

***Bacillus* responses to plant-associated fungal and bacterial communities**

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9 **Keywords: *Bacillus*, rhizosphere, bioactive secondary metabolites, microbial interaction,**
10 **biocontrol, molecular cross-talk, phenotype modulation**

Number of words: 3467

Number of Figure: 2

11 **Abstract**

12 Some members of root-associated *Bacillus* species have been developed as biocontrol agents due
13 to their contribution to plant protection by directly interfering with the growth of pathogens or by
14 stimulating systemic resistance in their host. As rhizosphere-dwelling bacteria, these bacilli are
15 surrounded and constantly interacting with other microbes via different types of
16 communications. With this review, we provide an updated vision of the molecular and
17 phenotypic responses of *Bacillus* upon sensing other rhizosphere microorganisms and/or their
18 metabolites. We illustrate how *Bacillus* spp. may react by modulating the production of
19 secondary metabolites such as cyclic lipopeptides or polyketides. On the other hand, some
20 developmental processes such as biofilm formation, motility and sporulation may also be
21 modified upon interaction, reflecting the adaptation of *Bacillus* multicellular communities to
22 microbial competitors for preserving their ecological persistence. This review also points out the
23 limited data available and a global lack of knowledge indicating that more research is needed in
24 order to, not only better understand the ecology of bacilli in their natural soil niche, but also to
25 better assess and improve their promising biocontrol potential.

26 **1 Introduction**

27 Some *Bacillus* species of the *B. subtilis* complex are plant-associated and important members of
28 the microbiome (Mendes et al., 2013; Müller et al., 2016; Fierer, 2017). During the last decades,
29 their potential use as biocontrol agents with protective activity toward economically important
30 plant pathogens has been highlighted thereby representing a promising alternative to chemical

1 pesticides (Expósito et al., 2017; Fan et al., 2017; Finkel et al., 2017; Fira et al., 2018; Köhl et
2 al., 2019). The efficacy of such bacilli in plant protection, as well as their constant presence in
3 the strongly competitive rhizosphere niche, are due to their high potential to synthesize a wide
4 range of volatile organic compounds (VOCs) and soluble bioactive secondary metabolites
5 (BSMs). High structural diversity is observed in the patterns of VOCs formed by *Bacillus* (Kai
6 2020; Caulier 2019) but also in BSMs which can be either ribosomally synthesized and post-
7 translationally-modified like bacteriocins and lantibiotics or enzymatically formed via multi-
8 modular mega-enzymes as in the case of polyketides (PKs), di-peptides or cyclic lipopeptides
9 (CLPs) (Harwood et al., 2018; Kaspar et al., 2019; Rabbee et al., 2019). A prime role of some
10 soluble BSMs and volatiles in plant protection is related to their strong antimicrobial activity
11 leading to direct antagonism against plant pathogens (Raaijmakers and Mazzola, 2012; Borriss,
12 2015; Chowdhury et al., 2015a; Fan et al., 2018; Caulier et al., 2019; Rabbee et al., 2019; Kai
13 2020). A second important biocontrol-related trait of those compounds is their ability to trigger
14 an immune reaction in the host plants which leads to systemic resistance (Induced SR) rendering
15 the plant less susceptible to pathogen infection (Pieterse et al., 2014; Chowdhury et al., 2015a;
16 Fan et al., 2018; Caulier et al., 2019; Rabbee et al., 2019). An additional role of BSMs is also
17 linked to an efficient plant root colonization ability of *Bacillus* which indirectly protects the plant
18 by decreasing the space and nutrient availability for pathogens (Raaijmakers et al., 2010;
19 Borriss, 2015; Nayak, 2020). Some BSMs also contribute to colonization since they are
20 involved in the developmental processes of *Bacillus* social motility and biofilm formation
21 (Raaijmakers and Mazzola, 2012; Borriss, 2015; Pandin et al., 2017).

22 As rhizosphere-dwelling bacteria, these plant-associated bacilli are influenced by various
23 environmental factors such as temperature, pH, moisture, light and nutrient composition dictated
24 by plant exudation (Santoyo et al., 2017). In this competitive niche, *Bacillus* species are also
25 surrounded by and constantly interacting with a myriad of other (micro)organisms, (Mendes et
26 al., 2013; Traxler and Kolter, 2015; Fierer, 2017; Schmidt et al., 2019). In this review, we
27 illustrate the diversity of BSMs produced by different *Bacillus* species and how this metabolome
28 and phenotypic traits dictating ecological fitness can be impacted upon interaction with other
29 fungal and bacterial microorganisms. The outcomes of volatile-based microbial interactions, in
30 general, have been recently reviewed (Schmidt et al., 2015; Tyc et al., 2017). However, when
31 dealing with interactions involving bacilli, information is scarce concerning possible changes in
32 VOCs production upon cross-talk or perception of volatiles produced by other microorganisms
33 (Chen et al., 2015; Tahir et al., 2017; Martínez-Cámara et al., 2019). Thus, we focus hereafter on
34 interactions based on cross-talks mediated by the perception of soluble metabolites.

35 **2 Diversity and bioactivities of *Bacillus* BSMs**

36 In the comparative genomic era, numerous adjustments have been done in the last years to clarify
37 the phylogeny of the *B. subtilis* complex, which includes, among others, species such as *B.*
38 *velezensis*, *B. amyloliquefaciens*, *B. atrophaeus*, *B. subtilis* subspecies *subtilis*, *B. licheniformis*,
39 *B. pumilus* and *B. siamensis* with potential as biocontrol agents (Expósito et al., 2017; Fira et al.,
40 2018; Maksimov et al., 2020), and which led to some confusion in species names but also to
41 misassignments (Dunlap et al., 2016; Fan et al., 2017; Harwood et al., 2018; Du et al., 2019;
42 Torres Manno et al., 2019). Many isolates such as strains FZB42, QST713 or SQR9 formerly
43 assigned to the *B. subtilis* or *B. amyloliquefaciens* species have been re-classified as *B. velezensis*
44 representing the model species for plant-associated bacilli (Dunlap et al., 2016; Fan et al., 2017).

1 A large part of the genome of these species is devoted to the production of antimicrobial
2 compounds with up to 12% annotated as involved in the synthesis of bioactive secondary
3 metabolites (Molinatto et al., 2016; Fan et al., 2017; Pandin et al., 2018).

4 Non-ribosomal metabolites are synthesized either by polyketide synthases (PKS) or non-
5 ribosomal peptide synthase (NRPS), both acting as assembly lines catalyzing different steps for
6 the incorporation of amino acid residues (Dutta et al., 2014; Winn et al., 2016; Bozhüyük et al.,
7 2019). The three main families of *Bacillus* CLPs are surfactins, fengycins and iturins (Figure 1).
8 According to this limited number of families identified so far, the structural diversity of *Bacillus*
9 CLPs may appear quite limited compared to other bacterial genera such as *Pseudomonas*, for
10 which many more different groups have been discovered (Geudens and Martins, 2018; Götze and
11 Stallforth, 2020). However, reduced specificity of adenylation domains allows substitutions at
12 specific places in the peptide chain and the NRPS machinery can bind different fatty acids with
13 various chain lengths in the initiation step leading to co-production of various homologs within
14 the three families as illustrated in Figure 1 (Kraas et al., 2010; Bozhüyük et al., 2019).
15 Interestingly, some CLP peptidic variants are synthesized through species-specific clusters, like
16 pumilacidin and lichenysin which are only produced respectively by *B. pumilus* and *B.*
17 *licheniformis* (Figure 1).

18 The three different types of CLPs retain specific but complementary functions considering
19 biocontrol efficiency and, more generally, ecological fitness of the producing strains. By
20 contributing to motility and biofilm formation, surfactins are involved in colonization of plant
21 tissues which indirectly allow *Bacillus* to outcompete phytopathogens for space and nutrients.
22 Surfactins are also involved in the molecular cross-talk with the host and it is well characterized
23 as an elicitor of plant immunity leading to ISR (Ongena and Jacques, 2008; Henry et al., 2011;
24 García-Gutiérrez et al., 2013; Cawoy et al., 2015; Chowdhury et al., 2015a). Direct antibiotic
25 activity of surfactins at biologically relevant concentrations toward soil-dwelling or plant-
26 associated microbes has been only occasionally reported (Qi et al., 2010; Liu et al., 2014). By
27 contrast, fengycins and iturins are best characterized for their antifungal activities against a wide
28 range of plant pathogens (Caulier et al., 2019; Rabbee et al., 2019). This is mainly due to their
29 ability to perturb fungal cell membrane integrity resulting in cytoplasm leakage and finally
30 hyphae death and inhibition of spore germination (Chitarra et al., 2003; Romero et al., 2007;
31 Deleu et al., 2008; Etchegaray et al., 2008; Gong et al., 2015; Gao et al., 2017; Zhang and Sun,
32 2018). The three CLPs retain some selectivity but may also act synergistically to inhibit fungal
33 growth (Liu et al., 2014). The lipid composition of the plasma membrane could explain
34 differences in the sensitivity of fungal targets to one or more CLPs (Wise et al., 2014; Fiedler
35 and Heerklotz, 2015).

36 Besides lipopeptides, most species of the *B. subtilis* group also produce other non-ribosomal
37 oligopeptide derivatives such as bacilysin, chlorotetaine, bacitracins and rhizocitcins which are
38 known to be efficient as antibacterial compounds targeting cell wall biosynthesis (Zhao and
39 Kuipers, 2016). The siderophore bacillibactin is highly conserved in the *B. subtilis* group (Figure
40 1) and is induced in response to iron limitation in the environment. It allows *Bacillus* to
41 efficiently acquire Fe³⁺ and other metals (Miethke et al., 2006, 2008; Li et al., 2014) thereby
42 depriving phytopathogens of this essential element (Miethke et al., 2006; Niehus et al., 2017).

1 Polyketide biosynthesis is performed by successive condensation of small carboxylic acids
2 mediated by core domains of the corresponding enzyme machinery but some PKs are
3 synthesized via hybrid NRPS/PKS systems leading to the integration of amino acid residues
4 (Piel, 2010; Olishavska et al., 2019). The three main PKs produced by *Bacillus* are difficidins,
5 macrolactins and bacillaenes, the latter being more widespread across species (Figure 1). The
6 main PKs role is related to their antibacterial activity via the ability to inhibit protein
7 biosynthesis in numerous phytopathogenic bacteria but certain antifungal activity has been
8 reported for bacillaenes and macrolactins (Caulier et al., 2019; Olishavska et al., 2019).

9 Ribosomally synthesized BSMs encompass bacteriocins and lantibiotics including plantazolicin,
10 subtilin, ericin, mersacidin, amylolysin and amylocyclacin that are specifically produced by some
11 species or strains (Brötz et al., 1998; van Kuijk et al., 2012; Arguelles Arias et al., 2013; Scholz
12 et al., 2014; Torres Manno et al., 2019). These BSMs are responsible for growth inhibition of
13 Gram-positive bacteria by acting via different modes of action (Abriouel et al., 2011; Acedo et
14 al., 2018).

15 **3 Perception of fungi triggers the production of appropriate BSMs**

16 Several works have illustrated the impact of phytopathogenic fungi on BSMs production by soil
17 bacilli. Some *B. amyloliquefaciens*, *B. velezensis* and *B. subtilis* strains respond to the presence
18 of antagonistic fungi by stimulating the production of the antifungal CLPs fengycins and/or
19 iturins (Table 1). Not only the production of specific CLPs varies in a species-dependent manner
20 but it is also highly dependent on the interacting fungal species. For example, much higher
21 production of iturins and fengycins by *B. subtilis* 98S was observed in confrontation with
22 *Pythium aphanidermatum* and *Fusarium oxysporum* but not with *Botrytis cinerea* (Cawoy et al.,
23 2015). Further, upon interaction with fungi, some *B. velezensis* strains (SQR9, FZB42 and S499)
24 overproduced either iturins or fengycins (Li et al., 2014; Chowdhury et al., 2015b; Kulimushi et
25 al., 2017). For instance, Li et al. (2014) showed that when confronted with *Sclerotinia*
26 *sclerotiorum*, *B. velezensis* SQR9 overproduces bacillomycin D (iturin family), but not
27 fengycins. An overproduction of bacillomycin along with a reduced production of fengycins was
28 also reported by Chowdhury et al. (2015b) upon *B. velezensis* FZB42 interaction with
29 *Rhizoctonia solani* in the rhizosphere of lettuce plants. Differentially, Kulimushi et al. (2017),
30 showed that strains S499 and FZB42 improved production of fengycin but not iturins upon
31 interaction with *Rhizomucor variabilis*. Most of these studies also indicated that fengycins and
32 iturins are the main BSMs responsible for antifungal activities (Table 1). Thus, *Bacillus* cells
33 could specifically sense the presence of fungal competitors and accordingly overproduce
34 appropriate antifungal BSMs to outcompete the interacting fungi. Moreover, besides modulating
35 the production of fengycins and iturins, some strains of *B. velezensis* (SQR9, FZB42 and
36 QST713) and *B. subtilis* (B9-5) may overproduce surfactins when sensing phytopathogenic fungi
37 (Li et al., 2014; Chowdhury et al., 2015b; DeFilippi et al., 2018; Pandin et al., 2019). In support
38 to this hypothesis, surfactin production of *B. velezensis* FZB42 was highly induced in the
39 presence of fungal pathogen *R. solani* in the lettuce rhizosphere where it was found as the main
40 produced compound (Chowdhury et al., 2015b). A similar response was recorded when *B.*
41 *velezensis* SQR9 was confronted with *S. sclerotiorum* and *Phytophthora parasitica* (Li et al.,
42 2014) or when *B. subtilis* B9-5 interacted in liquid medium with *Rhizopus stolonifer* (DeFilippi
43 et al., 2018). In contrast to fengycins and iturins, surfactins are not strong direct antifungal
44 metabolites in biologically relevant concentrations (Raaijmakers and Mazzola, 2012). Thus, it

1 stays unclear why *Bacillus* responded by surfactin overproduction to the presence of antagonistic
2 fungi. A possible explanation could be rooted in its global role promoting the rhizosphere and
3 thereby, contributing to competition for nutrients and space with the interacting fungi (Ongena
4 and Jacques, 2008; Rabbee et al., 2019).

5 Even though the siderophore bacillibactin is produced by all members of the *B. subtilis* species
6 complex (Figure 1), its possible overproduction upon microbial interactions has been poorly
7 investigated. Interestingly, the work of Li et al. (2014) showed that *B. velezensis* SQR9
8 overproduces bacillibactin when grown in presence of a range of fungi including *V. dahliae*, *S.*
9 *sclerotiorum*, *F. oxysporum*, *R. solani*, *F. solani* and *P. parasitica*. This may be interpreted as a
10 response of the bacterium to some iron-limitation in the medium caused by the fungi via the
11 release of their own chelatants.

12 In *B. subtilis*, the expression of many BSM biosynthesis genes is transcriptionally fine-tuned by
13 compound-specific regulation but also by global regulators governing the transition to crucial
14 developmental processes like motility, biofilm formation and sporulation (Inaoka et al., 2009;
15 López et al., 2009; Vargas-Bautista et al., 2014). Fungal triggers may affect both types of
16 regulatory systems involved in BSMs production. For instance, upon sensing *F. verticillioides*,
17 the global stress-related regulator SigB is activated in *B. subtilis* NCIB3610 which in return
18 enhances surfactin production (Bartolini et al., 2019). In interaction with *F. culmorum* under
19 biofilm-conducive conditions, *B. subtilis* Bs12 down-regulates the expression of the *sinR* gene
20 known as a repressor of biofilm formation which also negatively regulates surfactin production
21 (Kearns et al., 2005; Khezri et al., 2016; Zhi et al., 2017). These observations strongly suggest
22 that specific soluble signals, emitted by fungal pathogens, could be perceived by bacilli which in
23 turn modulate BSMs synthesis. As observed by Bartolini et al. (2019), cells of the *Bacillus*
24 colony, physically close to the fungal culture, responded to signals by over-expressing genes
25 coding for transcription factors involved in CLPs synthesis regulation. In contrast, colony cells
26 positioned on the opposite side of the fungi did not react to the fungus (Bartolini et al., 2019).
27 This phenomenon indicates that the specific fungal metabolite diffuses on a short distance and
28 has an influence on closely located *Bacillus* cells. Currently, no fungal compounds have been
29 identified as triggers of BSM stimulation in *Bacillus*. Nonetheless, few commonly produced
30 metabolites by *Fusarium* species were suggested to modify *Bacillus* behavior. It was shown that
31 two cyclic depsipeptides (enniatins B1 and enniatins A1) and a pyrone (lateropyrone) had an
32 antagonistic effect on *B. subtilis* growth (Ola et al., 2013). Fusaric acid also modified
33 antibacterial activity of *B. mojavensis* but it was not related to a decrease in the production of
34 specific BSMs (Bacon et al., 2004, 2006; Bani et al., 2014). These metabolites could also play a
35 triggering role at sub-inhibitory concentration and could have an inducible effect on the range of
36 *Bacillus* responses as has been shown for other signal metabolites (Bleich et al., 2015; Liu et al.,
37 2018).

38 **4 *Bacillus* phenotype is modulated upon a perception of bacterial competitors**

39 Some BSMs may also act as molecular determinants driving outcomes of interactions between *B.*
40 *subtilis* and bacterial competitors as illustrated for the bacillaene polyketide displaying an
41 essential protective role for survival in competition with *Streptomyces* soil isolates (Straight et
42 al., 2007; Barger et al., 2012). However, there are few direct evidences for enhanced expression
43 of BSMs upon interbacteria interactions. The only convincing examples involve the interaction

1 of plant-associated bacilli with plant pathogens such as *Ralstonia solanacearum* (Almoneafy et
2 al., 2014) and *Pseudomonas fuscovaginae* (Kakar et al., 2014). In these two studies, improved
3 expression of surfactin, bacilysin and iturin biosynthesis genes were observed when *Bacillus* and
4 pathogens were grown together in dual-cultures. Nevertheless, no clear indication about the
5 enhanced production of the aforementioned BSMs based on their quantification nor improved
6 antibacterial activities of *Bacillus* was presented as a result of this interaction.

7 Interestingly, at the phenotypical level, the development of soil bacilli is differentially altered
8 upon sensing other bacteria from the same natural environment. Some of these phenotypical
9 changes can be associated or due to a modulated production of specific BSMs. First, exogenous
10 antibiotics or signals may stimulate biofilm formation which depends, to some extent, on
11 surfactin production (López et al., 2009) and which may be viewed as a defensive response
12 against exogenous toxic compounds and/or infiltration by competitors (Flemming et al., 2016;
13 Townsley and Shank, 2017; Molina-Santiago et al., 2019). For instance, *B. subtilis* increased its
14 relative subpopulation of biofilm matrix-producing cells in response to small molecules secreted
15 by other bacterial species (López et al., 2009; Shank et al., 2011). The same phenomenon was
16 illustrated for thiazolyl peptides emitted by closely related species such as *B. cereus* and
17 putatively formed by other soil microbes such as *Streptomyces* isolates (Bleich et al., 2015).
18 However, no change in surfactin production associated with the stimulation of biofilm was
19 reported in these studies.

20 Besides biofilm formation, other mechanisms drive bacteria to initiate protective responses upon
21 the detection of competitors. The flagellum-independent sliding motility is considered as an
22 adaptive mechanism that allows bacterial cells to physically relocate in the context of a
23 competitive interaction (Wadhams and Armitage, 2004; Jones et al., 2017; McCully et al., 2019).
24 Upon sensing *S. venezuelae*, the *B. subtilis* ability to slide was increased (Liu et al., 2018). It
25 depends in part on the production of surfactin (Grau et al., 2015; van Gestel et al., 2015) but a
26 potential boost in lipopeptide synthesis upon the perception of the *Streptomyces* challenger was
27 not demonstrated. Chloramphenicol and derivatives produced by *S. venezuelae* were identified as
28 molecular triggers acting at subinhibitory concentrations for inducing *Bacillus* motility (Liu et
29 al., 2018).

30 Multiple bacteria promote sporulation in *B. subtilis* which represents another example of
31 alteration of the physiological development of this species. In a context of distant interactions,
32 exogenous siderophores accelerate the differentiation of *Bacillus* cells into spores. It was notably
33 shown for enterobactin from *E. coli* and for ferrioxamine E produced by *Streptomyces*
34 (Grandchamp et al., 2017). In iron-limited environments, *B. subtilis* cells would thus respond by
35 taking up those “piratable” siderophores and start sporulating. This is not a general response to
36 xenosiderophores since for instance, pyochelin from *Pseudomonas* does not affect *Bacillus*
37 sporulation (Molina-Santiago et al., 2019). Nevertheless, the ability of siderophores to alter
38 cellular differentiation in *B. subtilis* suggests that those molecules are likely to mediate complex
39 microbial interactions in iron-depleted conditions, as often met in a soil environment. However,
40 induction of *B. subtilis* sporulation by other bacteria may also occur in a cell-to-cell contact
41 situation. Upon interaction with *P. chlororaphis*, its type VI secretion system acted as a trigger
42 for sporulation, independently from its established role as cargo for delivering toxic effectors
43 into the target *Bacillus* cells (García-Bayona and Comstock, 2018; Molina-Santiago et al., 2019).

1 That said, interspecies interactions may also result in inhibition rather than in stimulation of key
2 developmental processes determining the fate of *Bacillus* multicellular communities. As an
3 example, 2,4-diacetylphloroglucinol, a broad-spectrum antibiotic synthesized by fluorescent
4 *Pseudomonas*, alters colony morphology, inhibits biofilm formation and sporulation in *B. subtilis*
5 populations grown adjacent to *P. protegens* colonies (Powers et al., 2015). This antibiotic seems
6 to act as an interspecific signaling molecule that inhibits bacterial differentiation at subinhibitory
7 concentrations (Powers et al., 2015).

8 **5 Conclusions**

9 Here we provide an overview of the phenotypic and molecular responses of plant-beneficial soil
10 bacilli upon sensing signals from other microorganisms that can be encountered in the
11 rhizosphere niche. It is clear that BSMs production by *Bacillus* can be modulated upon
12 interactions with other microbes and that key BSM-driven developmental processes may
13 undergo unsuspected changes. It somehow illustrates the flexibility of these bacteria in re-
14 directing their secondary metabolome to adapt environmental fitness upon sensing the presence
15 of neighboring microorganisms. Nevertheless, the molecular mechanisms integrating the
16 perception of exogenous triggers with a regulatory response leading to enhanced production of
17 BSMs still remain unclear.

18 A significant boost in BSMs production by soil bacilli has been reported in most cases as an
19 outcome from interactions with plant pathogenic fungi. This is of value in the context of
20 biocontrol of fungal pathogens since direct antagonism is considered as the most powerful mode
21 of action for suppression of plant diseases (Fravel, 2005; Frey-Klett et al., 2011; Köhl et al.,
22 2019). By contrast, direct evidence for an impact of interbacteria interactions on the expression
23 of the secondary metabolome in *Bacillus* is still globally missing. Nevertheless, interaction-
24 mediated variations in colony morphology, motility, biofilm formation, or sporulation illustrate
25 how soil bacilli can protect themselves from antimicrobials emitted by bacterial competitors.
26 Such an impact on those key developmental processes should thus be coupled with significant
27 modulation in the production of specific BSMs underpinning these phenotypes. Depending on
28 the concentration, these BSMs would then act as antimicrobials in interference competition or as
29 signals in cooperative interspecies communication processes not necessarily affecting the growth
30 of the partners (Bleich et al., 2015; Liu et al., 2018). However, this has yet to be thoroughly
31 demonstrated and future examination of developmental controls for BSMs biosynthesis will
32 likely bring light upon the key principles driving environmental fitness of soil bacilli as
33 intrinsically influenced by interspecies competition.

34 From an ecological viewpoint, further investigations would also help to better understand why
35 soil amendment with selected bacilli, even at high doses, do not durably impact the composition
36 of the rhizosphere microbiome despite their huge arsenal in antimicrobial weapons (Correa et al.,
37 2009; Chowdhury et al., 2013; Kröber et al., 2014; Qiao et al., 2017) and by contrast with some
38 other bacteria and fungi (Buddrus-Schiemann et al., 2010; Chowdhury et al., 2013; Erlacher et
39 al., 2014; Thomas et al., 2016; Wu et al. 2016). Those bacilli may thus provide protection to their
40 host plant toward microbial pathogen ingress but would avoid detrimental effect on its naturally
41 selected beneficial microbiome which is of prime interest for future application as biocontrol
42 agents.

1 **6 Conflict of Interest Statement**

2 The authors declare that the research was conducted in the absence of any commercial or
3 financial relationships that could be construed as a potential conflict of interest.

4 **7 Author Contributions Statement**

5 SA, TM and MO conceived the idea and designed the outlines of the review. SA, TM and MO
6 wrote the manuscript. All authors listed have made a substantial, direct and intellectual
7 contribution to the work, and approved it for publication.

8 **8 Funding**

9 Research in the laboratory is supported by the Interreg FWVL V portfolio project
10 SmartBiocontrol, and by the Excellence Of Science grant 30650620 (F.R.S.-FNRS (Fonds
11 National de la Recherche Scientifique).

12 **9 Acknowledgments**

13 We thank A. Argüelles-Arias, G. Hoff and A. Rigolet for reading the manuscript and for their
14 helpful suggestions.

15 **10 References**

16 Abriouel, H., Franz, C. M. A. P., Omar, N. Ben, and Gálvez, A. (2011). Diversity and
17 applications of *Bacillus* bacteriocins. *FEMS Microbiol. Rev.* 35, 201–232. doi:10.1111/j.1574-
18 6976.2010.00244.x.

19 Acedo, J. Z., Chiorean, S., Vederas, J. C., and van Belkum, M. J. (2018). The expanding
20 structural variety among bacteriocins from Gram-positive bacteria. *FEMS Microbiol. Rev.* 42,
21 805–828. doi:10.1093/femsre/fuy033.

22 Almoneafy, A. A., Kakar, K. U., Nawaz, Z., Li, B., Saand, M. A., Chun-lan, Y., et al. (2014).
23 Tomato plant growth promotion and antibacterial related-mechanisms of four rhizobacterial
24 *Bacillus* strains against *Ralstonia solanacearum*. *Symbiosis* 63, 59–70. doi:10.1007/s13199-014-
25 0288-9.

26 Arguelles Arias, A., Ongena, M., Devreese, B., Terrak, M., Joris, B., and Fickers, P. (2013).
27 Characterization of amylolysin, a novel lantibiotic from *Bacillus amyloliquefaciens* GA1. *PLoS*
28 *One* 8, e83037. doi:10.1371/journal.pone.0083037.

29 Bacon, C. W., Hinton, D. M., and Hinton, A. (2006). Growth-inhibiting effects of concentrations
30 of fusaric acid on the growth of *Bacillus mojavensis* and other biocontrol *Bacillus* species. *J.*
31 *Appl. Microbiol.* 100, 185–194. doi:10.1111/j.1365-2672.2005.02770.x.

32 Bacon, C. W., Hinton, D. M., Porter, J. K., Glenn, A. E., and Kuldau, G. (2004). Fusaric acid, a
33 *Fusarium verticillioides* metabolite, antagonistic to the endophytic biocontrol bacterium *Bacillus*
34 *mojavensis*. *Can. J. Bot.* 82, 878–885. doi:10.1139/B04-067.

- 1 Bani, M., Rispaill, N., Evidente, A., Rubiales, D., and Cimmino, A. (2014). Identification of the
2 main toxins isolated from *Fusarium oxysporum* f. sp. *pisi* race 2 and their relation with isolates'
3 pathogenicity. *J. Agric. Food Chem.* 62, 2574–2580. doi:10.1021/jf405530g.
- 4 Barger, S. R., Hoefler, B. C., Cubillos-Ruiz, A., Russell, W. K., Russell, D. H., and Straight, P.
5 D. (2012). Imaging secondary metabolism of *Streptomyces* sp. Mg1 during cellular lysis and
6 colony degradation of competing *Bacillus subtilis*. *Antonie van Leeuwenhoek, Int. J. Gen. Mol.*
7 *Microbiol.* 102, 435–445. doi:10.1007/s10482-012-9769-0.
- 8 Bartolini, M., Cogliati, S., Vileta, D., Bauman, C., Ramirez, W., and Grau, R. (2019). Stress
9 responsive alternative sigma factor SigB plays a positive role in the antifungal proficiency of
10 *Bacillus subtilis*. *Appl. Environ. Microbiol.* 85, 1–15. doi:10.1128/AEM.00178-19.
- 11 Bleich, R., Watrous, J. D., Dorrestein, P. C., Bowers, A. A., and Shank, E. A. (2015).
12 Thiopeptide antibiotics stimulate biofilm formation in *Bacillus subtilis*. *Proc. Natl. Acad. Sci. U.*
13 *S. A.* 112, 3086–3091. doi:10.1073/pnas.1414272112.
- 14 Borriss, R. (2015). “*Bacillus*, A Plant-Beneficial Bacterium,” in *Principles of Plant-Microbe*
15 *Interactions* (Cham: Springer International Publishing), 379–391. doi:10.1007/978-3-319-08575-
16 3_40.
- 17 Bozhüyük, K. A., Micklefield, J., and Wilkinson, B. (2019). Engineering enzymatic assembly
18 lines to produce new antibiotics. *Curr. Opin. Microbiol.* 51, 88–96.
19 doi:10.1016/j.mib.2019.10.007.
- 20 Brötz, H., Bierbaum, G., Leopold, K., Reynolds, P. E., and Sahl, H. G. (1998). The lantibiotic
21 mersacidin inhibits peptidoglycan synthesis by targeting lipid II. *Antimicrob. Agents Chemother.*
22 42, 154–60. doi:10.1128/AAC.42.1.154.
- 23 Buddrus-Schiemann, K., Schmid, M., Schreiner, K., Welzl, G., Hartmann, A. (2010). Root
24 colonization by *Pseudomonas* sp. DSMZ 13134 and impact on the indigenous rhizosphere
25 bacterial community of barley. *Microb. Ecol.* 60, 381–393. doi:10.1007/s00248-010-9720-8.
- 26 Caulier, S., Nannan, C., Gillis, A., Licciardi, F., Bragard, C., and Mahillon, J. (2019). Overview
27 of the antimicrobial compounds produced by members of the *Bacillus subtilis* group. *Front.*
28 *Microbiol.* 10, 1–19. doi:10.3389/fmicb.2019.00302.
- 29 Cawoy, H., Debois, D., Franzil, L., De Pauw, E., Thonart, P., and Ongena, M. (2015).
30 Lipopeptides as main ingredients for inhibition of fungal phytopathogens by *Bacillus*
31 *subtilis/amyloliquefaciens*. *Microb. Biotechnol.* 8, 281–295. doi:10.1111/1751-7915.12238.
- 32 Chen, Y., Gozzi, K., Yan, F., and Chai, Y. (2015). Acetic acid acts as a volatile signal to
33 stimulate bacterial biofilm formation. *MBio.* 6:e00392. doi: 10.1128/mBio.00392-15
34 Chitarra, G. S., Breeuwer, P., Nout, M. J. R., van Aelst, A. C., Rombouts, F. M., and Abee, T. (2003). An
35 antifungal compound produced by *Bacillus subtilis* YM 10-20 inhibits germination of
36 *Penicillium roqueforti* conidiospores. *J. Appl. Microbiol.* 94, 159–166. doi:10.1046/j.1365-
37 2672.2003.01819.x.

- 1 Chowdhury, S. P., Dietel, K., Rändler, M., Schmid, M., Junge, H., Borriss, R., et al. (2013).
2 Effects of *Bacillus amyloliquefaciens* FZB42 on lettuce growth and health under pathogen
3 pressure and its impact on the rhizosphere bacterial community. *PLoS ONE*. 8:e68818. doi:
4 10.1371/journal.pone.0068818.
- 5 Chowdhury, S. P., Hartmann, A., Gao, X. W., and Borriss, R. (2015a). Biocontrol mechanism by
6 root-associated *Bacillus amyloliquefaciens* FZB42 - A review. *Front. Microbiol.* 6.
7 doi:10.3389/fmicb.2015.00780.
- 8 Chowdhury, S. P., Uhl, J., Grosch, R., Alquéres, S., Pittroff, S., Dietel, K., et al. (2015b). Cyclic
9 lipopeptides of *Bacillus amyloliquefaciens* subsp. *plantarum* colonizing the lettuce rhizosphere
10 enhance plant defense responses toward the bottom rot pathogen *Rhizoctonia solani*. *Mol. Plant-
11 Microbe Interact.* 28, 984–995. doi:10.1094/mpmi-03-15-0066-r.
- 12 Correa, O. S., Montecchia, M. S., Berti, M. F., Ferrari, M. C. F., Pucheu, N. L., Kerber, N. L., et
13 al. (2009). *Bacillus amyloliquefaciens* BNM122, a potential microbial biocontrol agent applied
14 on soybean seeds, causes a minor impact on rhizosphere and soil microbial communities. *Appl.
15 Soil Ecol.* 41, 185–194. doi: 10.1016/j.apsoil.2008.10.007.
- 16 DeFilippi, S., Groulx, E., Megalla, M., Mohamed, R., and Avis, T. J. (2018). Fungal competitors
17 affect production of antimicrobial lipopeptides in *Bacillus subtilis* Strain B9–5. *J. Chem. Ecol.*
18 44, 374–383. doi:10.1007/s10886-018-0938-0.
- 19 Deleu, M., Paquot, M., and Nylander, T. (2008). Effect of fengycin, a lipopeptide produced by
20 *Bacillus subtilis*, on model biomembranes. *Biophys. J.* 94, 2667–79.
21 doi:10.1529/biophysj.107.114090.
- 22 Dutta, S., Whicher, J. R., Hansen, D. A., Hale, W. A., Chemler, J. A., Congdon, G. R., et al.
23 (2014). Structure of a modular polyketide synthase. *Nature* 510, 512–517.
24 doi:10.1038/nature13423.
- 25 Erlacher, A., Cardinale, C., Grosch, R., Grube, M., and Berg, G. (2014). The impact of the
26 pathogen *Rhizoctonia solani* and its beneficial counterpart *Bacillus amyloliquefaciens* on the
27 indigenous lettuce microbiome. *Front. Microbiol.* 5:175. doi:10.3389/fmicb.2014.00175.
- 28 Etchegaray, A., de Castro Bueno, C., de Melo, I. S., Tsai, S. M., de Fátima Fiore, M., Silva-
29 Stenico, M. E., et al. (2008). Effect of a highly concentrated lipopeptide extract of *Bacillus*
30 *subtilis* on fungal and bacterial cells. *Arch. Microbiol.* 190, 611–622. doi:10.1007/s00203-008-
31 0409-z.
- 32 Expósito, R. G., de Bruijn, I., Postma, J., and Raaijmakers, J. M. (2017). Current insights into the
33 role of rhizosphere bacteria in disease suppressive soils. *Front. Microbiol.* 8, 1–12.
34 doi:10.3389/fmicb.2017.02529.
- 35 Fan, B., Blom, J., Klenk, H. P., and Borriss, R. (2017). *Bacillus amyloliquefaciens*, *Bacillus*
36 *velezensis*, and *Bacillus siamensis* form an “Operational Group *B. amyloliquefaciens*” within the
37 *B. subtilis* species complex. *Front. Microbiol.* 8, 22. doi:10.3389/fmicb.2017.00022.

- 1 Fan, B., Wang, C., Song, X., Ding, X., Wu, L., Wu, H., et al. (2018). *Bacillus velezensis* FZB42
2 in 2018: the Gram-positive model strain for plant growth promotion and biocontrol. *Front.*
3 *Microbiol.* 9, 1–14. doi:10.3389/fmicb.2018.02491.
- 4 Fiedler, S., and Heerklotz, H. (2015). Vesicle leakage reflects the target selectivity of
5 antimicrobial lipopeptides from *Bacillus subtilis*. *Biophys. J.* 109, 2079–2089.
6 doi:10.1016/j.bpj.2015.09.021.
- 7 Fierer, N. (2017). Embracing the unknown: disentangling the complexities of the soil
8 microbiome. *Nat. Rev. Microbiol.* 15, 579–590. doi:10.1038/nrmicro.2017.87.
- 9 Finkel, O. M., Castrillo, G., Herrera Paredes, S., Salas González, I., and Dangl, J. L. (2017).
10 Understanding and exploiting plant beneficial microbes. *Curr. Opin. Plant Biol.* 38, 155–163.
11 doi:10.1016/j.pbi.2017.04.018.
- 12 Fira, D., Dimkić, I., Berić, T., Lozo, J., and Stanković, S. (2018). Biological control of plant
13 pathogens by *Bacillus* species. *J. Biotechnol.* 285, 44–55. doi:10.1016/j.jbiotec.2018.07.044.
- 14 Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., and Kjelleberg, S.
15 (2016). Biofilms: An emergent form of bacterial life. *Nat. Rev. Microbiol.* 14, 563–575.
16 doi:10.1038/nrmicro.2016.94.
- 17 Fravel, D. R. (2005). Commercialization and implementation of biocontrol. *Annu. Rev.*
18 *Phytopathol.* 43, 337–359. doi:10.1146/annurev.phyto.43.032904.092924.
- 19 Frey-Klett, P., Burlinson, P., Deveau, A., Barret, M., Tarkka, M., and Sarniguet, A. (2011).
20 Bacterial-Fungal interactions: Hyphens between agricultural, clinical, environmental, and food
21 microbiologists. *Microbiol. Mol. Biol. Rev.* 75, 583–609. doi:10.1128/mmbr.00020-11.
- 22 Gao, L., Han, J., Liu, H., Qu, X., Lu, Z., and Bie, X. (2017). Plipastatin and surfactin
23 coproduction by *Bacillus subtilis* pB2-L and their effects on microorganisms. *Antonie Van*
24 *Leeuwenhoek* 110, 1007–1018. doi:10.1007/s10482-017-0874-y.
- 25 García-Bayona, L., and Comstock, L. E. (2018). Bacterial antagonism in host-associated
26 microbial communities. *Science (80-)*. 361. doi:10.1126/science.aat2456.
- 27 García-Gutiérrez, L., Zerrouh, H., Romero, D., Cubero, J., de Vicente, A., and Pérez-García, A.
28 (2013). The antagonistic strain *Bacillus subtilis* UMAF6639 also confers protection to melon
29 plants against cucurbit powdery mildew by activation of jasmonate- and salicylic acid-dependent
30 defence responses. *Microb. Biotechnol.* 6, 264–274. doi:10.1111/1751-7915.12028.
- 31 Geudens, N., and Martins, J. C. (2018). Cyclic lipodepsipeptides from *Pseudomonas* spp. –
32 Biological swiss-army knives. *Front. Microbiol.* 9, 1867. doi:10.3389/fmicb.2018.01867.
- 33 Gong, A.-D., Li, H.-P., Yuan, Q.-S., Song, X.-S., Yao, W., He, W.-J., et al. (2015). Antagonistic
34 mechanism of iturin A and plipastatin A from *Bacillus amyloliquefaciens* S76-3 from wheat
35 spikes against *Fusarium graminearum*. *PLoS One* 10, e0116871.
36 doi:10.1371/journal.pone.0116871.

- 1 Götze, S., and Stallforth, P. (2020). Structure, properties, and biological functions of
2 nonribosomal lipopeptides from pseudomonads. *Nat. Prod. Rep.* 37, 29–54.
3 doi:10.1039/C9NP00022D.
- 4 Grandchamp, G. M., Caro, L., and Shank, E. A. (2017). Pirated siderophores promote
5 sporulation in *Bacillus subtilis*. *Appl. Environ. Microbiol.* 83, 1–17. doi:10.1128/AEM.03293-16.
- 6 Grau, R. R., De Oña, P., Kunert, M., Leñini, C., Gallegos-Monterrosa, R., Mhatre, E., et al.
7 (2015). A duo of potassium-responsive histidine kinases govern the multicellular destiny of
8 *Bacillus subtilis*. *MBio* 6, 1–16. doi:10.1128/mBio.00581-15.
- 9 Harwood, C. R., Mouillon, J.-M., Pohl, S., and Arnau, J. (2018). Secondary metabolite
10 production and the safety of industrially important members of the *Bacillus subtilis* group. *FEMS*
11 *Microbiol. Rev.* 42, 721–738. doi:10.1093/femsre/fuy028.
- 12 Henry, G., Deleu, M., Jourdan, E., Thonart, P., and Ongena, M. (2011). The bacterial lipopeptide
13 surfactin targets the lipid fraction of the plant plasma membrane to trigger immune-related
14 defence responses. *Cell. Microbiol.* 13, 1824–1837. doi:10.1111/j.1462-5822.2011.01664.x.
- 15 Inaoka, T., Wang, G., and Ochi, K. (2009). ScoC regulates bacilysin production at the
16 transcription level in *Bacillus subtilis*. *J. Bacteriol.* 191, 7367–7371. doi:10.1128/JB.01081-09.
- 17 Jones, S. E., Ho, L., Rees, C. A., Hill, J. E., Nodwell, J. R., and Elliot, M. A. (2017).
18 *Streptomyces* exploration is triggered by fungal interactions and volatile signals. *Elife* 6.
19 doi:10.7554/eLife.21738.
- 20 Kai, M. (2020). Diversity and distribution of volatile secondary metabolites throughout *Bacillus*
21 *subtilis* isolates. *Front. Microbiol.* 11. doi:10.3389/fmicb.2020.00559.
- 22 Kakar, K. U., Duan, Y. P., Nawaz, Z., Sun, G., Almoneafy, A. A., Hassan, M. A., et al. (2014).
23 A novel rhizobacterium Bk7 for biological control of brown sheath rot of rice caused by
24 *Pseudomonas fuscovaginae* and its mode of action. *Eur. J. Plant Pathol.* 138, 819–834.
25 doi:10.1007/s10658-013-0356-7.
- 26 Kaspar, F., Neubauer, P., and Gimpel, M. (2019). Bioactive secondary metabolites from *Bacillus*
27 *subtilis*: A comprehensive review. *J. Nat. Prod.* 82, 2038–2053.
28 doi:10.1021/acs.jnatprod.9b00110.
- 29 Kearns, D. B., Chu, F., Branda, S. S., Kolter, R., and Losick, R. (2005). A master regulator for
30 biofilm formation by *Bacillus subtilis*. *Mol. Microbiol.* 55, 739–749. doi:10.1111/j.1365-
31 2958.2004.04440.x.
- 32 Khezri, M., Jouzani, G. S., and Ahmadzadeh, M. (2016). *Fusarium culmorum* affects expression
33 of biofilm formation key genes in *Bacillus subtilis*. *Brazilian J. Microbiol.* 47, 47–54.
34 doi:10.1016/j.bjm.2015.11.019.

- 1 Köhl, J., Kolnaar, R., and Ravensberg, W. J. (2019). Mode of action of microbial biological
2 control agents against plant diseases: Relevance beyond efficacy. *Front. Plant Sci.* 10, 1–19.
3 doi:10.3389/fpls.2019.00845.
- 4 Kraas, F. I., Helmetag, V., Wittmann, M., Strieker, M., and Marahiel, M. A. (2010). Functional
5 dissection of surfactin synthetase initiation module reveals insights into the mechanism of
6 lipoinitiation. *Chem. Biol.* 17, 872–880. doi:10.1016/j.chembiol.2010.06.015.
- 7 Kröber, M., Wibberg, D., Grosch, R., Eikmeyer, F., Verwaaijen, B., Chowdhury, S. P., et al.
8 (2014). Effect of the strain *Bacillus amyloliquefaciens* FZB42 on the microbial community in the
9 rhizosphere of lettuce under field conditions analyzed by whole metagenome sequencing. *Front.*
10 *Microbiol.* 5:252. doi:10.3389/fmicb.2014.00252.
- 11 Kulimushi, P. Z., Arias, A. A., Franzil, L., Steels, S., and Ongena, M. (2017). Stimulation of
12 fengycin-type antifungal lipopeptides in *Bacillus amyloliquefaciens* in the presence of the maize
13 fungal pathogen *Rhizomucor variabilis*. *Front. Microbiol.* 8, 1–12.
14 doi:10.3389/fmicb.2017.00850.
- 15 Li, B., Li, Q., Xu, Z., Zhang, N., Shen, Q., and Zhang, R. (2014). Responses of beneficial
16 *Bacillus amyloliquefaciens* SQR9 to different soilborne fungal pathogens through the alteration
17 of antifungal compounds production. *Front. Microbiol.* 5, 636. doi:10.3389/fmicb.2014.00636.
- 18 Liu, Y., Kyle, S., and Straight, P. D. (2018). Antibiotic stimulation of a *Bacillus subtilis*
19 migratory response. *mSphere* 3, 1–13. doi:10.1128/mSphere.00586-17.
- 20 Liu, Y., Zhang, N., Qiu, M., Feng, H., Vivanco, J. M., Shen, Q., et al. (2014). Enhanced
21 rhizosphere colonization of beneficial *Bacillus amyloliquefaciens* SQR9 by pathogen infection.
22 *FEMS Microbiol. Lett.* 353, 49–56. doi:10.1111/1574-6968.12406.
- 23 López, D., Vlamakis, H., Losick, R., and Kolter, R. (2009). Cannibalism enhances biofilm
24 development in *Bacillus subtilis*. *Mol. Microbiol.* 74, 609–618. doi:10.1111/j.1365-
25 2958.2009.06882.x.
- 26 Maksimov, I. V., Singh, B. P., Cherepanova, E. A., Burkhanova, G. F., and Khairullin, R. M.
27 (2020). Prospects and applications of lipopeptide-producing bacteria for plant protection
28 (Review). *Appl. Biochem. Microbiol.* 56, 15–28. doi:10.1134/S0003683820010135.
- 29 Martínez-Cámara, R., Montejano-Ramírez, V., Moreno-Hagelsieb, G. et al. (2019). The volatile
30 organic compound dimethylhexadecylamine affects bacterial growth and swarming motility of
31 bacteria. *Folia Microbiol. (Praha)*. doi:10.1007/s12223-019-00756-6.
- 32 McCully, L. M., Bitzer, A. S., Seaton, S. C., Smith, L. M., and Silby, M. W. (2019). Interspecies
33 Social Spreading: Interaction between two sessile soil bacteria leads to emergence of surface
34 motility. *mSphere* 4, 1–16. doi:10.1128/msphere.00696-18.
- 35 Mendes, R., Garbeva, P., and Raaijmakers, J. M. (2013). The rhizosphere microbiome:
36 significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS*
37 *Microbiol. Rev.* 37, 634–663. doi:10.1111/1574-6976.12028.

- 1 Miethke, M., Klotz, O., Linne, U., May, J. J., Beckering, C. L., and Marahiel, M. A. (2006).
2 Ferri-bacillibactin uptake and hydrolysis in *Bacillus subtilis*. *Mol. Microbiol.* 61, 1413–1427.
3 doi:10.1111/j.1365-2958.2006.05321.x.
- 4 Miethke, M., Schmidt, S., and Marahiel, M. A. (2008). The Major Facilitator Superfamily-Type
5 Transporter YmfE and the Multidrug-Efflux Activator Mta Mediate Bacillibactin Secretion in
6 *Bacillus subtilis*. *J. Bacteriol.* 190, 5143–5152. doi:10.1128/JB.00464-08.
- 7 Molinatto, G., Puopolo, G., Sonego, P., Moretto, M., Engelen, K., Viti, C., et al. (2016).
8 Complete genome sequence of *Bacillus amyloliquefaciens* subsp. *plantarum* S499, a
9 rhizobacterium that triggers plant defences and inhibits fungal phytopathogens. *J. Biotechnol.*
10 238, 56–59. doi:10.1016/j.jbiotec.2016.09.013.
- 11 Molina-Santiago, C., Pearson, J. R., Navarro, Y., Berlanga-Clavero, M. V., Caraballo-Rodriguez,
12 A. M., Petras, D., et al. (2019). The extracellular matrix protects *Bacillus subtilis* colonies from
13 *Pseudomonas* invasion and modulates plant co-colonization. *Nat. Commun.* 10.
14 doi:10.1038/s41467-019-09944-x.
- 15 Müller, D. B., Vogel, C., Bai, Y., and Vorholt, J. A. (2016). The plant microbiota: Systems-level
16 insights and perspectives. *Annu. Rev. Genet.* 50, 211–234. doi:10.1146/annurev-genet-120215-
17 034952.
- 18 Nayak, S. K., Nayak, S., and Patra, J. K. (2020). Rhizobacteria and its biofilm for sustainable
19 agriculture: A concise review. *New Futur. Dev. Microb. Biotechnol. Bioeng. Microb. Biofilms*,
20 165–175. doi:10.1016/B978-0-444-64279-0.00013-X.
- 21 Niehus, R., Picot, A., Oliveira, N. M., Mitri, S., and Foster, K. R. (2017). The evolution of
22 siderophore production as a competitive trait. *Evolution (N. Y.)*. 71, 1443–1455.
23 doi:10.1111/evo.13230.
- 24 Ola, A. R. B., Thomy, D., Lai, D., Brötz-Oesterhelt, H., and Proksch, P. (2013). Inducing
25 secondary metabolite production by the endophytic fungus *Fusarium tricinctum* through
26 coculture with *Bacillus subtilis*. *J. Nat. Prod.* 76, 2094–2099. doi:10.1021/np400589h.
- 27 Olishkevskaya, S., Nickzad, A., and Déziel, E. (2019). *Bacillus* and *Paenibacillus* secreted
28 polyketides and peptides involved in controlling human and plant pathogens. *Appl. Microbiol.*
29 *Biotechnol.* 103, 1189–1215. doi:10.1007/s00253-018-9541-0.
- 30 Ongena, M., and Jacques, P. (2008). *Bacillus* lipopeptides: versatile weapons for plant disease
31 biocontrol. *Trends Microbiol.* 16, 115–125. doi:10.1016/j.tim.2007.12.009.
- 32 Pandin, C., Darsonval, M., Mayeur, C., Le Coq, D., Aymerich, S., and Briandet, R. (2019).
33 Biofilm formation and synthesis of antimicrobial compounds by the biocontrol agent *Bacillus*
34 *velezensis* QST713 in an *Agaricus bisporus* compost micromodel. *Appl. Environ. Microbiol.* 85,
35 1–13. doi:10.1128/AEM.00327-19.

- 1 Pandin, C., Le Coq, D., Canette, A., Aymerich, S., and Briandet, R. (2017). Should the biofilm
2 mode of life be taken into consideration for microbial biocontrol agents? *Microb. Biotechnol.* 10,
3 719–734. doi:10.1111/1751-7915.12693.
- 4 Pérez-García, A., Romero, D., and de Vicente, A. (2011). Plant protection and growth
5 stimulation by microorganisms: biotechnological applications of Bacilli in agriculture. *Curr.*
6 *Opin. Biotechnol.* 22, 187–193. doi:10.1016/J.COPBIO.2010.12.003.
- 7 Piel, J. (2010). Biosynthesis of polyketides by trans-AT polyketide synthases. *Nat. Prod. Rep.*
8 27, 996–1047. doi:10.1039/b816430b.
- 9 Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M., and
10 Bakker, P. A. H. M. (2014). Induced systemic resistance by beneficial microbes. *Annu. Rev.*
11 *Phytopathol.* 52, 347–375. doi:10.1146/annurev-phyto-082712-102340.
- 12 Powers, M. J., Sanabria-Valentín, E., Bowers, A. A., and Shank, E. A. (2015). Inhibition of cell
13 differentiation in *Bacillus subtilis* by *Pseudomonas protegens*. *J. Bacteriol.* 197, 2129–2138.
14 doi:10.1128/JB.02535-14.
- 15 Qi, G., Zhu, F., Du, P., Yang, X., Qiu, D., Yu, Z., et al. (2010). Lipopeptide induces apoptosis in
16 fungal cells by a mitochondria-dependent pathway. *Peptides* 31, 1978–1986.
17 doi:10.1016/J.PEPTIDES.2010.08.003.
- 18 Qiao, J. Q., Yu, X., Liang, X. J., Liu, Y. F., Borriss, R., and Liu, Y. Z. (2017). Addition of plant-
19 growth-promoting *Bacillus subtilis* PTS-394 on tomato rhizosphere has no durable impact on
20 composition of root microbiome. *BMC Microbiol.* 17:131. doi: 10.1186/s12866-017-1039-x
- 21 Raaijmakers, J. M., de Bruijn, I., Nybroe, O., and Ongena, M. (2010). Natural functions of
22 lipopeptides from *Bacillus* and *Pseudomonas*: More than surfactants and antibiotics. *FEMS*
23 *Microbiol. Rev.* 34, 1037–1062. doi:10.1111/j.1574-6976.2010.00221.x.
- 24 Raaijmakers, J. M., and Mazzola, M. (2012). Diversity and natural functions of antibiotics
25 produced by beneficial and plant pathogenic bacteria. *Annu. Rev. Phytopathol.* 50, 403–424.
26 doi:10.1146/annurev-phyto-081211-172908.
- 27 Rabbee, M., Ali, M., Choi, J., Hwang, B., Jeong, S., Baek, K., et al. (2019). *Bacillus velezensis*:
28 A valuable member of bioactive molecules within plant microbiomes. *Molecules* 24, 1046.
29 doi:10.3390/molecules24061046.
- 30 Romero, D., de Vicente, A., Rakotoaly, R. H., Dufour, S. E., Veening, J.-W., Arrebola, E., et al.
31 (2007). The Iturin and Fengycin families of lipopeptides are key factors in antagonism of
32 *Bacillus subtilis* toward *Podosphaera fusca*. *Mol. Plant-Microbe Interact.* 20, 430–440.
33 doi:10.1094/MPMI-20-4-0430.
- 34 Santoyo, G., Hernández-Pacheco, C., Hernández-Salmerón, J., and Hernández-León, R. (2017).
35 The role of abiotic factors modulating the plant-microbe-soil interactions: toward sustainable
36 agriculture. A review. *Spanish J. Agric. Res.* 15, 1–15.

- 1 Schmidt, R., Cordovez, V., De Boer, W., Raaijmakers, J., and Garbeva, P. (2015). Volatile
2 affairs in microbial interactions. *ISME J.* 9, 2329–2335. doi:10.1038/ismej.2015.42.
- 3 Schmidt, R., Ulanova, D., Wick, L., Bode, H., and Garbeva, P. (2019). Microbe-driven chemical
4 ecology: past, present and future. *ISME J.* 13, 2656–2663. doi:10.1038/s41396-019-0469-x.
- 5 Scholz, R., Vater, J., Budiharjo, A., Wang, Z., He, Y., Dietel, K., et al. (2014). Amylocyclicin, a
6 novel circular bacteriocin produced by *Bacillus amyloliquefaciens* FZB42. *J. Bacteriol.* 196,
7 1842–52. doi:10.1128/JB.01474-14.
- 8 Shank, E. A., Klepac-Ceraj, V., Collado-Torres, L., Powers, G. E., Losick, R., and Kolter, R.
9 (2011). Interspecies interactions that result in *Bacillus subtilis* forming biofilms are mediated
10 mainly by members of its own genus. *Proc. Natl. Acad. Sci. U. S. A.* 108.
11 doi:10.1073/pnas.1103630108.
- 12 Straight, P. D., Fischbach, M. A., Walsh, C. T., Rudner, D. Z., and Kolter, R. (2007). A singular
13 enzymatic megacomplex from *Bacillus subtilis*. *Proc. Natl. Acad. Sci.* 104, 305–310.
14 doi:10.1073/pnas.0609073103.
- 15 Tahir, H.A.S., Gu, Q., Wu, H. et al. (2017). Effect of volatile compounds produced by *Ralstonia*
16 *solanacearum* on plant growth promoting and systemic resistance inducing potential of *Bacillus*
17 *volatiles*. *BMC Plant. Biol.* 17:133. doi:10.1186/s12870-017-1083-6.
- 18 Thomas, P., and Sekhar, A. C. (2016). Effects due to rhizospheric soil application of an
19 antagonistic bacterial endophyte on native bacterial community and its survival in soil: A case
20 study with *Pseudomonas aeruginosa* from banana. *Front. Microbiol.* 7:493.
21 doi:10.3389/fmicb.2016.00493.
- 22 Torres Manno, M. A., Pizarro, M. D., Prunello, M., Magni, C., Daurelio, L. D., and Espariz, M.
23 (2019). GeM-Pro: a tool for genome functional mining and microbial profiling. *Appl. Microbiol.*
24 *Biotechnol.* 103, 3123–3134. doi:10.1007/s00253-019-09648-8.
- 25 Townsley, L., and Shank, E. A. (2017). Natural-product antibiotics: Cues for modulating
26 bacterial biofilm formation. *Trends Microbiol.* 25, 1016–1026. doi:10.1016/j.tim.2017.06.003.
- 27 Traxler, M. F., and Kolter, R. (2015). Natural products in soil microbe interactions and
28 evolution. *Nat. Prod. Rep.* 32, 956–970. doi:10.1039/c5np00013k.
- 29 Tyc, O., Song, C., Dickschat, J. S., Vos, M., and Garbeva, P. (2017). The ecological role of
30 volatile and soluble secondary metabolites produced by soil bacteria. *Trends Microbiol.* 25, 280–
31 292. doi:10.1016/j.tim.2016.12.002.
- 32 van Gestel, J., Vlamakis, H., and Kolter, R. (2015). From cell differentiation to cell collectives:
33 *Bacillus subtilis* uses division of labor to migrate. *PLoS Biol.* 13, 1–29.
34 doi:10.1371/journal.pbio.1002141.

1 van Kuijk, S., Noll, K. S., and Chikindas, M. L. (2012). The species-specific mode of action of
2 the antimicrobial peptide subtilisin against *Listeria monocytogenes* Scott A. *Lett. Appl.*
3 *Microbiol.* 54, 52–58. doi:10.1111/j.1472-765X.2011.03170.x.

4 Vargas-Bautista, C., Rahlwes, K., and Straight, P. (2014). Bacterial competition reveals
5 differential regulation of the *pks* genes by *Bacillus subtilis*. *J. Bacteriol.* 196, 717–728.
6 doi:10.1128/JB.01022-13.

7 Wadhams, G. H., and Armitage, J. P. (2004). Making sense of it all: Bacterial chemotaxis. *Nat.*
8 *Rev. Mol. Cell Biol.* 5, 1024–1037. doi:10.1038/nrm1524.

9 Winn, M., Fyans, J. K., Zhuo, Y., and Micklefield, J. (2016). Recent advances in engineering
10 nonribosomal peptide assembly lines. *Nat. Prod. Rep.* 33, 317–347. doi:10.1039/c5np00099h.

11 Wise, C., Falardeau, J., Hagberg, I., and Avis, T. J. (2014). Cellular lipid composition affects
12 sensitivity of plant pathogens to Fengycin, an antifungal compound produced by *Bacillus subtilis*
13 strain CU12. *Phytopathology* 104, 1036–1041. doi:10.1094/PHYTO-12-13-0336-R.

14 Wu, B., Wang, X., Yang, L., Yang, H., Zeng, H., Qiu, Y. M., et al. (2016). Effects of *Bacillus*
15 *amyloliquefaciens* ZM9 on bacterial wilt and rhizosphere microbial communities of tobacco.
16 *Appl. Soil Ecol.* 103, 1–12. doi:10.1016/j.apsoil.2016.03.002.

17 Zhang, L., and Sun, C. (2018). Fengycins, cyclic lipopeptides from marine *Bacillus subtilis*
18 Strains, kill the plant-pathogenic fungus *Magnaporthe grisea* by inducing reactive oxygen
19 species production and chromatin condensation. *Appl. Environ. Microbiol.* 84.
20 doi:10.1128/AEM.00445-18.

21 Zhao, X., and Kuipers, O. P. (2016). Identification and classification of known and putative
22 antimicrobial compounds produced by a wide variety of Bacillales species. *BMC Genomics* 17,
23 1–18. doi:10.1186/s12864-016-3224-y.

24 Zhi, Y., Wu, Q., and Xu, Y. (2017). Genome and transcriptome analysis of surfactin biosynthesis
25 in *Bacillus amyloliquefaciens* MT45. *Sci. Rep.* 7, 1–13. doi:10.1038/srep40976.

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1 **11 Figures and legends**

2 **Figure 1:** Main non-ribosomal BSMs produced by the various species in the *B. subtilis* complex.

3 The BSMs production is indicated for the following species *B. subtilis*, *B. siamensis*, *B. velezensis*, *B. atrophaeus*, *B.*
4 *amyloliquefaciens*, *B. pumilus* and *B. licheniformis* by a green square whereas red square indicates an absence of
5 production of the BSMs in this species (Zhao and Kuipers, 2016; Fan et al., 2017; Harwood et al., 2018; Du et al.,
6 2019; Torres Manno et al., 2019). The surfactins, iturins and fengycins groups include lichenycin (1;AA1:L-Gln)
7 and pumilacidin (1;AA4: L-Leu, AA7=I-Ile); mycosubtilin (2;AA6: D-Ser, AA7=L-Thr) and bacillomycin (2; AA6:
8 D-Ser, AA7=L-Asn); maltacin (4;AA1: L-Ser), agrastatin (4;AA10: L-Val) and plipastatin (4;AA9: D-Tyr)
9 respectively. The structure of the representative metabolite is indicated by a number and represented below. The
10 possible variations in the PKs structure are highlighted in red. For the macrolactin family, the main variants are
11 R=H; CO-CH₂-COOH; CO-CH₂-CH₂-COOH or 6-*O*-succinyl-β-glucose (for review see Piel, 2010).

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1 Table 1. Change in expression and bioactivity of BSMs produced by members of *B. subtilis*
2 group, upon interaction with fungal species.
3 ‘‘0’’ indicates no changes, ‘‘+’’ enhanced and ‘‘-’’ decreased BSMs production by *Bacillus* upon interaction with
4 fungi. ‘‘Yes’’ indicates fungitoxic activity, ‘‘No’’ no antifungal activity, ‘‘ND’’ indicates that BSM with antifungal
5 activity are not detected.

BSMs	Change in expression	Involvement in antifungal activity	<i>Bacillus</i> species (strains)	Fungal species	References	
ngycins	0	Yes	<i>B. subtilis</i> (98S)	<i>B. cinerea</i>	Cawoy et al., 2014	
	+	Yes	<i>B. subtilis</i> (98S)	<i>F. oxysporum</i>	Cawoy et al., 2014	
	+	No	<i>B. subtilis</i> (98S)	<i>P. aphanidermatum</i>	Cawoy et al., 2014	
	+	Yes	<i>B. velezensis</i> (S499)	<i>R. variabilis</i>	Kulimushi et al., 2017	
	+	Yes	<i>B. velezensis</i> (FZB42)	<i>R. variabilis</i>	Kulimushi et al., 2017	
	0	Yes	<i>B. velezensis</i> (QST713)	<i>R. variabilis</i>	Kulimushi et al., 2017	
	+	Yes	<i>B. velezensis</i> (SQR9)	<i>Verticillium dahliae</i>	Li et al., 2014	
	+	Yes	<i>B. velezensis</i> (SQR9)	<i>F. oxysporum</i>	Li et al., 2014	
	+	Yes	<i>B. velezensis</i> (SQR9)	<i>Phytophthora parasitica</i> var. <i>nicotianae</i>	Li et al., 2014	
	-	mediating the plant defense expression		<i>B. velezensis</i> (FZB42)	<i>R. solani</i>	Chowdhury et al., 2015
	+	ND	<i>B. subtilis</i> (B9-5)	<i>R. stolonifer</i>	DeFilippi et al., 2018	
	+	ND	<i>B. subtilis</i> (B9-5)	<i>Fusarium sambucinum</i>	DeFilippi et al., 2018	
	+	ND	<i>B. subtilis</i> (B9-5)	<i>V. dahliae</i>	DeFilippi et al., 2018	
	+	ND	<i>B. velezensis</i> (QST713)	<i>Trichoderma aggressivum</i> f. <i>europaeum</i>	Pandin et al., 2019	
trins	0	Yes	<i>B. subtilis</i> (98S)	<i>B. cinerea</i>	Cawoy et al., 2014	
	+	Yes	<i>B. subtilis</i> (98S)	<i>F. oxysporum</i>	Cawoy et al., 2014	
	+	No	<i>B. subtilis</i> (98S)	<i>P. aphanidermatum</i>	Cawoy et al., 2014	
	+	No	<i>B. velezensis</i> (SQR9)	<i>V. dahliae</i>	Li et al., 2014	
	+	No	<i>B. velezensis</i> (SQR9)	<i>S. sclerotiorum</i>	Li et al., 2014	
	+	Yes	<i>B. velezensis</i> (SQR9)	<i>F. oxysporum</i>	Li et al., 2014	
	+	Yes	<i>B. velezensis</i> (SQR9)	<i>P. parasitica</i>	Li et al., 2014	
	+	mediating the plant defense expression		<i>B. velezensis</i> (FZB42)	<i>R. solani</i>	Chowdhury et al., 2015
rfactins	+	Yes	<i>B. velezensis</i> (SQR9)	<i>S. sclerotiorum</i>	Li et al., 2014	
	+	Yes	<i>B. velezensis</i> (SQR9)	<i>R. solani</i>	Li et al., 2014	
	+	Yes	<i>B. velezensis</i> (SQR9)	<i>Fusarium solani</i>	Li et al., 2014	
	+	Yes	<i>B. velezensis</i> (SQR9)	<i>P. parasitica</i>	Li et al., 2014	
	+	mediating the plant defense expression		<i>B. velezensis</i> (FZB42)	<i>R. solani</i>	Chowdhury et al., 2015
	+	ND	<i>B. subtilis</i> (B9-5)	<i>R. solani</i>	DeFilippi et al., 2018	
	+	ND	<i>B. subtilis</i> (B9-5)	<i>F. sambucinum</i>	DeFilippi et al., 2018	
	+	ND	<i>B. subtilis</i> (B9-5)	<i>V. dahliae</i>	DeFilippi et al., 2018	
	+	ND	<i>B. velezensis</i> (QST713)	<i>T. aggressivum</i> f. <i>europaeum</i>	Pandin et al., 2019	
icillibactin	+	Yes	<i>B. velezensis</i> (SQR9)	<i>V. dahliae</i>	Li et al., 2014	
	+	No	<i>B. velezensis</i> (SQR9)	<i>S. sclerotiorum</i>	Li et al., 2014	
	+	No	<i>B. velezensis</i> (SQR9)	<i>F. oxysporum</i>	Li et al., 2014	

+	Yes	<i>B. velezensis</i> (SQR9)	<i>R. solani</i>	Li et al., 2014
+	Yes	<i>B. velezensis</i> (SQR9)	<i>F. solani</i>	Li et al., 2014
+	Yes	<i>B. velezensis</i> (SQR9)	<i>P. parasitica</i>	Li et al., 2014

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