

CHARACTERIZATION OF A NEW *BACILLUS NAKAMURAI* SOIL ISOLATE WITH STRONG ANTIMICROBIAL AND BIOCONTROL POTENTIAL

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PRESSURE OF DISEASES AND PESTS ON AGRICULTURE WORLDWIDE AND BURUNDI



Tomatoes plantation infested by *Alternaria* sp. (Nimbeshaho, 2020)
Production losses: 35–78%



Maize plantation infested by *Helminthosporium* sp.
<https://images.app.goo.gl/uwkDQSX1Kj3jkmP9>



Banana Xanthomonas wilt (Hashim & Mabagala, 2013)
Production losses : up to 100%

- Managed inefficiently by local methods or conventional pesticides
- Hazardous effects of pesticides on environment and concern of pesticide resistance

BACILLUS BASED BIOPESTICIDES AS ALTERNATIVES TO PESTICIDES

- Biopesticides as good alternatives to conventional pesticides are lacking in many developing countries including Burundi
- *Bacillus* based biopesticides are leaders on the market of biocontrol agents and have proven their efficacy
- As insecticide: eg. *B. thuringiensis*
- as fungicide/bactericide/ISR trigger. eg. *B. velezensis* QST713 (SERENADE® ASO) and *B. velezensis* FZB42 (RHIZOVITAL®42)



10l bottle of *B. velezensis* QST713 suspension



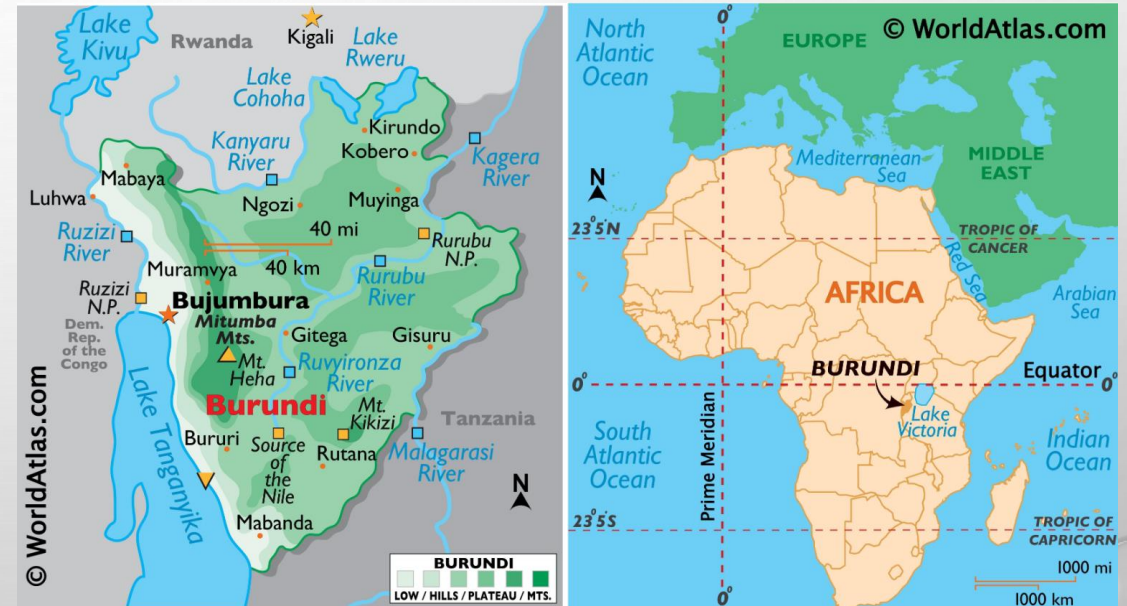
B. thuringiensis spores
https://maluttebio.com/181-home_default/traitement-pyrale-du-buis-bacillus-thuringiensis.jpg

OBJECTIVES

- Implementing the use of microorganisms (*Bacillus* genus particularly) as biocontrol agents in Burundian agricultural context (86% of households with < 0.5 ha)
- Valorize Burundi local microbial biodiversity by prospecting for novel strains with high potential as biocontrol agent



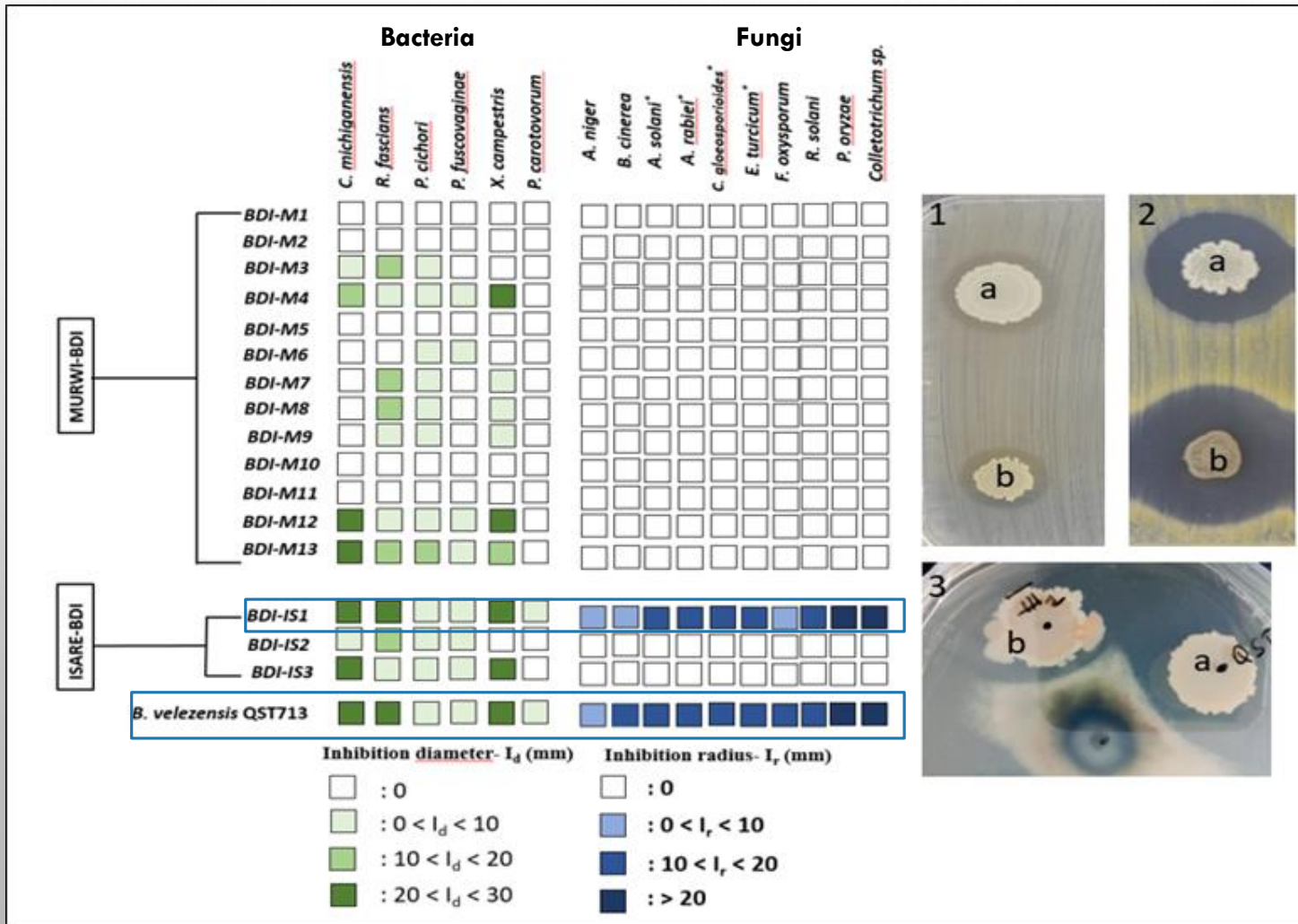
Agricultural context-Small farming lands
(adapted from <https://aadmi.com/burundi/>)



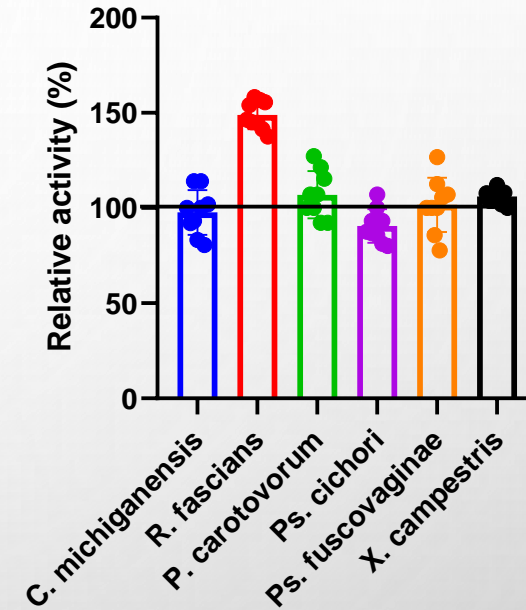
Burundi map and its location in Africa
(<https://www.worldatlas.com/maps/burundi>)

ANTIMICROBIAL ACTIVITIES OF BACTERIAL ISOLATES

- Sampling arable soils from different agroecological niches
- Isolating *Bacillus* resembling colonies



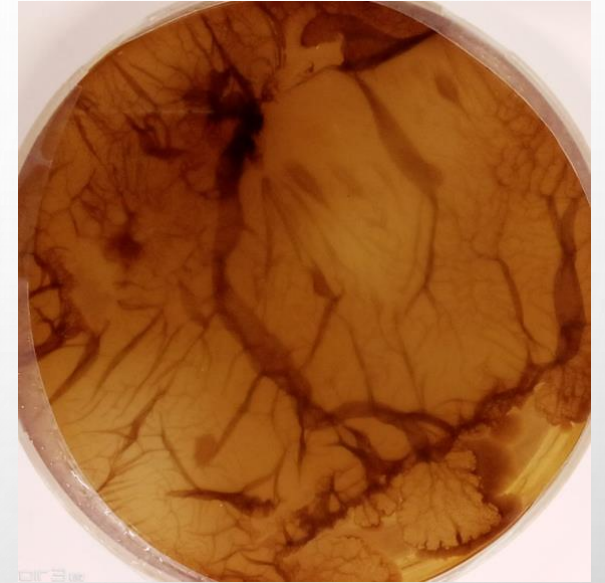
Antibacterial activity



- Isolate BDI-IS1 and *B. velezensis* QST713 are active at comparable level in general (90%-140%)

IDENTIFICATION OF THE PROMISING ISOLATE BDI-IS1

- DNA genome of the isolate BDI-IS1 was sequenced (NCBI acc. number **JAJJBV0000000000**)
- Identified as a strain of the rare species, *Bacillus nakamurai* (first described in 2016)
- It belongs to the *Bacillus amyloliquefaciens* group together with *Bacillus velezensis*
- only 2 strains (NRRL B-41091 and NRRL B-41092) were sequenced so far
- Phenotypical identification: **Black-brown pigment** on Tryptic Soy Agar (TSA), peculiar to *B. nakamurai* strains



Black-brown pigment produced by BDI-IS1 on TSA

***B. nakamurai* BDI-IS1, a house of biosynthetic gene clusters (BGCS) for antimicrobial biactive secondary metabolites (BSMs)**

- Genome mining using antismash 6.01

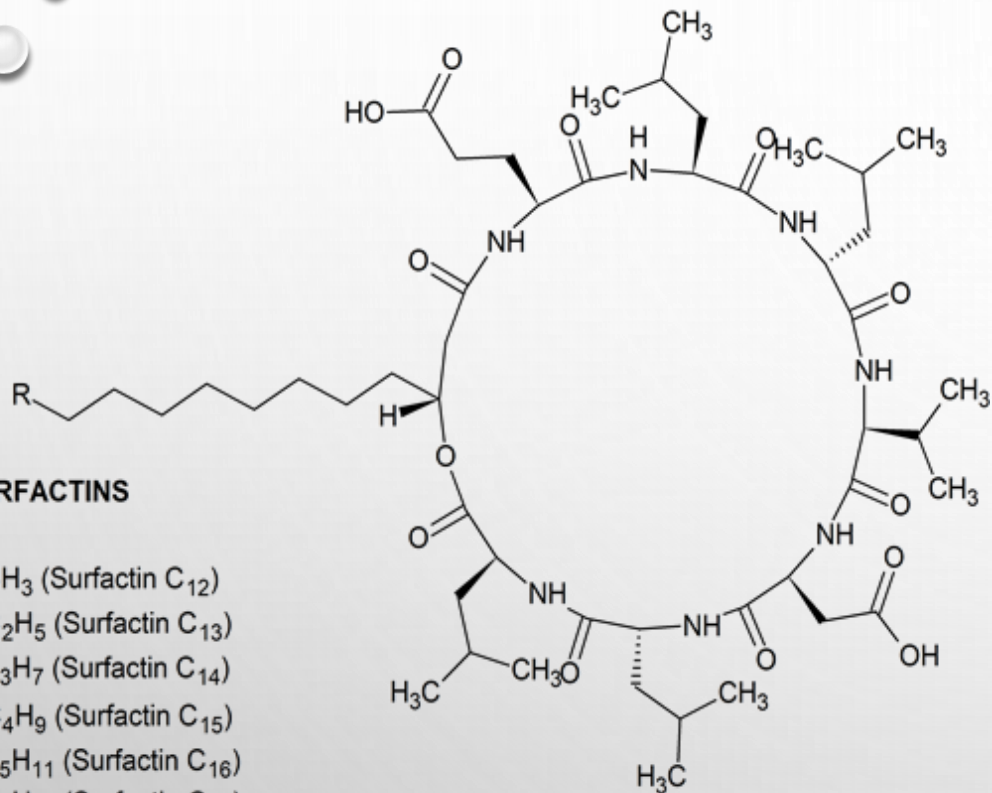
N°	PREDICTED BSM	PERCENTAGE OF SIMILARITY	NATURE OF THE COMPOUND
1	Surfactin	86%	Cyclic lipopeptide (NRPS)
2	Iturin A	44%	Cyclic lipopeptide (NRPS)
3	Bacilysin	100%	Dipeptide
4	Amylocyclicin	100%	Lanthipeptide
5	Bacillibactin	69%	Siderophore (NRPS)
6	Bacillaene	100%	Polyketide (NRPS-PkS)
7	Plantazolicin	100%	Lanthipeptide

- Known BSMs produced by *Bacillus* spp. are predicted: cyclic lipopeptides, polyketide and lanthipeptide, siderophore and dipeptide
- But an unusual compound, plantazolicin (described only in *B. velezensis* FZB42)

IDENTIFICATION OF BSMs BY UPLC-qTOF-MS

SURFACTINS

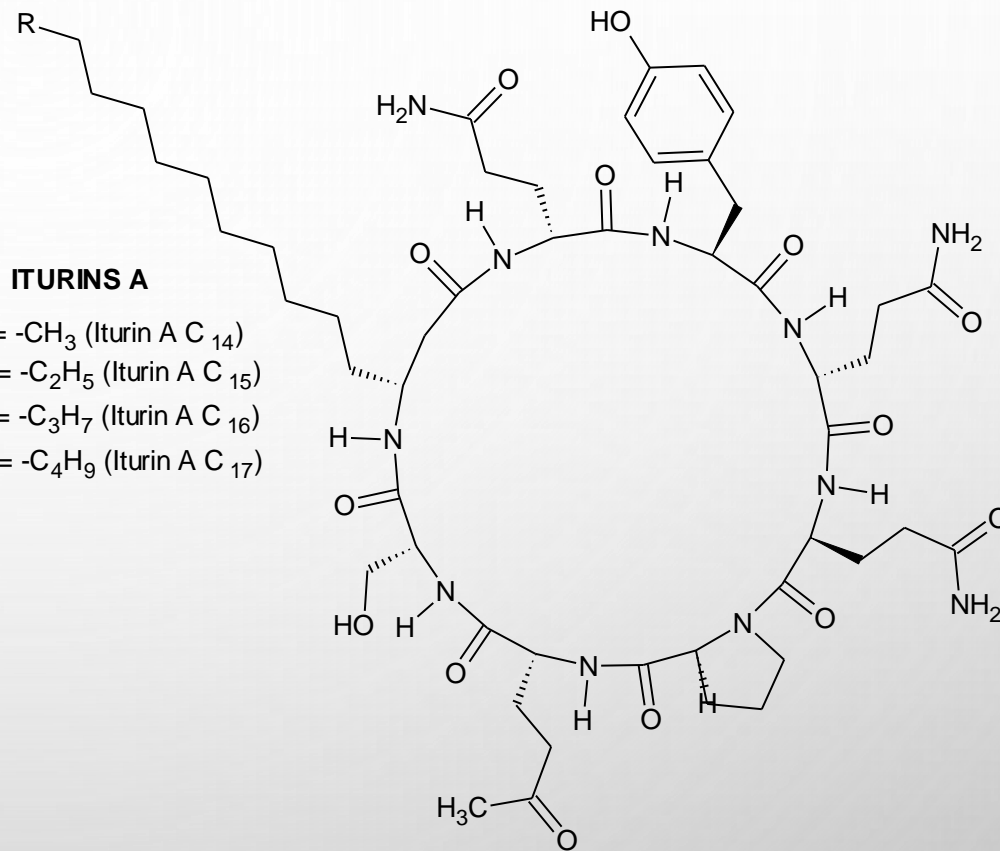
- R = CH₃ (Surfactin C₁₂)
- R = C₂H₅ (Surfactin C₁₃)
- R = C₃H₇ (Surfactin C₁₄)
- R = C₄H₉ (Surfactin C₁₅)
- R = C₅H₁₁ (Surfactin C₁₆)
- R = C₆H₁₁ (Surfactin C₁₇)



- Antibacterial activity at high concentration, but no antifungal activity in general.
- Involved in the mediation of Induced Systemic Resistance (ISR) in plants

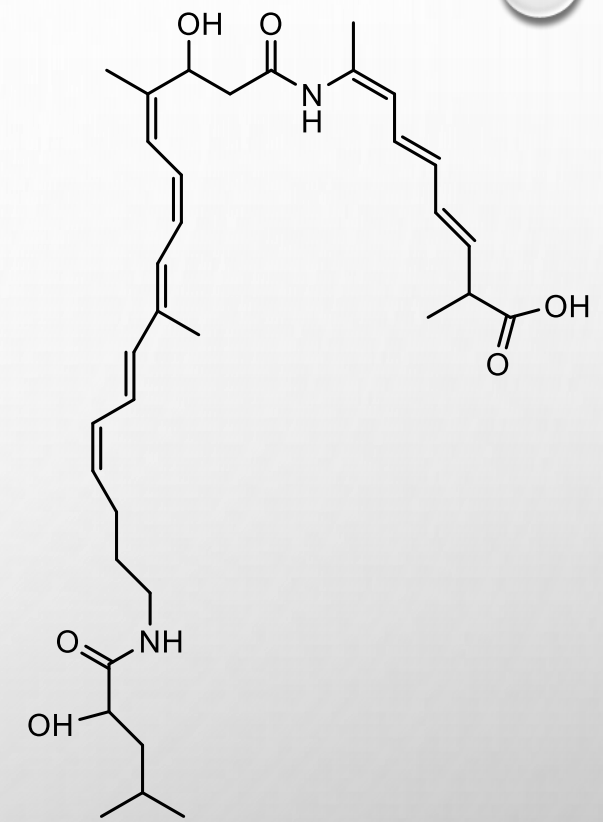
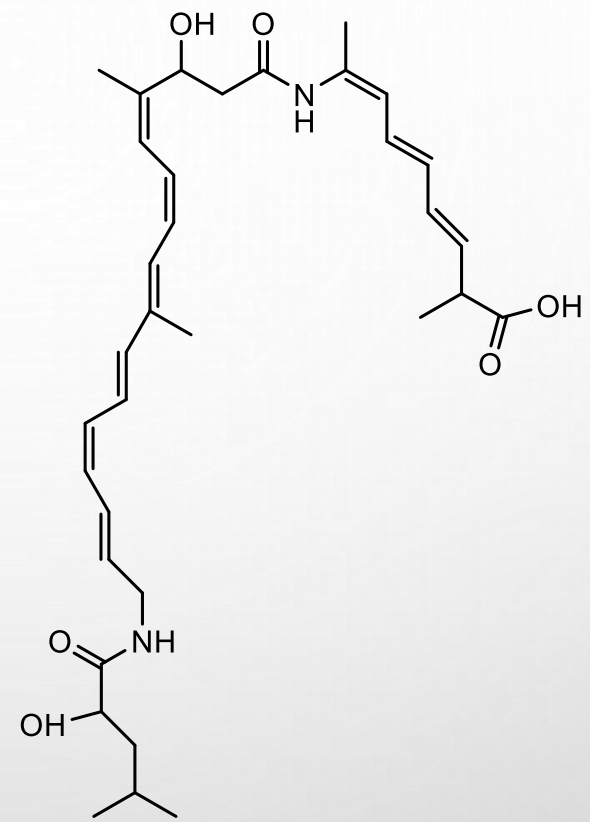
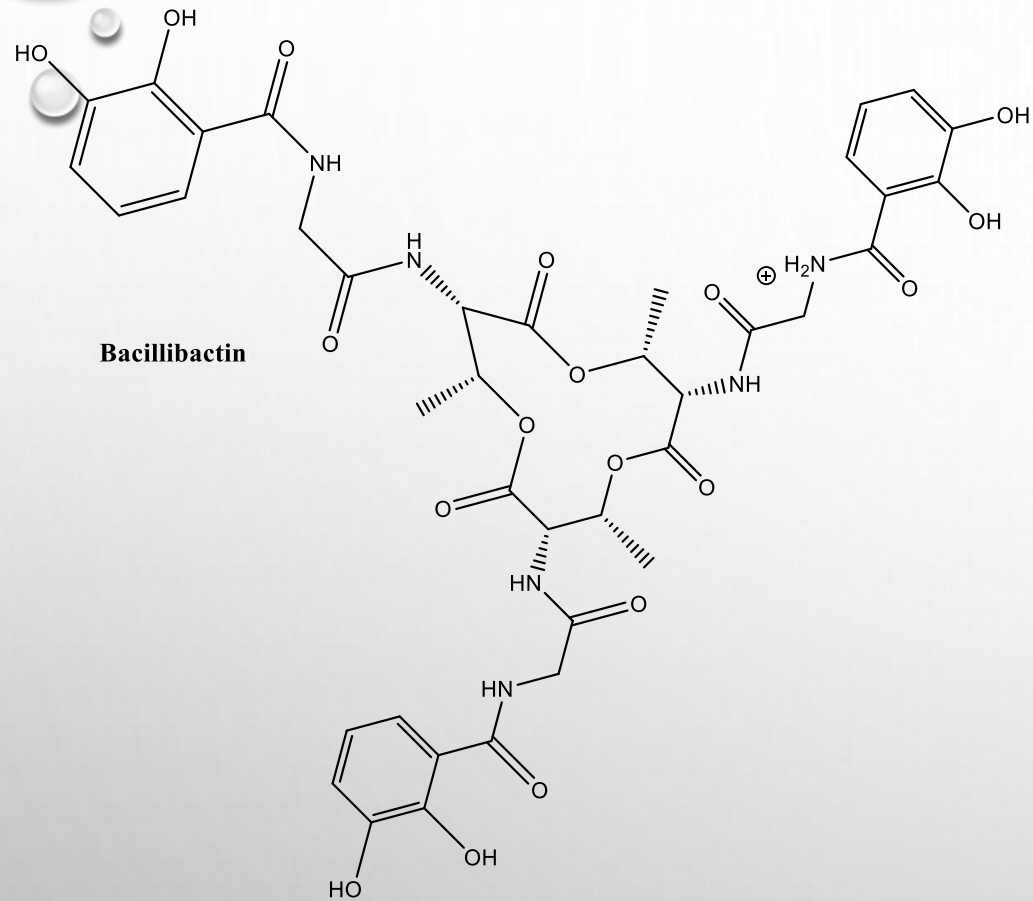
ITURINS A

- R = -CH₃ (Iturin A C₁₄)
- R = -C₂H₅ (Iturin A C₁₅)
- R = -C₃H₇ (Iturin A C₁₆)
- R = -C₄H₉ (Iturin A C₁₇)



Good antifungals against a broad range of phytopathogenic fungi and could justify the antifungal activity observed with this strain.

IDENTIFICATION OF BSMs BY UPLC-qTOF-MS (...)



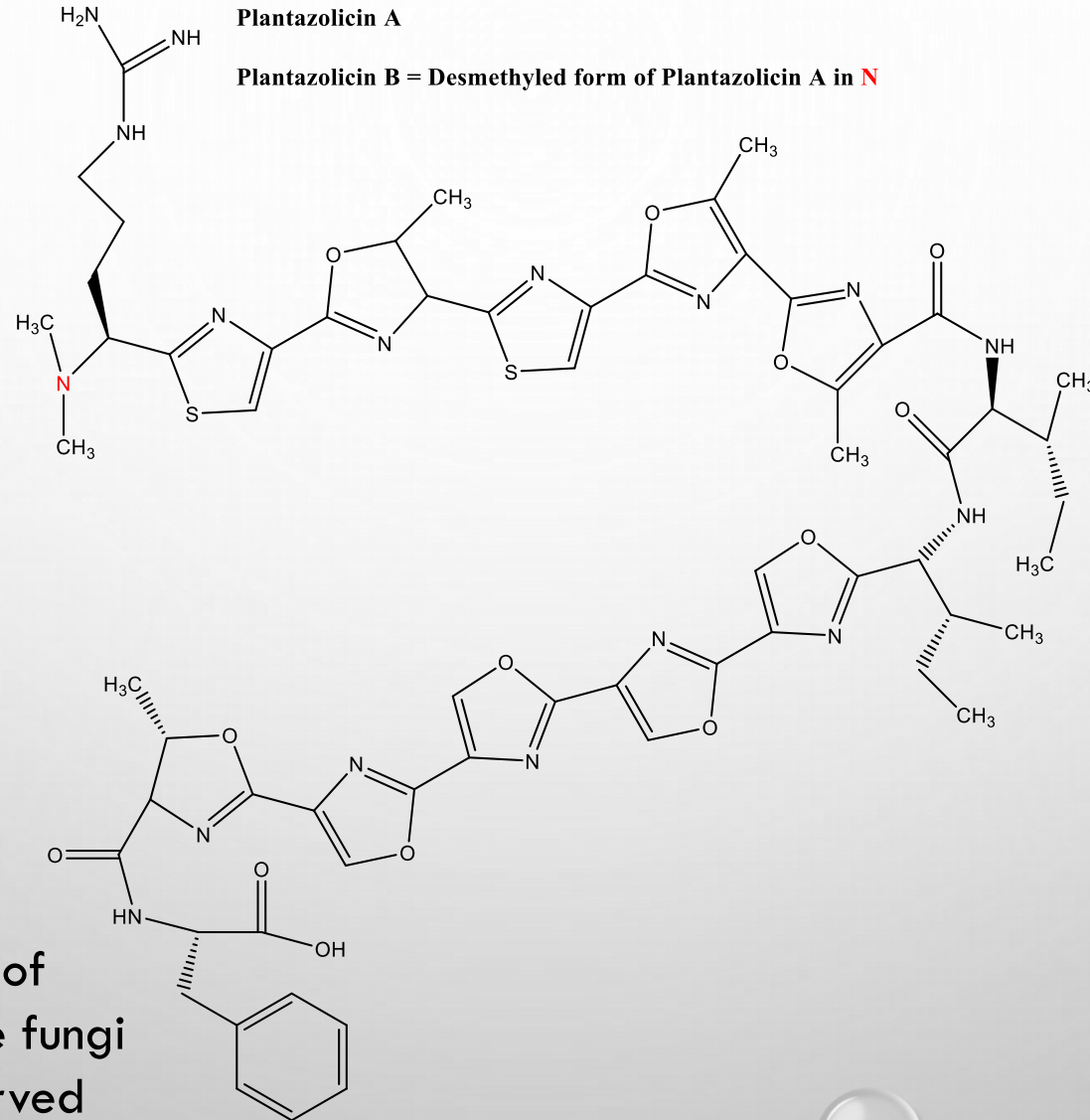
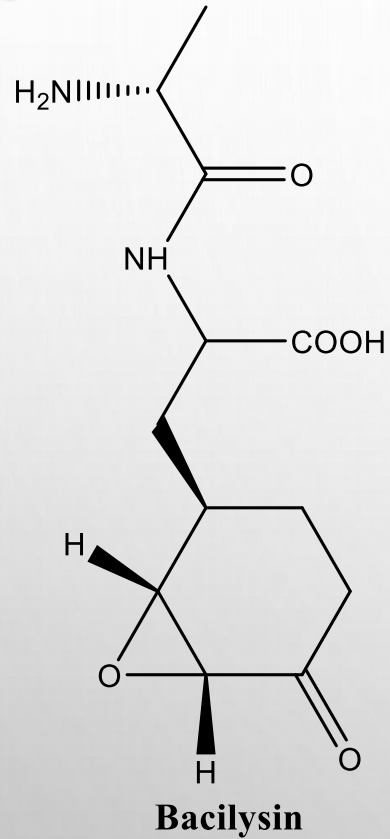
Bacillaene

Dihydroxybacillaene

- A siderophore, secreted by most of *Bacillus* spp.
- Play a key role in **competition** with other microorganisms by its Fe chelation property

- Good antibacterial activity against both Gram positive and Gram negative pathogens.
- Could be behind the antibacterial activity observed with this isolate

IDENTIFICATION OF BSMs BY UPLC-qTOF-MS (...)



- **Plantazolicin**, detected for the first time in culture supernatant of *B. velezensis* FZB42
- Great activity against the **only obligate pathogen within Bacillus genus, *Bacillus anthracis***
- A moderate nematocidal activity
- Active against some members of the Gram positive *Bacillus* genus,
- But inactive against Gram negative bacteria

- Active against a broad range of pathogenic bacteria and some fungi
- Could be involved in the observed activities

PREDICTED vs DETECTED BSMs

N°	BSM name	PREDICTED	DETECTED
1	Surfactin	+	+
2	Iturin A	+	+
3	Bacilysin	+	+
4	Amylocyclycin	+	-
5	Bacillibactin	+	+
6	Bacillaene	+	+
7	Plantazolicin	+	+

- One of the **predicted** compounds is not **detected** (large peptide, high molecular mass)
- This requires optimization of the LC/MS q-TOF method

Isolate BDI-IS1, a novel strain of the species *B. nakamurai*

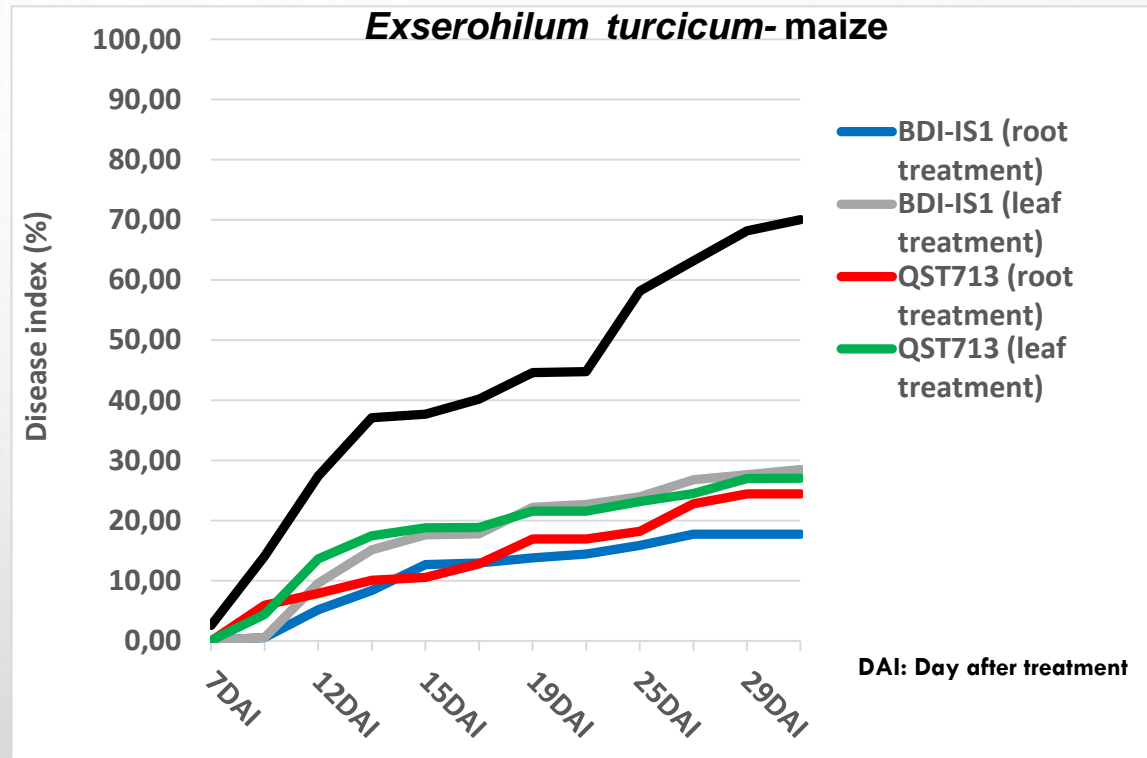
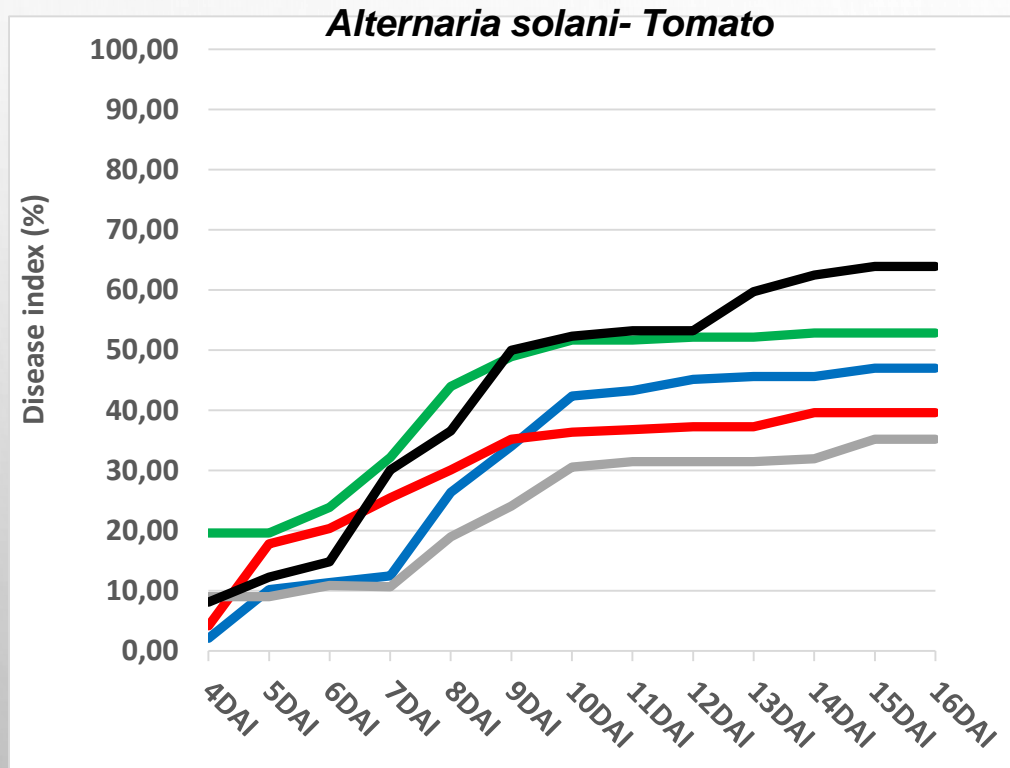
Based on the BGCs found out by the Antismash software on the 3 strains' genomes:

N°	BSM name	BDI-IS1	NRRL B-41091	NRRL B-41092
1	Surfactin	P	P	P
2	Iturin A	P	P	P
3	Bacillaene	P	P	P
4	Bacilysin	P	P	P
5	Bacillibactin	P	P	P
6	Plantazolicin	P	A	P
7	Amylocyclcin	P	A	A
8	Subtilin	A	A	P

- The 3 strains are different.
- Then, a novel strain within the species *B. nakamurai*, ***B. nakamurai* BDI-IS1**

Biocontrol benefits of *B. nakamurai* BDI-IS1

- *B. nakamurai* BDI-IS1 was evaluated in greenhouse experiment on tomato and maize infested with *Alternaria solani* (tomato early blight (A)) and *Exserohilum turcicum* (northern leaf blight of maize (B)) respectively
- Application was done on roots or on leaves (more details in Nihorimbere et al., poster in this symposium)



- ✓ Against tomato early blight, BDI-IS1 upon leaf treatment is **more efficient** than QST 713
- ✓ Against northern leaf blight of maize, BDI-IS1 and QST 713 are **efficient at comparable levels**, upon root treatment.

PERSPECTIVES

- Determine the role of each metabolite in the bioactivity spectrum by specific gene silencing (design of mutants)
- Study of the effects of abiotic factors on growth and metabolome production of the strain
- Understand the mechanisms underlying the biocontrol effect (antagonism or plant immunity stimulation)
- Test of the efficacy of the strain in field under local conditions

THANK YOU FOR YOUR ATTENTION

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