbiology*, *7*(JUN). https://doi.org/10.3389/fmicb.2016.00993

Yin, Q. J., Ying, T. T., Zhou, Z. Y., Hu, G. A., Yang, C. L., Hua, Y., Wang, H., & Wei, B. (2023). Species-specificity of the secondary biosynthetic potential in *Bacillus*.

Frontiers in Microbiology, *14*, 1271418. https://doi.org/10.3389/fmicb.2023.1271418

Yin, Y., Wang, X., Zhang, P., Wang, P., & Wen, J. (2024). Strategies for improving fengycin production: A review. *Microbial Cell Factories*, *23*(1), 144. https://doi.org/10.1186/s12934-024-02425-x

Youssef, H. F. B. (2024). Bactericidal performance of some biotic and abiotic factors for controlling soft-rot bacteria (*Pectobacterium carotovorum* subsp. *carotovorum*) on potato plants. *Egyptian Journal of Agricultural Research*, *102*(3), 464–478. https://doi.org/10.21608/ejar.2024.281851.1534

Yu, C., Wang, H., Blaustein, R. A., Guo, L., Ye, Q., Fu, Y., Fan, J., Su, X., Hartmann, E. M., & Shen, C. (2022a). Pangenomic and functional investigations for dormancy and biodegradation features of an organic pollutant-degrading bacterium Rhodococcus biphenylivorans TG9. *Science of the Total Environment*, *809*. https://doi.org/10.1016/j.scitotenv.2021.151141

Yu, C., Wang, H., Blaustein, R. A., Guo, L., Ye, Q., Fu, Y., Fan, J., Su, X., Hartmann, E. M., & Shen, C. (2022b). Pangenomic and functional investigations for dormancy and biodegradation features of an organic pollutant-degrading bacterium *Rhodococcus biphenylivorans* TG9. *Science of The Total Environment*, *809*, 151141. https://doi.org/10.1016/j.scitotenv.2021.151141

Yu, X., Ai, C., Xin, L., & Zhou, G. (2011). The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on *Fusarium* wilt and promotes the growth of pepper. *European Journal of Soil Biology*, *47*, 138–145. https://doi.org/10.1016/j.ejsobi.2010.11.001

Yuan, J., Li, B., Zhang, N., Waseem, R., Shen, Q., & Huang, Q. (2012). Production of bacillomycin- and macrolactin-type antibiotics by *Bacillus amyloliquefaciens* NJN-6 for suppressing soilborne plant pathogens. *Journal of Agricultural and Food Chemistry*, *60*(12), 2976–2981. https://doi.org/10.1021/jf204868z

Yuan, J., Raza, W., Shen, Q., & Huang, Q. (2012). Antifungal activity of *Bacillus amyloliquefaciens* NJN-6 volatile compounds against *Fusarium oxysporum* f. sp. *cubense*. *Applied and Environmental Microbiology*, *78*(16), 5942–5944. https://doi.org/10.1128/AEM.01357-12

Zaid, D. S., Cai, S., Hu, C., Li, Z., & Li, Y. (2022). Comparative genome analysis reveals phylogenetic identity of *Bacillus velezensis* HNA3 and genomic insights into its plant growth promotion and biocontrol effects. *Microbiology Spectrum*, *10*(1), e02169-21. https://doi.org/10.1128/spectrum.02169-21

Zalila-Kolsi, I., Ben-Mahmoud, A., & Al-Barazie, R. (2023). *Bacillus amyloliquefaciens* : Harnessing its potential for industrial, medical, and agricultural applications- a comprehensive review. *Microorganisms*, *11*(9), 2215. https://doi.org/10.3390/microorganisms11092215

Zanon, M. S. A., Cavaglieri, L. R., Palazzini, J. M., Chulze, S. N., & Chiotta, M. L. (2024). *Bacillus velezensis* RC218 and emerging biocontrol agents against *Fusarium graminearum* and *Fusarium poae* in barley: *in vitro*, greenhouse and field

conditions. *International Journal of Food Microbiology*, *413*, 110580. https://doi.org/10.1016/j.ijfoodmicro.2024.110580

Zarrabian, M., Adhikary, L., Nita, M., Sriyanka, L., & Sherif, S. M. (2025). Toxicological and functional Assessment of minicell-encapsulated dsRNA on biocontrol agents in agriculture. *ACS Environmental Au*, *5*(4), 427–441. https://doi.org/10.1021/acsenvironau.5c00067

Zeng, Q., Ding, X., Wang, J., Han, X., Iqbal, H. M. N., & Bilal, M. (2022). Insight into soil nitrogen and phosphorus availability and agricultural sustainability by plant growth-promoting rhizobacteria. *Environmental Science and Pollution Research*, *29*(30), 45089–45106. https://doi.org/10.1007/s11356-022-20399-4

Zerrouk, I. Z., Rahmoune, B., Auer, S., Rößler, S., Lin, T., Baluska, F., Dobrev, P. I., Motyka, V., & Ludwig-Müller, J. (2020). Growth and aluminum tolerance of maize roots mediated by auxin- and cytokinin-producing *Bacillus toyonensis* requires polar auxin transport. *Environmental and Experimental Botany*, *176*, 104064. https://doi.org/10.1016/j.envexpbot.2020.104064

Zhang, B., Xu, L., Ding, J., Wang, M., Ge, R., Zhao, H., Zhang, B., & Fan, J. (2022). Natural antimicrobial lipopeptides secreted by *Bacillus* spp. and their application in food preservation, a critical review. *Trends in Food Science & Technology*, *127*, 26–37. https://doi.org/10.1016/j.tifs.2022.06.009

Zhang, D., Yu, S., Yang, Y., Zhang, J., Zhao, D., Pan, Y., Fan, S., Yang, Z., & Zhu, J. (2020). Antifungal Effects of Volatiles Produced by Bacillus subtilis Against Alternaria solani in Potato. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.01196

Zhang, J., Huang, X., Hou, Y., Xia, X., Zhu, Z., Huang, A., Feng, S., Li, P., Shi, L., & Dong, P. (2023). Isolation and screening of antagonistic endophytes against *Phytophthora infestans* and preliminary exploration on anti-oomycete mechanism of *Bacillus velezensis* 6-5. *Plants*, *12*(4), 909. https://doi.org/10.3390/plants12040909

Zhang, J., Huang, X., Yang, S., Huang, A., Ren, J., Luo, X., Feng, S., Li, P., Li, Z., & Dong, P. (2023). Endophytic *Bacillus subtilis* H17-16 effectively inhibits *Phytophthora infestans*, the pathogen of potato late blight, and its potential application. *Pest Management Science*, *79*(12), 5073–5086. https://doi.org/10.1002/ps.7717

Zhang, N., Wang, D., Liu, Y., Li, S., Shen, Q., & Zhang, R. (2014). Effects of different plant root exudates and their organic acid components on chemotaxis, biofilm formation and colonization by beneficial rhizosphere-associated bacterial strains. *Plant and Soil*, *374*(1–2), 689–700. https://doi.org/10.1007/s11104-013-1915-6

Zhang, N., Wang, Z., Shao, J., Xu, Z., Liu, Y., Xun, W., Miao, Y., Shen, Q., & Zhang, R. (2023). Biocontrol mechanisms of *Bacillus* : Improving the efficiency of green agriculture. *Microbial Biotechnology*, *16*(12), 2250–2263. https://doi.org/10.1111/1751-7915.14348

Zhang, Q., Kobras, C. M., Gebhard, S., Mascher, T., & Wolf, D. (2022). Regulation of heterologous subtilin production in Bacillus subtilis W168. *Microbial Cell Factories*, *21*(1). https://doi.org/10.1186/s12934-022-01782-9

Zhang, Y., Chen, M., Bruner, S. D., & Ding, Y. (2018). Heterologous production of microbial ribosomally synthesized and post-translationally modified peptides. *Frontiers in Microbiology*, *9*, 1801. https://doi.org/10.3389/fmicb.2018.01801

Zhang, Y., Hong, Z., Zhou, L., Zhang, Z., Tang, T., Guo, E., Zheng, J., Wang, C., Dai, L., Si, T., & Wang, H. (2022). Biosynthesis of gut-microbiota-derived lantibiotics reveals a subgroup of S8 family proteases for class III leader removal. *Angewandte Chemie International Edition*, *61*(6), e202114414. https://doi.org/10.1002/anie.202114414

Zhang, Z., Chen, Z., Zhang, J., Liu, Y., Chen, L., Yang, M., Osman, A. I., Farghali, M., Liu, E., Hassan, D., Ihara, I., Lu, K., Rooney, D. W., & Yap, P. S. (2024). Municipal solid waste management challenges in developing regions: A comprehensive review and future perspectives for Asia and Africa. *Science of the Total Environment*, *930*, 172794. https://doi.org/10.1016/j.scitotenv.2024.172794

Zhao, D., Ding, Y., Cui, Y., Zhang, Y., Liu, K., Yao, L., Han, X., Peng, Y., Gou, J., Du, B., & Wang, C. (2022). Isolation and genome sequence of a novel phosphate-solubilizing rhizobacterium *Bacillus altitudinis* GQYP101 and its effects on rhizosphere microbial community structure and functional traits of corn seedling. *Current Microbiology*, *79*(9), 249. https://doi.org/10.1007/s00284-022-02944-z

Zhao, J., Zhou, Z., Bai, X., Zhang, D., Zhang, L., Wang, J., Wu, B., Zhu, J., & Yang, Z. (2022). A novel of new class II bacteriocin from *Bacillus velezensis* HN-Q-8 and its antibacterial activity on *Streptomyces scabies*. *Frontiers in Microbiology*, *13*, 943232. https://doi.org/10.3389/fmicb.2022.943232

Zhao, K., Ma, R., Cheng, M., Guo, T., Wu, W., Song, Y., Xu, H., Tan, A., Qin, B., & Wei, S. (2025). Isolation of macrolactin A from a new *Bacillus amyloliquefaciens* and its aphicidal activity against *Rhopalosiphum padi*. *Pest Management Science*, *81*(4), 1882–1893. https://doi.org/10.1002/ps.8589

Zhao, M., Liu, D., Liang, Z., Huang, K., & Wu, X. (2022). Antagonistic activity of *Bacillus subtilis* CW14 and its β-glucanase against *Aspergillus ochraceus*. *Food Control*, *131*, 108475. https://doi.org/10.1016/j.foodcont.2021.108475

Zhao, P., Li, P., Wu, S., Zhou, M., Zhi, R., & Gao, H. (2019). Volatile organic compounds (VOCs) from *Bacillus subtilis* CF-3 reduce anthracnose and elicit active defense responses in harvested litchi fruits. *AMB Express*, *9*(1), 119. https://doi.org/10.1186/s13568-019-0841-2

Zhao, P., Quan, C., Wang, Y., Wang, J., & Fan, S. (2014). *Bacillus amyloliquefaciens* Q-426 as a potential biocontrol agent against *Fusarium oxysporum* f. sp. *spinaciae*. *Journal of Basic Microbiology*, *54*(5), 448–456. https://doi.org/10.1002/jobm.201200414

Zhao, S., Hu, N., Huang, J., Liang, Y., & Zhao, B. (2008). High-yield spore production from *Bacillus licheniformis* by solid state fermentation. *Biotechnology Letters*, *30*(2), 295–297. https://doi.org/10.1007/s10529-007-9540-1

Zhao, X., He, C., Liu, W. S., Liu, W. X., Liu, Q. Y., Bai, W., Li, L. J., Lal, R., & Zhang, H. L. (2022). Responses of soil pH to no-till and the factors affecting it: A global

meta-analysis. *Global Change Biology*, *28*(1), 154–166. https://doi.org/10.1111/gcb.15930

Zheng, G., Hehn, R., & Zuber, P. (2000). Mutational analysis of the *sbo-alb* locus of *Bacillus subtilis*: Identification of genes required for subtilosin production and immunity. *Journal of Bacteriology*, *182*(11), 3266–3273. https://doi.org/10.1128/jb.182.11.3266-3273.2000

Zheng, J., Ge, Q., Yan, Y., Zhang, X., Huang, L., & Yin, Y. (2023). DbCAN3: Automated carbohydrate-active enzyme and substrate annotation. *Nucleic Acids Research*, *51*(W1), W115–W121. https://doi.org/10.1093/nar/gkad328

Zheng, X., Wang, J., Chen, M., Chen, Y., Chen, Z., Wang, M. K., & Liu, B. (2024). Testing a biocontrol agent consortium for suppression of tomato bacterial wilt through rhizosphere microecological regulation. *Applied Soil Ecology*, *193*, 105155. https://doi.org/10.1016/j.apsoil.2023.105155

Zhou, C., Ma, Z., Zhu, L., Xiao, X., Xie, Y., Zhu, J., & Wang, J. (2016). Rhizobacterial strain *Bacillus megaterium* BOFC15 induces cellular polyamine changes that improve plant growth and drought resistance. *International Journal of Molecular Sciences*, *17*(6), 976. https://doi.org/10.3390/ijms17060976

Zhu, L., Huang, J., Lu, X., & Zhou, C. (2022). Development of plant systemic resistance by beneficial rhizobacteria: Recognition, initiation, elicitation and regulation. *Frontiers in Plant Science*, *13*, 952397. https://doi.org/10.3389/fpls.2022.952397

Zhu, M.-L., Wang, Y.-H., Dai, Y., Wu, X.-Q., & Ye, J.-R. (2020). Effects of different culture conditions on the biofilm formation of *Bacillus pumilus* HR10. *Current Microbiology*, *77*(8), 1405–1411. https://doi.org/10.1007/s00284-020-01944-1

Zimina, M., Babich, O., Prosekov, A., Sukhikh, S., Ivanova, S., Shevchenko, M., & Noskova, S. (2020). Overview of global trends in classification, methods of preparation and application of bacteriocins. *Antibiotics*, *9*(9), 1–21. https://doi.org/10.3390/antibiotics9090553

Zumkeller, M., Yu, R., Torres, N., Marigliano, L. E., Zaccaria, D., & Kurtural, S. K. (2022). Site characteristics determine the effectiveness of tillage and cover crops on the net ecosystem carbon balance in California vineyard agroecosystems. *Frontiers in Plant Science*, *13*, 1024606. https://doi.org/10.3389/fpls.2022.1024606

Supplementary materials

1. Supplemntary tables

Table S1: Registered pesticides in Burundi, DRC, and Rwanda

Class of pesticides	Active ingredients	Burundi	DRC	Rwanda
Insecticides + Acaricides	Abamectin	R	R	NR
	Acephate	R	NR	NR
	Acetamiprid	R	R	R
	Acrinathrin	NR	NR	R
	Alpha-cypermethrin	R	NR	R
	Aluminium phosphide	R	NR	R
	Azocyclotin	NR	NR	R
	Benfuracarb	R	NR	NR
	Beta-cyfluthrin	R	NR	R
	Bifenthrin	R	R	R
	Bromopropylate	NR	NR	R
	Buprofenzin	NR	NR	R
	Carbofuran	NR	NR	R
	Carbosulfan	NR	NR	R
	Chlorfenapyr	NR	NR	R

	Chlorpyriphos	R	NR	NR
	Chlorpyriphos-ethyl	R	R	NR
	Chlorpyriphos-methyl	R	NR	NR
	Clofentezin	NR	NR	R
	Copper oxychloride	NR	R	NR
	Cyfluthrin	R	NR	NR
	Cypermethrin	R	R	R
	Cyphenothrin	NR	R	NR
	Cyromazine	NR	NR	R
	Deltamethrin	R	R	R
	Diafenthiuron	NR	NR	R
	Diazinon	R	NR	NR
	Dichlorvos	R	NR	NR
	Diflubenzuron	NR	NR	R
	Dimethoate	R	R	NR
	Esfenvalerate	R	R	NR
	Fenamiphos	NR	NR	R
	Fenaxaquin	NR	NR	R
	Fenbutatin oxide	NR	NR	R
	Fenitrothion	R	R	NR
	Fenthion	R	NR	NR

	Fenvalerate	R	NR	NR
	Fipronil	R	R	R
	Flufenoxuron	NR	NR	R
	Flumethrin	NR	NR	R
	Imidacloprid	R	R	R
	Indoxacarb	NR	NR	R
	Isoxathion	R	NR	NR
	Lambda-cyhalothrin	R	R	R
	Lufenuron	R	NR	NR
	Magnesium Phosphide	R	NR	R
	Malathion	R	NR	R
	Methomyl	NR	NR	R
	Methoxyfenozide	NR	NR	R
	Nicosulfuron	NR	R	NR
	Novaluron	NR	NR	R
	Omethoate	R	NR	NR
	Oxydemeton-methyl	R	NR	NR
	Permethrin	NR	NR	R
	Piperonyl Butoxide (PBO)	NR	NR	R
	Pirimiphos	R	NR	R
	Pirimiphos-Methyl	NR	NR	R

	Profenofos	NR	NR	R
	Prosuler Oxamatrine	R	NR	NR
	Pymetrozine	R	NR	NR
	Pyridaphenthion	NR	R	NR
	Pyrimicarb	R	R	R
	Snake repellent	NR	NR	R
	Spiromesifen	NR	NR	R
	Tau-fluvalinat	NR	NR	R
	Teflubenzuron	NR	NR	R
	Tetradifon	NR	NR	R
	Tetramethrin	NR	R	NR
	Thiacloprid	NR	NR	R
	Thiamethoxam	NR	R	R
	Tralomethrin	R	NR	NR
	Triazophos	R	NR	NR
	Zeta-cypermethrin	R	NR	NR
Fungicides	Albesilate	NR	NR	R
	Azoxystrobin	R	NR	R
	Benalaxyl	NR	NR	R
	Benomyl	R	NR	R
	Bitertanol	NR	NR	R

	bromoxinyl-octanoate	NR	NR	R
	Bupimate	NR	NR	R
	Calcium Hypochlorite	NR	NR	R
	Captan	NR	NR	R
	Carbendazim	NR	NR	R
	Carboxin	NR	NR	R
	Chlorothalonil	R	NR	R
	Copper	NR	NR	R
	Copper ammonium acetate	NR	NR	R
	Copper hydroxide	R	R	R
	Copper oxychloride	R	R	R
	Cuprous Oxide	NR	NR	R
	Cymoxanil	NR	NR	R
	Cyproconazole	NR	NR	R
	Dichlofluanid	NR	NR	R
	Didecyldimethylammonium chloride	NR	NR	R
	Difenoconazole	NR	R	R
	Dimethomorphe	NR	NR	R
	Dithianon	NR	NR	R
	Dodemorph acetate	NR	NR	R
	Epoxiconazole	NR	NR	R

	Fenarimol	NR	NR	R
	Fenamidone	NR	NR	R
	Fenhexamid	NR	NR	R
	Flutriafol	NR	NR	R
	Fluzilazol	NR	NR	R
	Fosetyl - Aluminium	R	NR	R
	Hexaconazole	NR	R	R
	Imidachloriprid	NR	NR	R
	Iprobenfos	R	NR	R
	Iprodione	R	NR	R
	Iprovalicarb	NR	NR	R
	Isoxadifen-ethyl	NR	NR	R
	Kresoxim-methyl	NR	NR	R
	Mancozeb	R	R	R
	Maneb	R	NR	NR
	Mefenaxam	NR	NR	R
	Metalaxyl	R	R	R
	Metiram	NR	NR	R
	Micronised Sulphur	NR	NR	R
	Penconazole	NR	NR	R
	Pencycuron	NR	NR	R

	Prochloraz	R	NR	NR
	Propamocarb hydrochloride	NR	NR	R
	Propineb	NR	NR	R
	Pyrimethanil	NR	NR	R
	Spiroxamine	NR	NR	R
	Sulphur	NR	R	R
	Tebuconazole	R	NR	R
	Thiabendazole	NR	NR	R
	Thiophanate methyl	R	R	R
	Thiram	R	R	R
	Tolclofos Methyl	NR	NR	R
	Triadimefon	NR	R	NR
	Triadimenol	NR	R	NR
	Tricyclazole	NR	NR	R
	Trifloxystrobin	NR	NR	R
	Triforine	NR	NR	R
	Vinchlozoline	NR	NR	R
Herbicides	2,4-D	R	NR	R
	2,4-DB	R	NR	NR
	Ametryn	R	NR	NR
	Atrazine	R	R	NR

	Bispyribac sodium	NR	R	NR
	Clethodim	NR	NR	R
	Dalapon	R	NR	R
	Dimethamethryn	R	NR	NR
	Diuron	R	NR	R
	Fluazifop butyl	NR	R	NR
	Fluazifop-p-butyl	R	NR	NR
	Fluroxypyr	NR	R	NR
	Fluroxypyr ester-methylheptyl	NR	R	NR
	Glyphosate	R	R	R
	Hexazinone	R	NR	NR
	Isoproturon	R	NR	NR
	Linuron	NR	NR	R
	Methribuzin	NR	NR	R
	Metolachlor	R	NR	R
	Metribuzin	R	NR	NR
	MSMA	R	NR	NR
	Nicosulfuron	NR	R	NR
	Oxadiazon	NR	R	NR
	Oxyfluorfen	NR	NR	R
	Penoxsulam	NR	R	NR

	Promethrin	R	NR	NR
	Propanil	NR	NR	R
	Tembrotrione	NR	NR	R
	Terbutryn	NR	NR	R
	Thiobencarb	NR	NR	R
	Triclopyr	NR	R	NR
Rodenticides	Brodifacoum	R	NR	R
	Bromadiolone	R	R	R
	Chlorophacinone	R	NR	NR
	Coumatetralyl	R	NR	R
	Difenacoum	R	NR	R
	Diphacinone	R	NR	R
	Flocoumafen	R	NR	NR
Nematicides	Dazomet	R	NR	R
	Fenamiphos	NR	NR	R
	Oxamyl	NR	R	NR
	Terbufos	R	NR	NR
Molluscicides	Mercaptodimethur	NR	NR	R
	Metaldehyde	R	NR	R
Growth regulators	Serricornine	R	NR	NR
	Etherphon	NR	R	NR

	Daminozide	NR	NR	R
Biopesticides	Azadirachtin	NR	NR	R
	Bacillus thuringiensis	R	R	R
	Beauveria bassiana	NR	NR	R
	Pyrethrins	NR	NR	R
	Spinosad	NR	NR	R
	Trichoderma harzianum	NR	NR	R
	Emamectine benzoate	NR	R	NR

R : Registered, NR : Not registered, Red colour: EU banned status

Table S2: Antibacterial activity (mm of inhibition diameter ± SE) of the isolated bacterial strains from Burundian soils

Samples/ pathogen	***Xanthomonas campestris***	***Clavibacter michiganensis***	***Pseudomonas cichorii***	***Pseudomonas fuscovaginae***	***Rhodococcus fascians***	***Pectobacterium carotovorum***
BDI-M1	0.0	0.0	0.0	0.0	0.0	0.0
BDI-M2	0.0	0.0	0.0	0.0	0.0	0.0
BDI-M3	6.0 ± 0.6	3.2 ± 0.2	5.5 ± 1.0	0.0	12.5 ± 0.3	0.0
BDI-M4	25.5 ± 0.3	19.7 ± 0.4	8.3 ± 0.3	8.6 ± 0.2	9.3 ± 0.2	0.0
BDI-M5	0.0	0.0	0.0	0.0	0.0	0.0
BDI-M6	0.0	0.0	8.3 ± 0.4	8.7 ± 0.4	0.0	0.0
BDI-M7	3.5 ± 0.4	0.0	7.7 ± 0.6	0.0	11.0 ± 0.3	0.0
BDI-M8	5.5 ± 0.4	0.0	4.3 ± 0.2	0.0	12.5 ± 0.6	0.0
BDI-M9	4.8 ± 0.4	0.0	5.5 ± 0.9	0.0	8.5 ± 0.3	0.0
BDI-M10	0.0	0.0	0.0	0.0	0.0	0.0
BDI-M11	0.0	0.0	0.0	0.0	0.0	0.0

BDI-M12	24.8 ± 0,2	20.0 ± 0.3	6.6 ± 0.3	8.7 ± 0.4	5.8 ± 0.2	0.0
BDI-M13	14.0 ± 0.5	17.3 ± 0.4	8.2 ± 0.7	8.7 ± 0,4	10.3 ± 0.2	0.0
BDI-IS1	27.8 ± 0.5	26.7 ± 0.6	6.8 ± 0.2	8.8 ± 0.3	28.6 ± 0.3	7.0 ± 0.3
BDI-IS2	5.8 ± 0.2	7.0 ± 0.3	5.3 ± 0.4	0.0	4.5 ± 0.3	0.0
BDI-IS3	26.3 ± 0.8	24.3 ± 0.3	8.0 ± 0.5	8.0	5.7 ± 0.2	0.0
***Bacillus velezensis* QST713**	26.2 ± 0.3	27.6 ± 1.2	7.6 ± 0.1	8.7 ± 0.4	19.2 ± 0.3	6.6 ± 0.2

BDI-M and BDI-IS imply the sites where bacteria were isolated from, Burundi-Murwi and Burundi-Isare respectively and 1,2… stands for the isolate's number (BDI-IS1: isolate 1 from Burundi-Isare)

Table S3: Antifungal activity (mm of inhibition radius ± SE) of the isolated bacterial strain BDI-IS1 against nine phytopathogenic fungi

Samples/ pathogen	***Aspergillus niger***	***Botrytis cinerea***	***Alternaria solani***	***Aschochyta rabiei***	***Exserohilum turcicum***	***Fusarium oxysporum***	***Rhizoctonia solani***	***Pyricularia oryzae***	***Colletotrichum sp.***
BDI-IS1	5.4 ± 0.2	8.8 ± 0.2	14.0 ± 0.1	12.0 ± 0.4	16.0 ± 0.3	8.3 ± 0.2	14.0 ± 0.7	21.0 ± 0.5	21.0 ± 0.4
***B. velezensis* QST713**	9.4 ± 0.2	14.0 ± 0.7	17.0 ± 0.3	15.0 ± 0.4	20.0 ± 0.3	12.0 ± 0.3	16.0 ± 0.6	21.0 ± 0.4	21.0 ± 0.4

Table S4: Primers utilized in the *Bacillus nakamurai* BDI-IS1 mutant strains construction

Targeted molecule (s)	Primer name	Oligonucleotide sequence
Bacillaene	baeJ verif	TACATGGACTCGCTGAACGAC
	up baeJ F	TCAGACCGGGATAATCAAGC
	up baeJ R CHL M13	caggaaacagctatgaCTTTCATAGAGCTGCCTCCAT
	dwn baeJ F CHL M13	gtaaaacgacggccagTGAGGTTTAATGTGAAGAACTTC
	dwn baeJ R	CCTTCAAGAGATGCGGAGAC
Iturin A	ituA verif	GGAGAATATTCAGCTCTTGTCT
	up ituA F	TTGATGCAAAATGCAGATCC
	Up ituA R CHL M13	caggaaacagctatgaCAAACATAGGTTTCCTCCAAGG
	dwn ituA F CHL M13	gtaaaacgacggccagTAACTTTTAGGAAGCAAGGGGA
	dwn ituA R	AGTTCGGCTTCCAGGGTAAT
Bacillibactin	dhbC verif	TAATCAGCCGTGCCCTGCGG
	up dhbC F	TCGGACTAAAAACGGCTGAT
	Up dhbC R CHL M13	caggaaacagctatgaCTGACATGTCTGTACCTCCTT
	dwn dhbC F CHL M13	gtaaaacgacggccagTATGTAATGGAGAAAGGGGAA
	dwn dhbC R	AGCACTTGGAGGCTGGATAA
Surfactin	srfAA verif	TGATCTCGGAGGACGGGGTT
	up srfAA F	CCATTCCGGAGCTTGTGTCC
	up srfAA R CHL M13	caggaaacagctatgacCGCCTCCGGCTTTCAAAATC
	down srfAA F CHL M13	gtaaaacgacggccagtGCTTGGACCAGCTCCCTCTT
	down srfAA R	GGGATTTGCCTTTCCATGCG
NRPs	sfp verif	cgattcagcctgagctgatg
	up sfp F	ggctggacaatggttgtcgt

	up sfp R CHL M13	caggaaacagctatgacgtccaacatcctccgtctgc
	down sfp F CHL M13	gtaaaacgacggccagtgcacagcccgatttttgtga
	down sfp R	cgatgcagtccccggattat
Bacilysin or NRPs + bacilysin	bacA verif	GCTTGCTTTAATTTTCGGCG
	up bacA F	ATGGACGTTTTGGATTTTCG
	up bacA R CHL M13	caggaaacagctatgaCAATCATGAGCACCAACCAAT
	up bacA R PHLEO M13	caggaaacagctatgacCATGAGCACCAACCAATCTT
	dwn bacA F CHL M13	gtaaaacgacggccagTACTGAACAAGATTTGCAGG
	dwn bacA F PHLEO M13	gtaaaacgacggccagtGAGTGGGAAAACGGGGTAAG
	dwn bacA R	CGTATCAATCGCTGACTGGA
Plantazolicin or NRPs + plantazolicin	pznA verif	CCCGGAGTTAAATACCTATGT
	up pznA F	GCCATCAACAATAACGCAAA
	Up pznA R CHL M13	caggaaacagctatgaCAACCATATTGTGATTTATAT
	Up pznA R PHLEO M13	caggaaacagctatgacAGGCAACATTGTCAACCTAA
	dwn pznA F CHL M13	gtaaaacgacggccagTACGTTCTAACCTCTAAGACAAA
	dwn pznA F PHLEO M13	gtaaaacgacggccagtCCGAATGTAATGAAGCTCCC
	dwn pznA R	ACCGCTGTAGAATTGGGACA
Amylocyclicin or NRPs + amylocyclicin	acnA verif	AGTGTTCCCGTAACAATTACTGA
	up acnA F	TTACGGAATGGGTTTTGAGC
	up acnA R CHL M13	caggaaacagctatgaCacctatcatATTACCATCCT
	up acnA R PHLEO M13	caggaaacagctatgacCGAATAGGGTGTTTTCTCGA
	dwn acnA F CHL M13	gtaaaacgacggccagTatttagcccgggggagagca
	dwn acnA F PLEO M13	gtaaaacgacggccagttaagatttagcccgggggag

	dwn acnA R	tgactgcccgataaacacag
bacinapeptin or NRPs + bacinapeptin	bcnKC verif	gctgttgctttgtcgcataa
	up bcnKC F	tgagtaaaagcaacggcggt
	up bcnKC R CHL M13	caggaaacagctatgacgccttccataaaatccccc
	up bcnKC R PHLEO M13	caggaaacagctatgacgccttccataaaatccccc
	dwn bcnKC F CHL M13	gtaaaacgacggccagtgatcagcacccaagagttcc
	dwn bcnKC F PHLEO M13	gtaaaacgacggccagtgatcagcacccaagagttcc
	dwn bcnKC R	agctgacgaaatccattcgt
LCI or NRPs + amylocyclicin	LCI verif	CACTACTTCGAATGTACGGG
	up LCI F	GGAGCATTCGTTTGACGGAA
	up LCI R CHL M13	caggaaacagctatgacAATGCTGACATCGTCTTTGG
	up LCI R PHLEO M13	caggaaacagctatgacAATGGCGTTAATGCTGACAT
	dwn LCI F CHL M13	gtaaaacgacggccagtATTAAACTTCTTCAGGCTGC
	dwn LCI F PHLEO M13	gtaaaacgacggccagtTTCTTCAGGCTGCCGGGAAA
	down LCI R	ACGGCACCTGTGCTATATTT

Table S5: Analysis of the daily variation (DAI) in the severity of early blight in tomato using a general linear mixed model by trial and by treatment method (trial 1&2)

Trial	Days post inoculation	Treatment method	BDI-IS1	QST713	Control	Trial	Days post inoculation	Treatment method	BDI-IS1	QST713	Control
1	4DAI	R	2.08a	4.17a	8.10a	2	3DAI	R	18.52a	16.67a	23.15 a
1		L	9.03a	19.60a	8.10a	2		L	20.37a	25.31a	23.15 a
1	5 DAI	R	10.19a	17.82a	12.27a	2	4 DAI	R	22.84ab	19.75a	30.86 b
1		L	9.03a	19.60a	12.27a	2		L	22.22a	28.40a	30.86 a
1	6DAI	R	11.34a	20.37a	14.81a	2	5DAI	R	31.48a	33.95a	40.74 a
1		L	10.88a	23.84a	14.81a	2		L	33.33a	45.06a	40.74 a
1	7DAI	R	12.50a	25.46a	30.09a	2	6DAI	R	32.72a	35.19ab	43.83 b
1		L	10.6a5	32.18a	30.09a	2		L	33.95a	46.30aa	43.83
1	8DAI	R	26.39a	30.09a	36.57a	2	7DAI	R	35.19a	35.19a	45.68 a
1		L	18.98a	43.98a	36.57a	2		L	38.89a	50.62a	45.68 a
1	9DAI	R	34.03a	35.19a	50.00a	2	8DAI	R	37.04a	41.36a	52.47 b
1		L	24.07a	48.92ab	50.00b	2		L	41.98a	56.79a	52.47 a
1	10DAI	R	42.36a	36.34a	52.31a	2	9DAI	R	40.74a	44.44a	59.88 b
1		L	30.56a	51.70a	52.31a	2		L	46.30a	59.88a	59.88 a
1	11DAI	R	43.29a	36.81a	53.24a	2	10DAI	R	46.91a	50.62a	63.58 b
1		L	31.48a	51.70a	53.24a	2		L	50.62a	61.73a	63.58 a

1	12DAI	R	45.14a	37.27a	53.24a	2	11DAI	R	51.23a	50.62a	67.28 b
1		L	31.48a	52.16a	53.24a	2		L	54.94a	67.28a	67.28 a
1	13DAI	R	45.60a	37.27a	59.72a	2	12DAI	R	56.17a	50.62a	69.14 b
1		L	31.48a	52.16ab	59.72b	2		L	59.26a	67.90a	69.14 a
1	14DAI	R	45.60a	39.58a	62.50a	2	13DAI	R	56.79a	51.23a	69.75 b
1		L	31.94a	52.85ab	62.50b	2		L	59.26a	68.52a	69.75 a
1	15DAI	R	46.99a	39.58a	63.89b	2	14DAI	R	56.79a	52.47a	70.99 b
1		L	35.19a	52.85ab	63.89b	2		L	60.49a	69.14a	70.99 a
1	16DAI	R	46.99a	39.58a	63.89b	2	15DAI	R	58.02a	53.09a	71.60 b
		L	35.19a	52.85ab	63.89b	2		L	61.73a	69.75a	71.60 a
						2	16DAI	R	58.02a	53.09a	71.60 b
						2		L	61.73a	69.75a	71.60 a

Legend: R: Root treatment, L: Leaf treatment. Values with different letters are significantly different at 0.05 threshold.

Table S6: Analysis of the daily variation in the severity of the northern corn leaf blight disease using a general linear mixed model by trial and by treatment method (trial 1 & 2)

Trial	Days post inoculation	Treatment method	BDI-IS1	QST713	Control
1	23DAI	R	23.44a	26.70a	56.71b
1		L	27.08a	22.38a	56.71b
1	38DAI	R	47.19a	55.94a	78.02b
1		L	54.06a	53.44a	78.02b
2	7DAI	R	0.10a	0.00a	2.58a
2		L	0.08a	0.08a	2.58a
2	10 DAI	R	0.58a	5.92a	14.08a
2		L	0.58a	4.42a	14.08a
2	12DAI	R	5.17a	7.92a	27.42a
2		L	9.63a	13.67a	27.42a
2	13DAI	R	8.38a	10.08a	37.1b
2		L	15.13a	17.50a	37.1a
2	15DAI	R	12.67a	10.54a	37.1b
2		L	17.63a	18.83a	37.1a
2	17DAI	R	12.96a	12.79a	40.21b
2		L	17.79a	18.88b	40.21b
2	19DAI	R	13.83a	16.96a	44.58b
2		L	22.22a	21.58a	44.58b
2	21DAI	R	14.46a	16.96a	44.75b
2		L	22.69b	21.58a	44.75b
2	25DAI	R	15.88a	18.21a	58.17b

2		L	23.99a	23.17a	58.17 b
2	27DAI	R	17.75a	22.79a	63.17 b
2		L	26.78a	24.50a	63.17b
2	29DAI	R	17.75a	24.46a	68.17b
2		L	27.64a	27.00a	68.17b
2	33DAI	R	17.75a	24.46a	70.04b
2		L	28.47a	27.04a	70.04b

Legend: R: Root treatment, L: Leaf treatment. Values with different letters are significantly different at 0.05 threshold.

Table S7: Area under the disease progression curve (AUDPC) and protection index (PI) of BDI-IS1 and QST713 against northern corn leaf blight and tomato early blight

		Northern leaf blight				**Tomato early blight**			
Trial	Treatment	AUDPCR	AUDPCL	PIR	PIL	AUDPCR	AUDPCL	PIR	PIL
1	BDI-IS1	799.31±97.24	920.01±62.09	51.93±5.85	44.67±3.73	388.19±48.30	287.85±34.93	23.59±12.96	43.70±9.39
1	QST713	926.91±146.90	825.94±45.27	44.25±8.84	50.32±2.72	377.66±100.11	518.40±114.78	26.67±19.80	-0.15±23.96
2	BDI-IS1	316.08±48.75	487.44±22.68	72.76±4.20	58.00±1.95	564.81±35.02	604.01±41.98	22.96±4.78	17.62±5.73
2	QST713	395.75±103.14	494.83±107.49	65.90±8.89	57.36±9.26	553.40±23.52	738.89±27.61	24.52±3.21	-0.78±3.77

Legend: AUDPCR: Area Under Disease Progress Curve after root treatment. AUDPCL: Area Under Disease Progress Curve after leaf treatment. PIR: Protection Index after root treatment. PIL: Protection Index after foliar treatment.

Table S8: Temperature and relative humidity in greenhouses during the experimental period

Pathosystem	Trial	Period	Temperature (°C)			Relative humidity (%)		
			minima	mean	maxima	minima	mean	maxima
Maize + *E. turcicum*	1	Day	14.40	20. 8	26.00	59.00	72.00	85.00
		Night	14. 40	16. 05	17. 60	73.00	80.00	86.00
	2	Day	15. 40	21. 95	26	55.00	70.00	85.00
		Night	15. 80	17.06	18. 90	71.00	78.00	85.00
Tomato + *A. solani*	1	Day	13. 90	19. 91	24.00	62.00	81.00	96.00
		Night	14,00	16.00	20.00	73.00	86.00	96.00
	2	Day	17. 30	20. 85	24.00	69.00	82.00	99.00
		Night	17. 20	17. 5	18.00	83.00	88.00	95.00

2. Supplementary figures

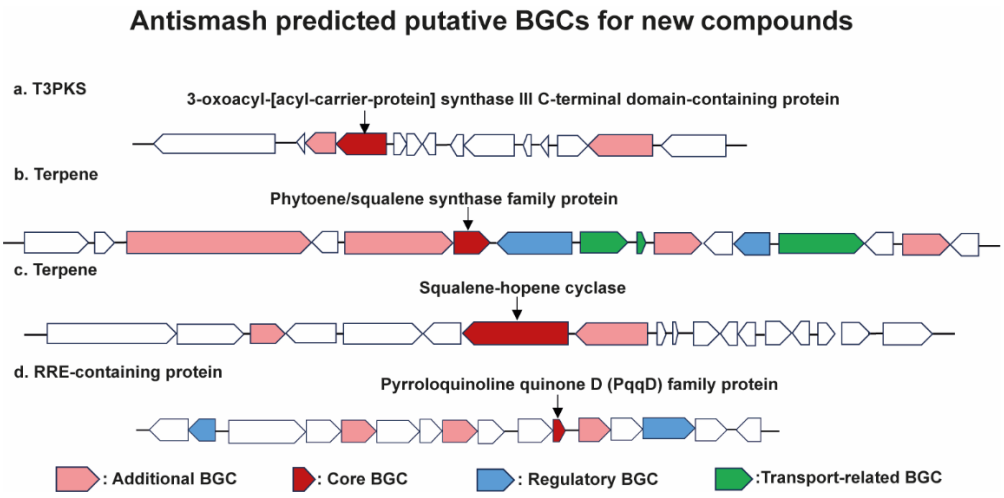


Figure S1: Putative biosynthetic gene clusters for new compounds in BDI-IS1 DNA genome. Prediction was performed with AntiSMASH 7.0 in a relaxed detection strictness mode. The architecture of the genes was adapted in line with the information provided by the software.

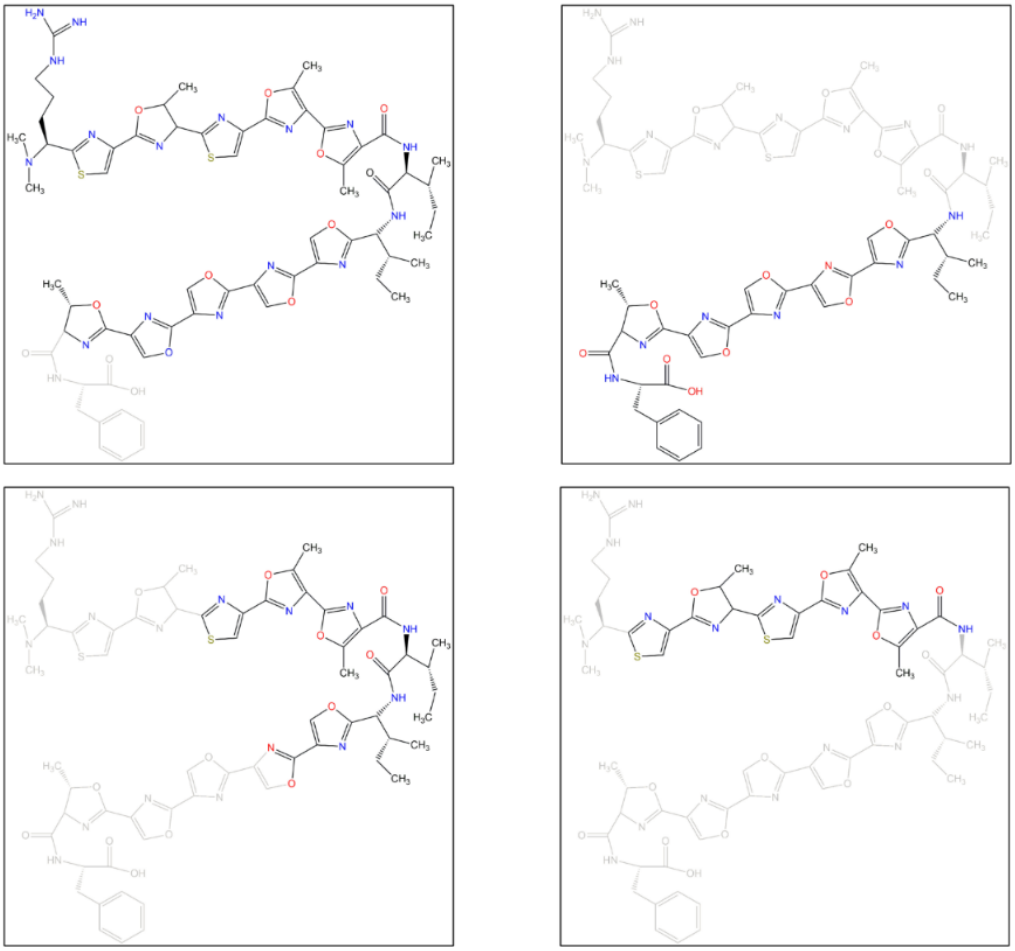


Figure S2: Chemical structures of the different MS^2 fragmentation patterns of plantazolicin. Each fragment is illustrated in bold font on the structure, while the remaining parts of the structure are less visible.

Figure S3: Prediction-based heterogeneity of some RiPPs across different *B. nakamurai* strains and related *Bacillus* strains. A. Amino acid sequences of different predicted class III

lanthipeptide compounds across different *Bacillus subtilis* group trains, for which bacinapeptin A and bacinapeptin B from *B. nakamurai* NRRL B-41092 are already characterized on experimental basis (Xue et al., 2022). B. Amino acid sequence variations of LCI-like compounds across some *Bacillus* spp. compared to the core amino acid sequence of already described LCI from *B. subtilis* A014 (Gong et al., 2011). C. Heterogeneity of amino acids sequence of the head-to-tail cyclized amylocyclicin-like metabolites compared to the precursor peptide sequence of the *novo* described amylocyclicin from *B. velezensis* FZB42 (formerly *B. amyloliquefaciens* subsp. *plantarum* FZB42) (Scholz et al., 2014).

Figure S4: Scale developed for the evaluation of tomato early blight (A) and maize northern leaf blight (B). The scale is based on necrotic leaf area, with tomato early blight scored on a scale of 1 to 9, where 1 = 0-5% of leaf area affected by the disease, 3 = 5-10%, 5 = 10-20%, 7 = 20-50% and 9 = 50-100%. Northern leaf blight was also scored on a scale from 1 to 9, where 1 = 0% of leaf area affected, 2 = 10%, 3 = 20%, 4 = 30%, 5 = 40%, 6 = 50%, 7 = 60%, 8 = 70%, 9 = 80-100%. Asymptomatic samples are not included in the maize northern leaf blight scale but will obviously have 0% necrotic area.

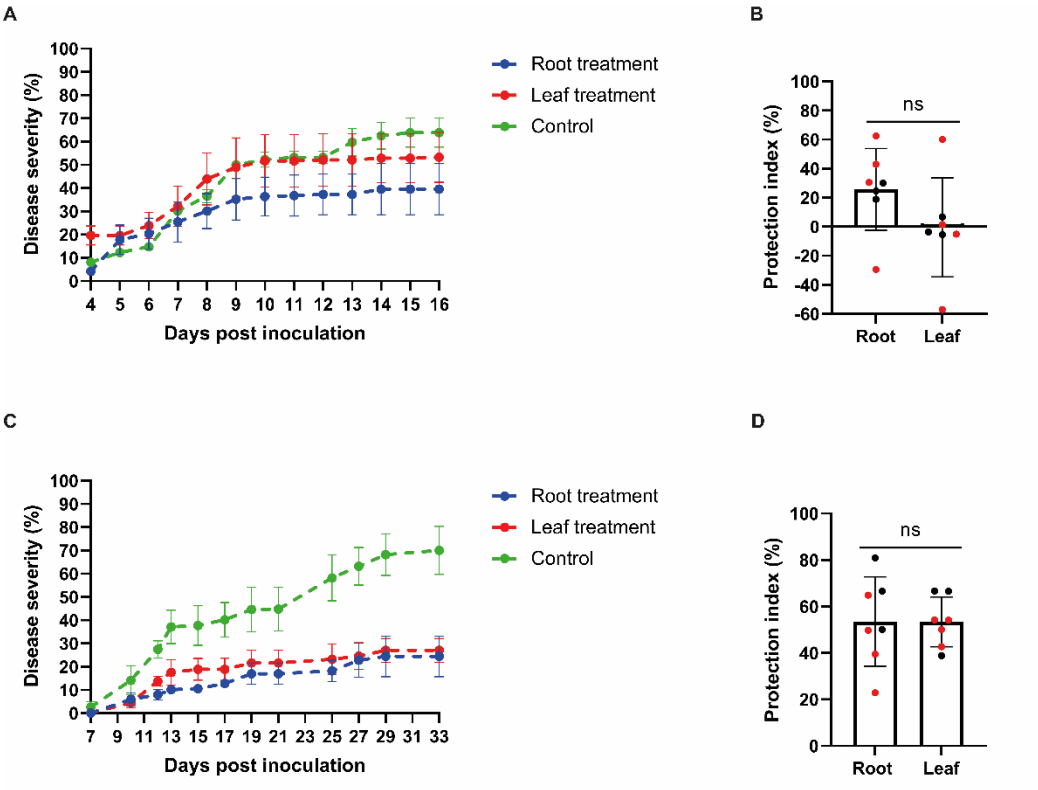


Figure S5: Biocontrol efficacy of QST713 against tomato early blight (TEB) and nothern corn leaf blight (NLB). A&C. Reduction in disease severity of TEB and NLB on plants treated in the roots (blue) or leaves (red) with QST713 followed by inoculation with *Alternaria solani* or *Exserohilum turcicum*, respectively, and control plants (in green) inoculated with the respective fungus, under greenhouse conditions. Each point in graphs A and C represents the mean (±SE) of disease severity from four replicates (n=4) and three replicates (n=3) for experiments one and two, respectively. B&D. Protection index (PI) of QST713 when applied to roots or leaves of tomato (B) and maize (D) plants inoculated with *A. solani* and *E. turcicum*, respectively. PIs represent means (± SE) of two independent experiments with four (red) and three (black) replicates per treatment for experiment one (n = 4) and two (n = 3), respectively. Means of PI are calculated from area under progress curve (AUDPC) data, which in turn depend on disease severity scores and the time interval between successive recording points (see formula in methods). The differences between PI of BDI-IS1 obtained from root and leaf treatment were analyzed using t-test method and there was no significant difference (ns) at *p-value* ≤5%.

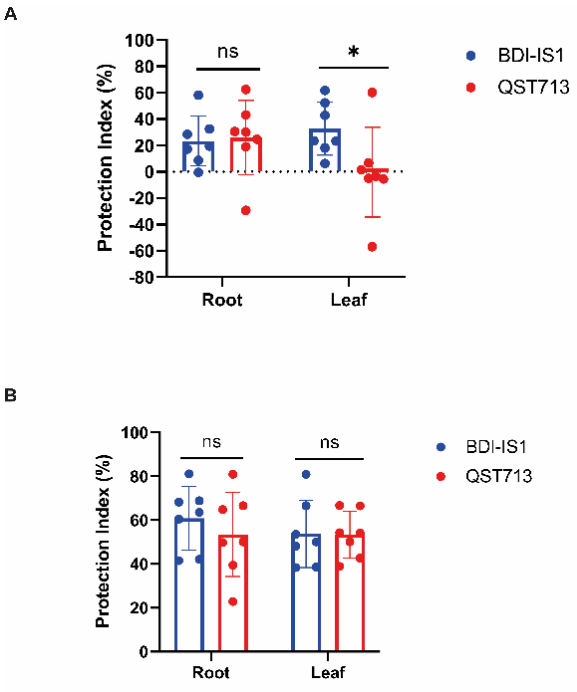


Figure S6: Comparison of protection provided by BDI-IS1 and QST713 against TEB and NLB. Protection index (PI) of BDI-IS1 and QST713 when applied to roots or leaves of tomato (A) and maize (B) plants inoculated with *A. solani* and *E. turcicum*, respectively. PIs represent means (± SE) of two independent experiments (n = 7) with four and three replicates per treatment for experiment one and two, respectively . Means of PI are calculated from area under progress curve (AUDPC) data, which in turn depend on disease severity scores and the time interval between successive recording points (see formula in Chapter 4). The differences between PI of BDI-IS1 and QST713 obtained from root and leaf treatment were analyzed using t-test method, ns implies that there was no significant difference, while * shows significant statistical difference at p-value ≤0.05.

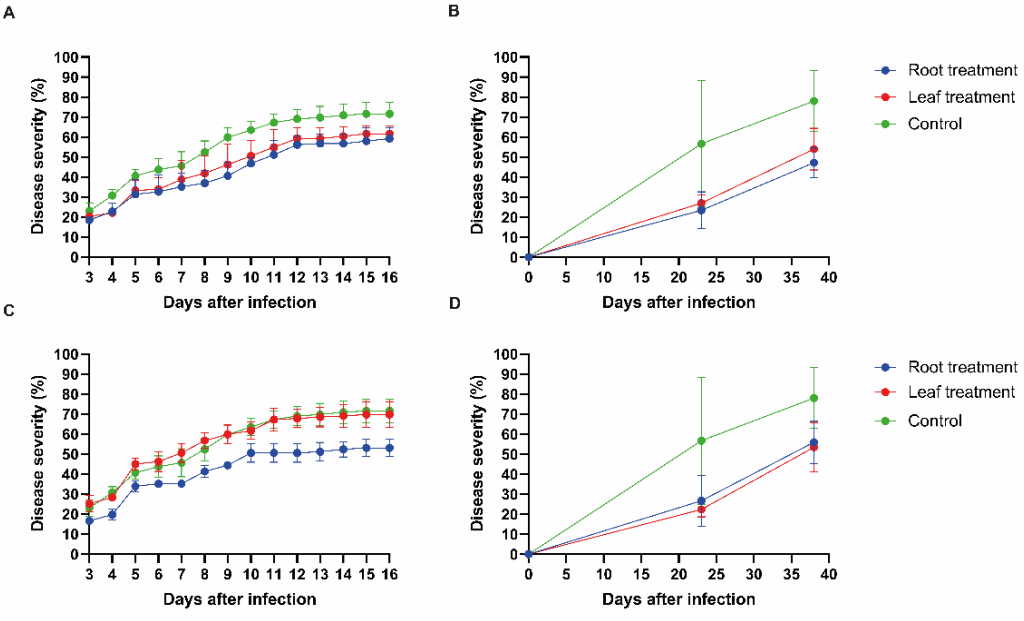


Figure S7: Biocontrol efficacy of BDI-IS1 and QST713 against tomato early blight (TEB) and nothern corn leaf blight (NLB). Reduction in disease severity of TEB (A&C) and NLB (B&D) on plants treated in the roots (blue) or leaves (red) with BDI-IS1 (A & B) or QST713 (C&D) followed by inoculation with *A. solani* or *E. turcicum*, respectively, and control plants (in green) inoculated with the respective fungus under greenhouse conditions. Each time-point represents the mean (± SE) of disease severity from three replicates (n = 3) and four replicates (n = 4) for TEB and NLB in experiments two and one, respectively. The difference between DS was analyzed using general linear mixed models at *p-value* ≤0.05 (Table S5 & S6).

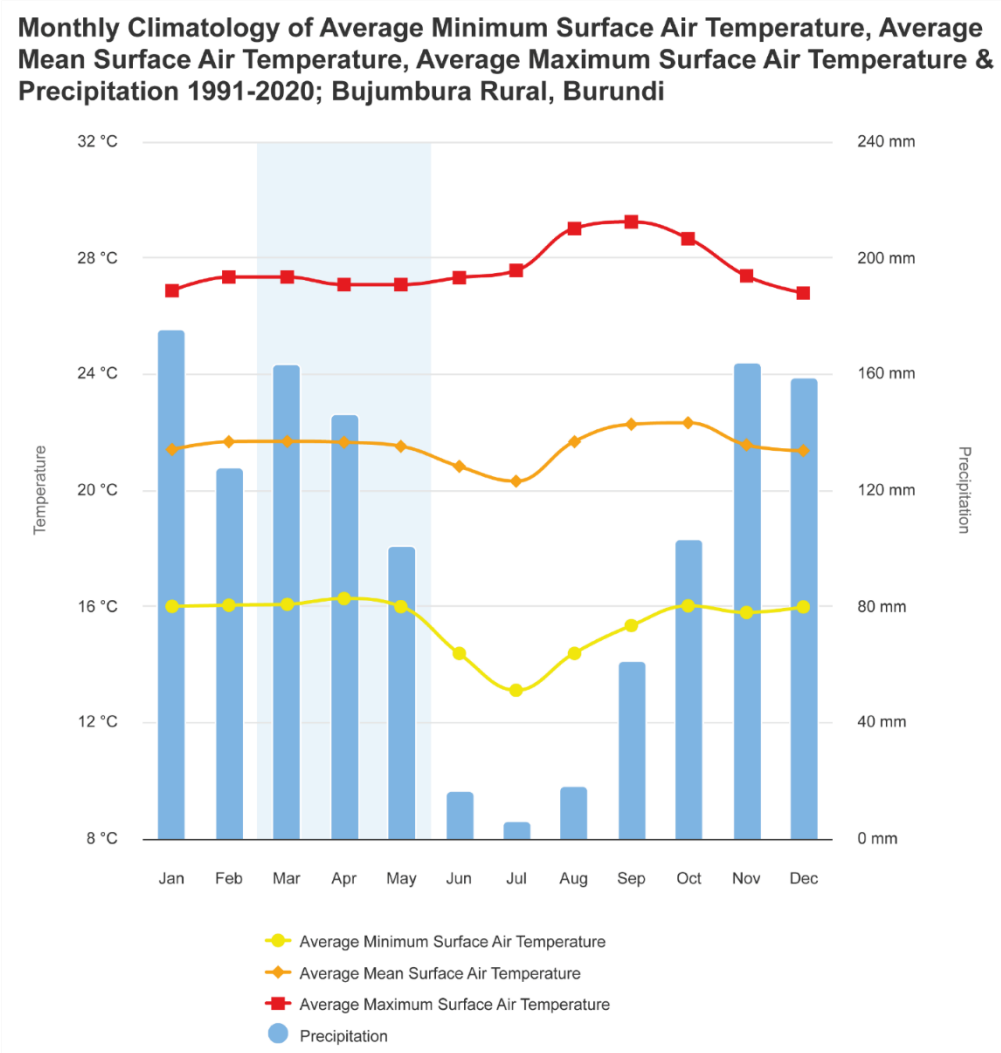


Figure S8: Temperature and rainfall variations in Bujumbura Rural, Burundi. The plotted graph evolve from means of complied data over the last 30 years (1990-2020) (World Bank Group, 2021).

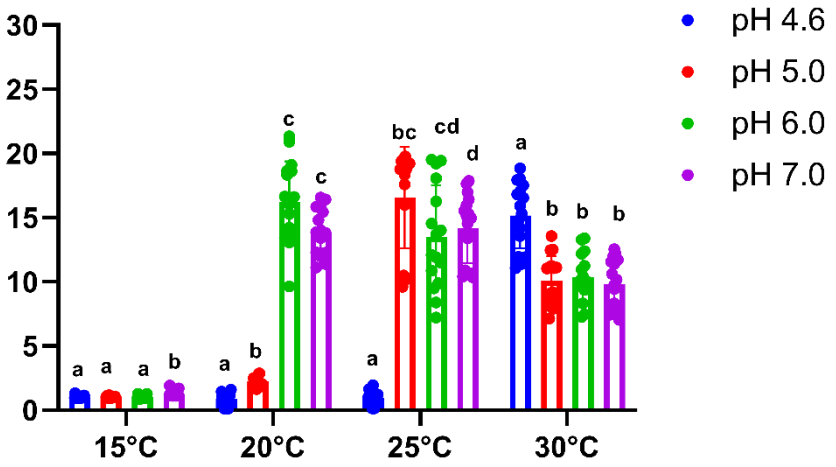


Figure S9: pH effect on the biofilm formation potential of *B. velezensis* QST713 at different temperatures. Fresh bacterial suspension (OD_{600nm} 0.1) in REM set at different initial pHs (pH 4.6 - 5.0 - 6.0 - 7.0), was partitioned into round bottom 96-wells microplate (150µL per well) and incubated for 72h without shaking at different temperatures (15°C-20°C-25°C-30°C). Medium and planktonic cells were gently removed by pipetting and wells were twice washed with phosphate buffered saline (PBS). Biofilm pellicles were stained with crystal violet (0.1% v/v) during 10 min, and wells underwent two successive washings with PBS after gentle removal of the dye. Stained biofilm pellicles were then dissolved for 30 min into glacial acetic acid (30% v/v) and absorbance readings were performed in TECAN machine at 595 nm. The assay was carried in two independent experiments, with eight technical replicates per each tested pH (n = 16). Comparison of means of the data collected at different pHs per each temperature was performed by two-way ANOVA coupled with multi-test Tukey ($\alpha = 0.05$), and bars with same or different letters imply that non-significant or significant differences between the means of considered variables.

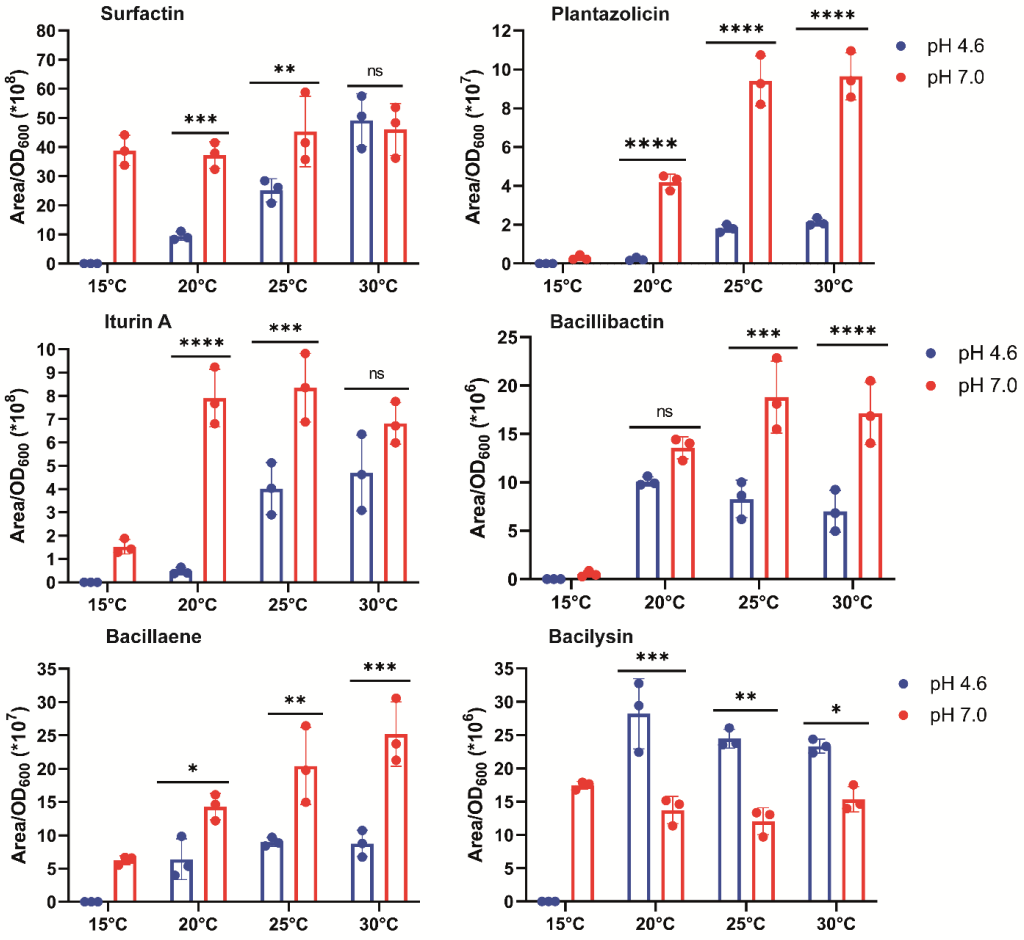


Figure S10: Impact of temperature on BSMs production by BDI-IS1 at different pH. Cell-free supernatants (22µm pore size) from a 48h-old-cultures whose initial pH of the starting culture (OD_{600nm} 0.1) was set at pHi 4.6 and pHi 7.0 and incubated separately at different temperatures (15°C-20°C-25°C-30°C; 150rpm, 48 wells-microplates), were analysed by UPLC/MS q-TOF. The plotted data are mean (of the cumulative normalized area under the extracted ion chromatogram (EIC) ($Area/OD_{600nm}$ max.) of the different detected chemical variants of each BSM (where applicable). The experiment was carried out in triplicates and statistical comparison of means was performed with two-way ANOVA coupled with Tukey's test ($\alpha = 0.05$) and ns represent non-significant difference, while asterisks *, **, ***, **** imply significant differences at $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.0001$ respectively.

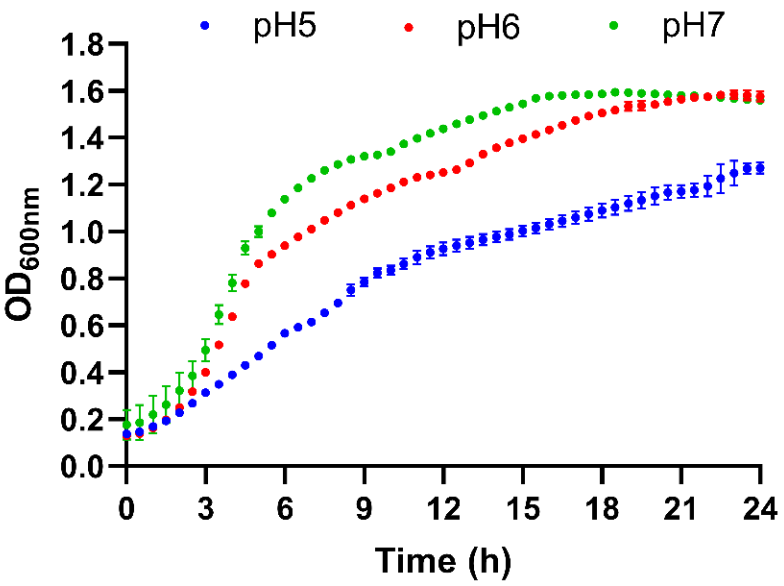


Figure S11: pH impact on *P. carotovorum* growth. The experiment was carried out into 48 well-microplates with continuous shaking and automated OD_{600nm} monitoring (400µL per well,30°C, 24h). Initial medium pH was set at different pH points i.e. pH 5, pH 6, pH 7. The assay was performed in triplicates (n = 3).