Bacillus subtilis 30B-B6, a promising bacterium for the biocontrol of early and late blight

Gil Colau, Simon Caulier, Claude Bragard, Jacques Mahillon and Anne Legrève

UCLouvain

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Subgroup: ***Alternaria***

EARTH & LIFE INSTITUTE

Can biopesticides replace chemical fungicides?

Food safety

Efficacy

Effects on non-targets

Environmental threats

Cost

Human health

"Easy use"

Resistances appearance

Knowledges

Less resistances

Effects on non-targets

Environmentally friendly

Survival during storage

Delay chemical pesticide use

Homologation process

Less dangerous for humans

Efficacy in field

Toxicity

Diversity of modes of action

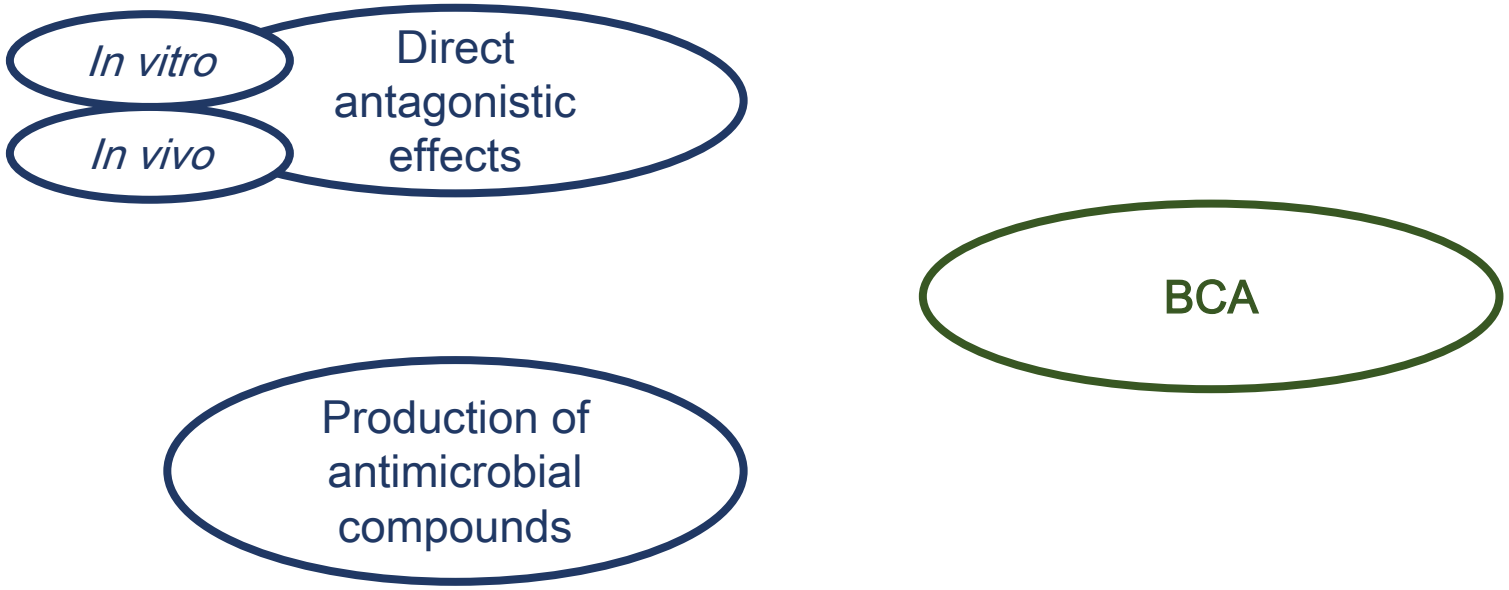
Everything needs to be qualified !

Many questions remain !

The context

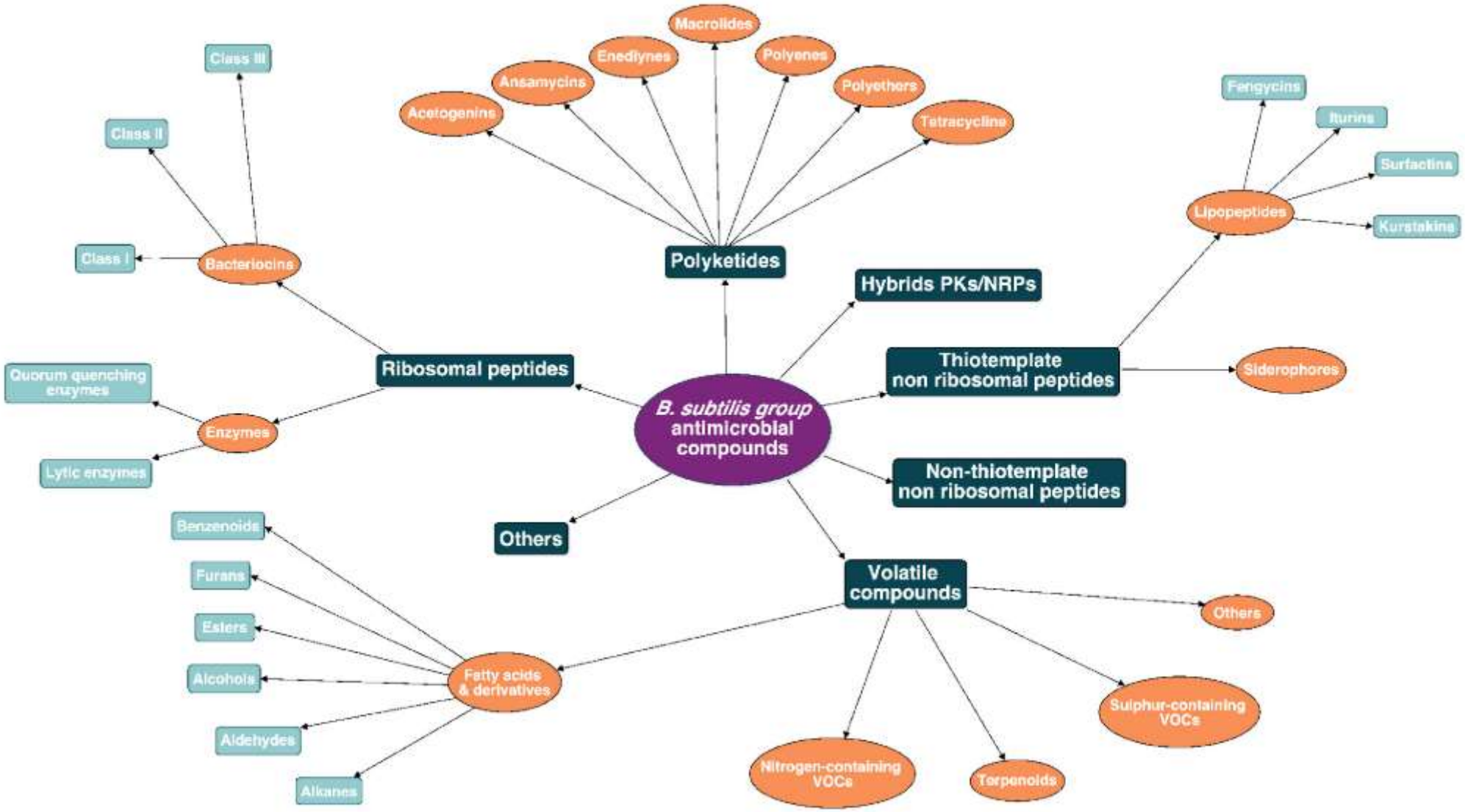
- Searching for alternatives to chemical pesticides
- Using indigenous microflora in an integrated pest management strategy
- Finding biocontrol agents (BCA) against *Phytophthora infestans* and other pathogens of solanaceae
- Understanding the mechanisms involved in the antagonism

Characteristics of a good biocontrol agent (BCA)



Characteristics of a good biocontrol agent (BCA)

Diversity of the antimicrobial molecules classes from the *Bacillus subtilis* group. The subdivision between the classes is based on the biosynthetic pathway.



Caulier *et al.*, 2019, Frontiers in Microbiology, 10, 1-19

Characteristics of a good biocontrol agent (BCA)

In vitro

In vivo

Direct antagonistic effects

Production of antimicrobial compounds

Indigenous

BCA

Plant defences stimulation

Safety for humans, environment

Industrial part

Production, storage optimization

Strategy to select biological control agents (BCA)

Sampling of Belgian soils from agroecosystems and isolation of bacteria (*Pseudomonas* and *Bacillus* spp.) — **2826 strains**

High-throughput screening for antagonistic activities and identification of candidate BCA (RNAr 16S) — **60 strains**

Screening for genes related to potential human toxins and for antagonist compound related genes (PCR assays)

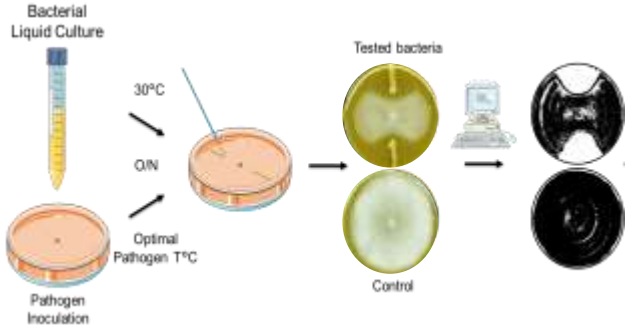
Characterization of enzymatic activities and detection of bioactive compounds using specific media

Efficacy of BCA against pathogens in plants under controlled conditions — **11 strains**

Efficacy of BCA against potato late blight under field conditions — **4 strains**

Characteristics of the strain 30B-B6

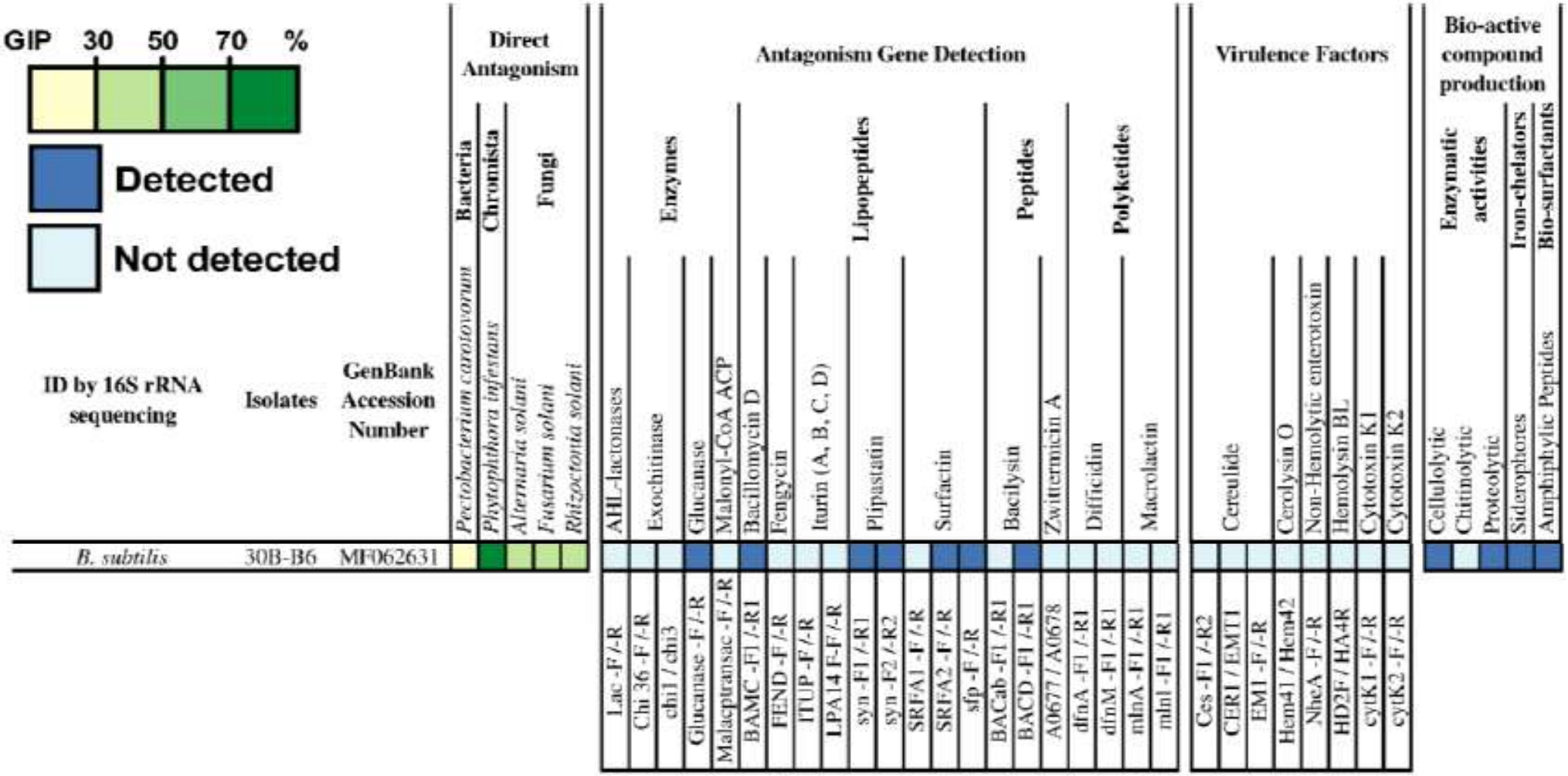
ID by 16S rRNA sequencing	Isolates	GenBank Accession Number	Direct Antagonism				
			Bacteria	Chromista	Fungi		
			Pectobacterium carotovorum	*Phytophthora infestans*	*Alternaria solani*	*Fusarium solani*	*Rhizoctonia solani*
B. subtilis	30B-B6	MF062631					



$$\textbf{Growth Inhibition Percentage} = \left(1 - \frac{S_{bacteria}}{S_{control}}\right) * 100$$

Caulier *et al.*, 2018, Frontiers in Microbiology, 9, 143-158

Characteristics of the strain 30B-B6



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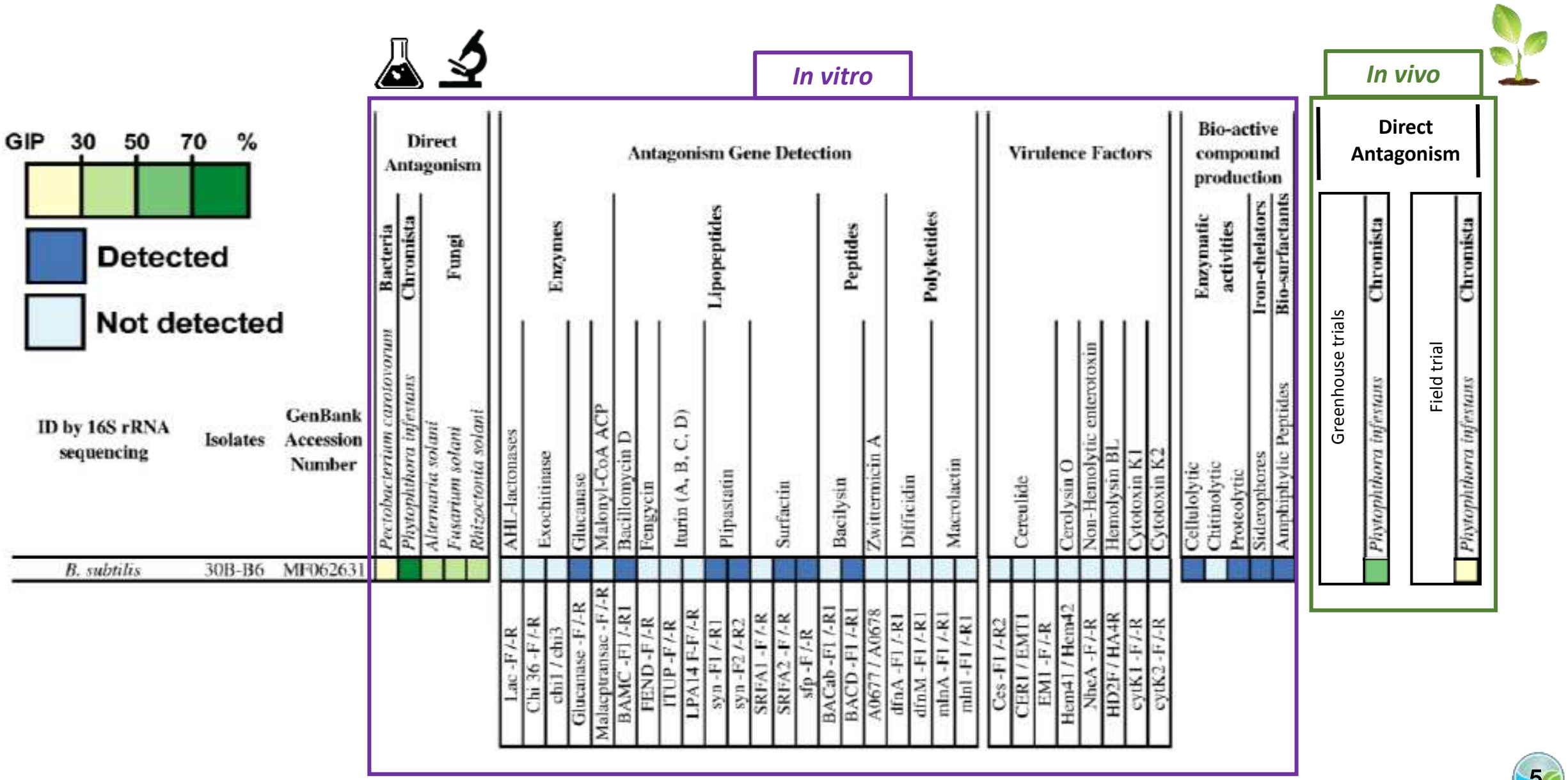
Characteristics of the strain 30B-B6



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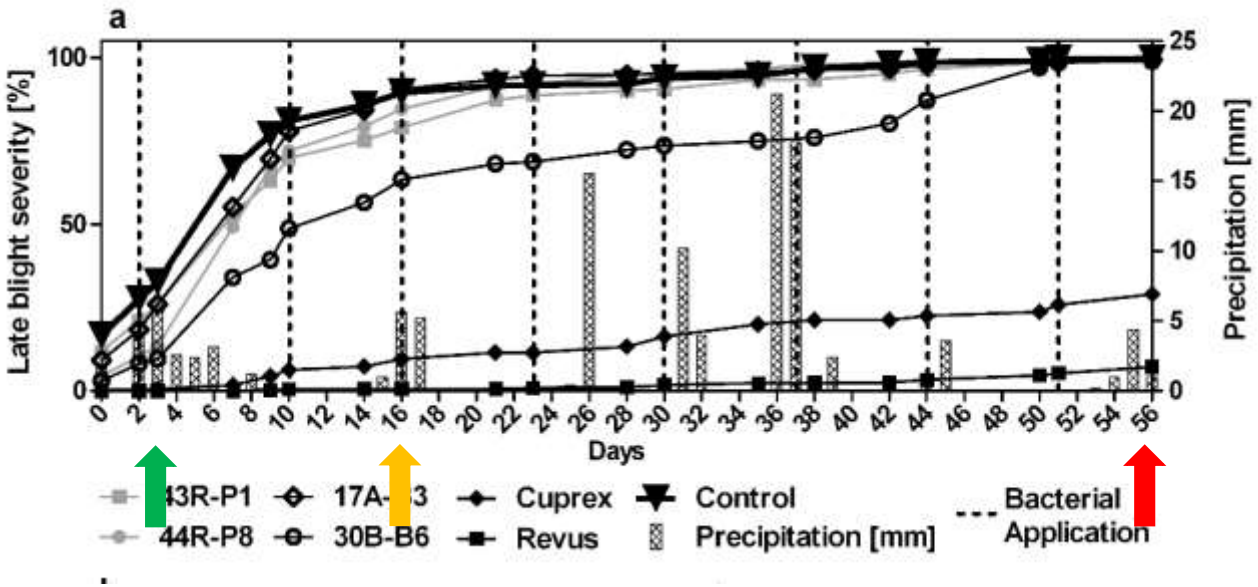
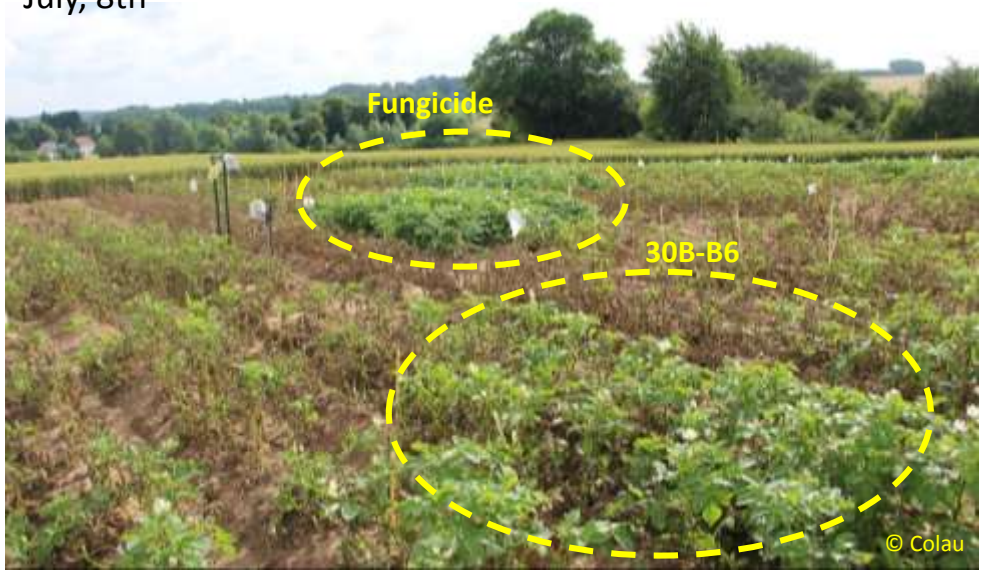
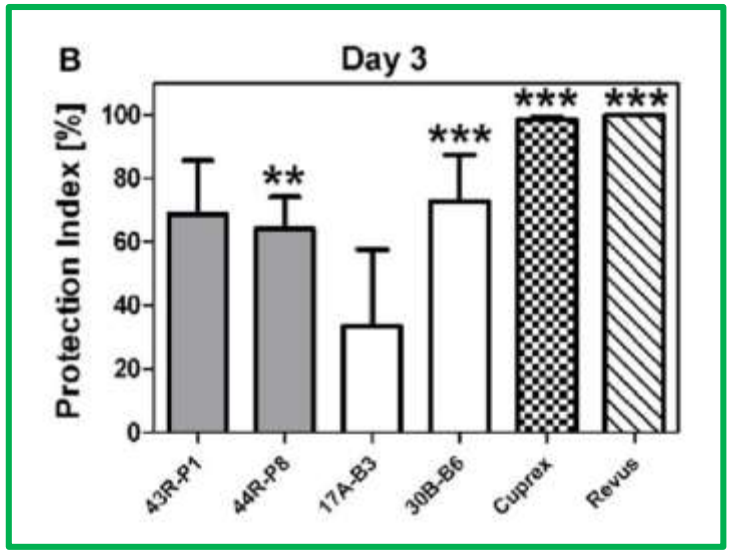
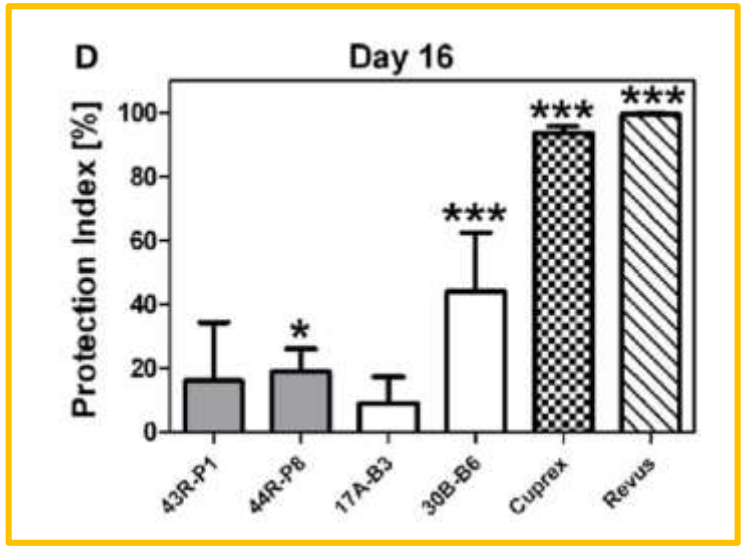
Pilot field trial (2016)

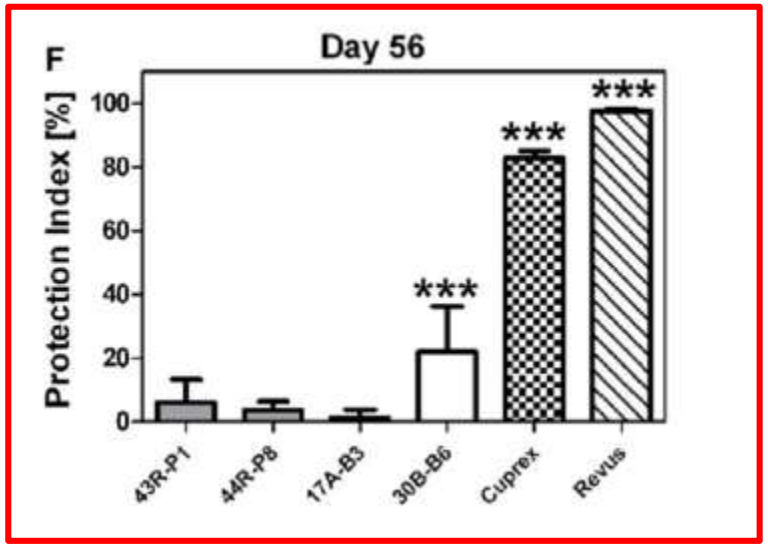


July, 8th









Caulier *et al.*, 2018, Frontiers in Microbiology, 9, 143-158

frontiers in Microbiology

ORIGINAL RESEARCH
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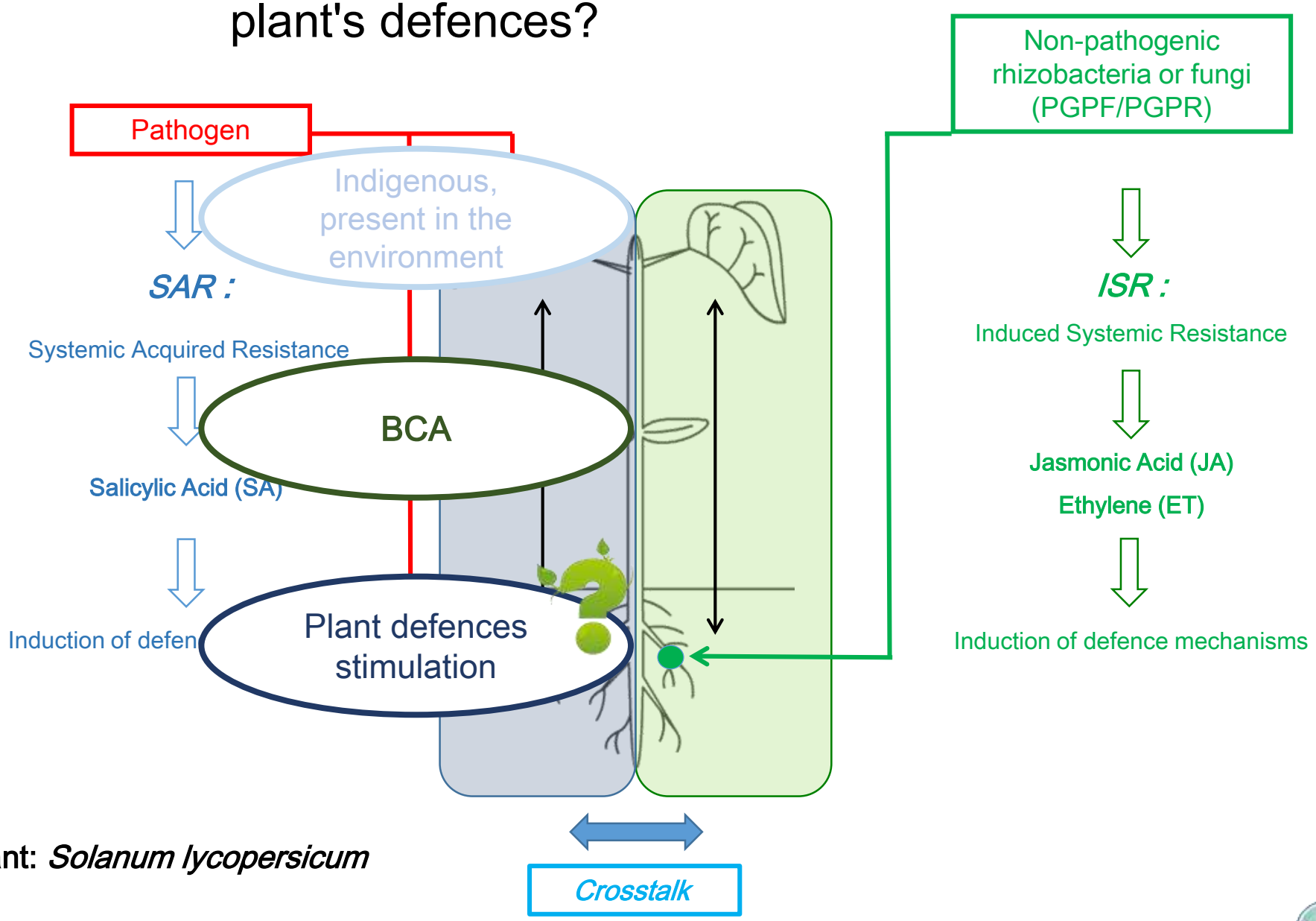
Versatile Antagonistic Activities of Soil-Borne *Bacillus* spp. and *Pseudomonas* spp. against *Phytophthora infestans* and Other Potato Pathogens

Simon Caulier[1,2†], Annika Gillis[2†], Gil Colau[1], Florent Licciardi[2], Maxime Liépin[1], Nicolas Desoignies[1], Pauline Modrie[2], Anne Legrève[1], Jacques Mahillon[2] and Claude Bragard[1*]*

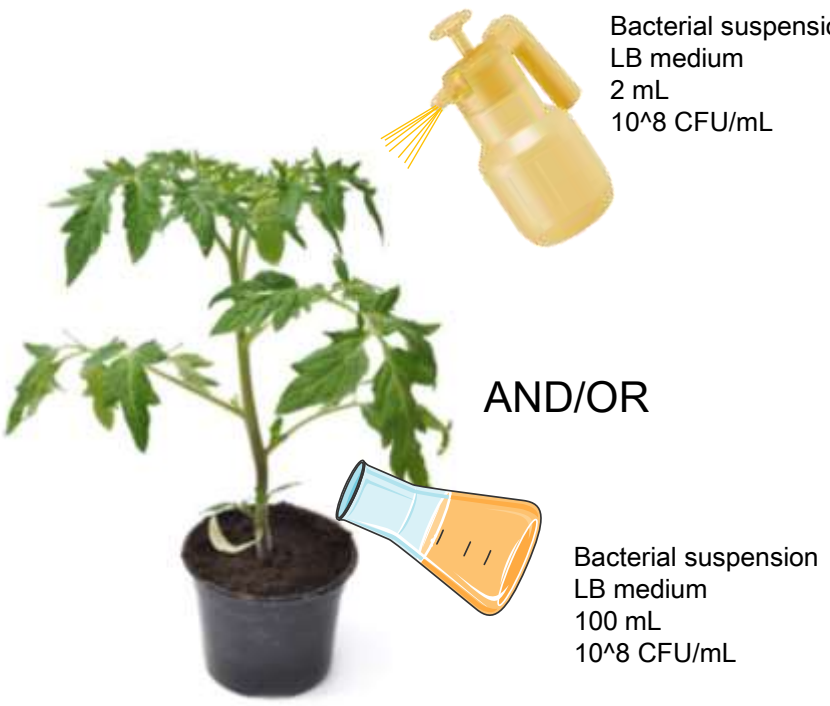
[1] Phytopathology-Applied Microbiology, Earth and Life Institute, Université Catholique de Louvain, Louvain-la-Neuve, Belgium, [2] Laboratory of Food and Environmental Microbiology, Earth and Life Institute, Université Catholique de Louvain, Louvain-la-Neuve, Belgium

Can *Bacillus subtilis* 30B-B6 stimulate the plant's defences?



➡ Study on a model plant: *Solanum lycopersicum*

Stimulation of systemic plant resistance



Bacterial suspension
LB medium
2 mL
10^8 CFU/mL

AND/OR

Bacterial suspension
LB medium
100 mL
10^8 CFU/mL

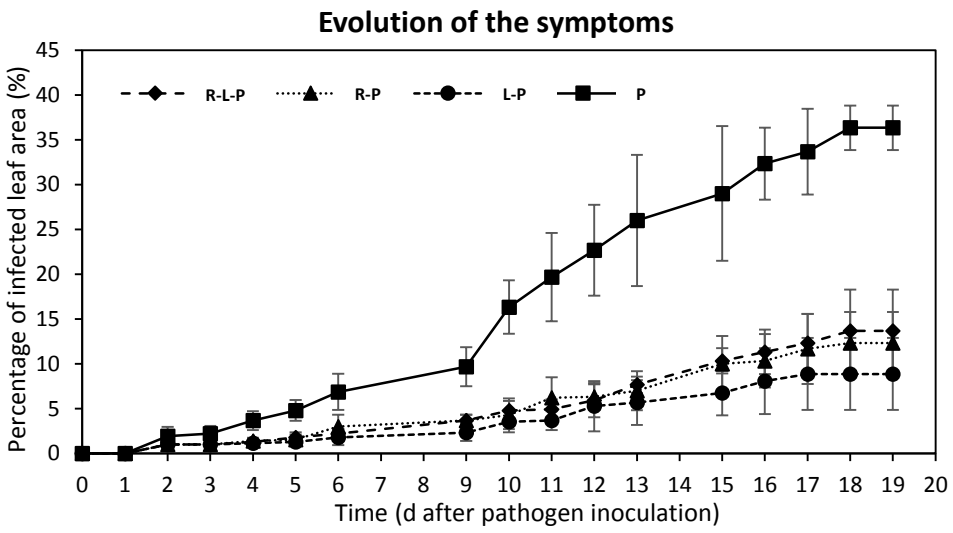
5 old leaves plant
Compost
Variety:moneymaker

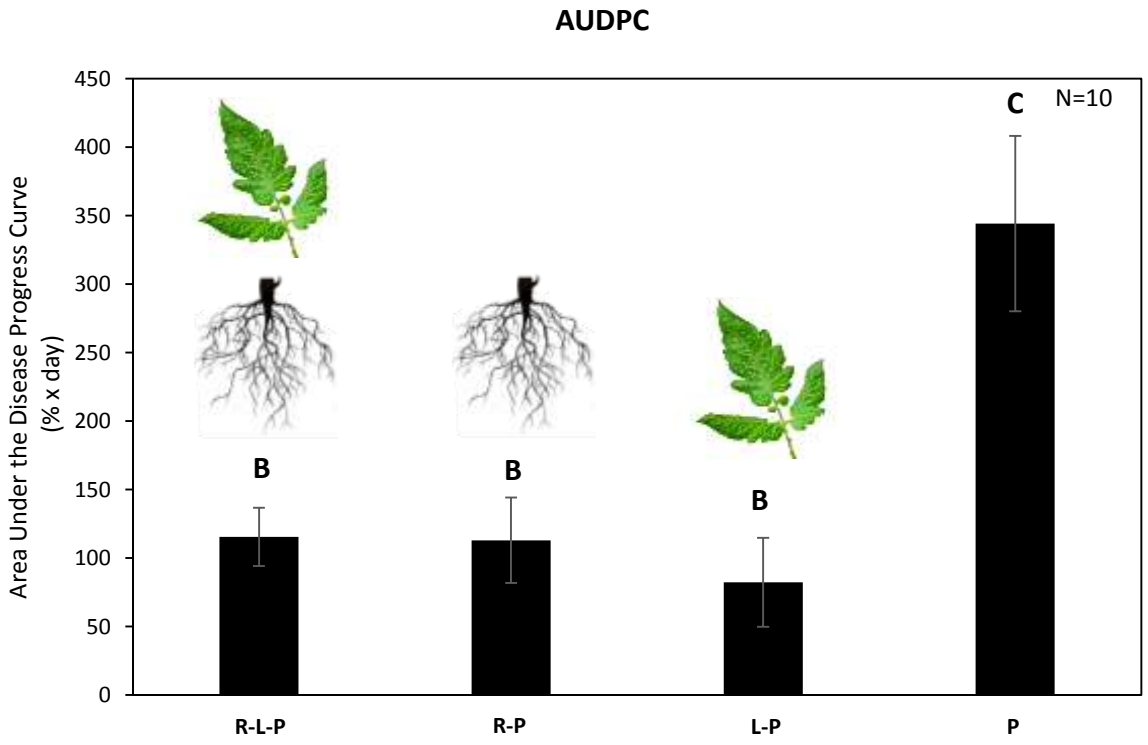
6 h

A. solani
Conidia suspension
15 000 c/mL

Symptoms quantification

Stimulation of systemic plant resistance



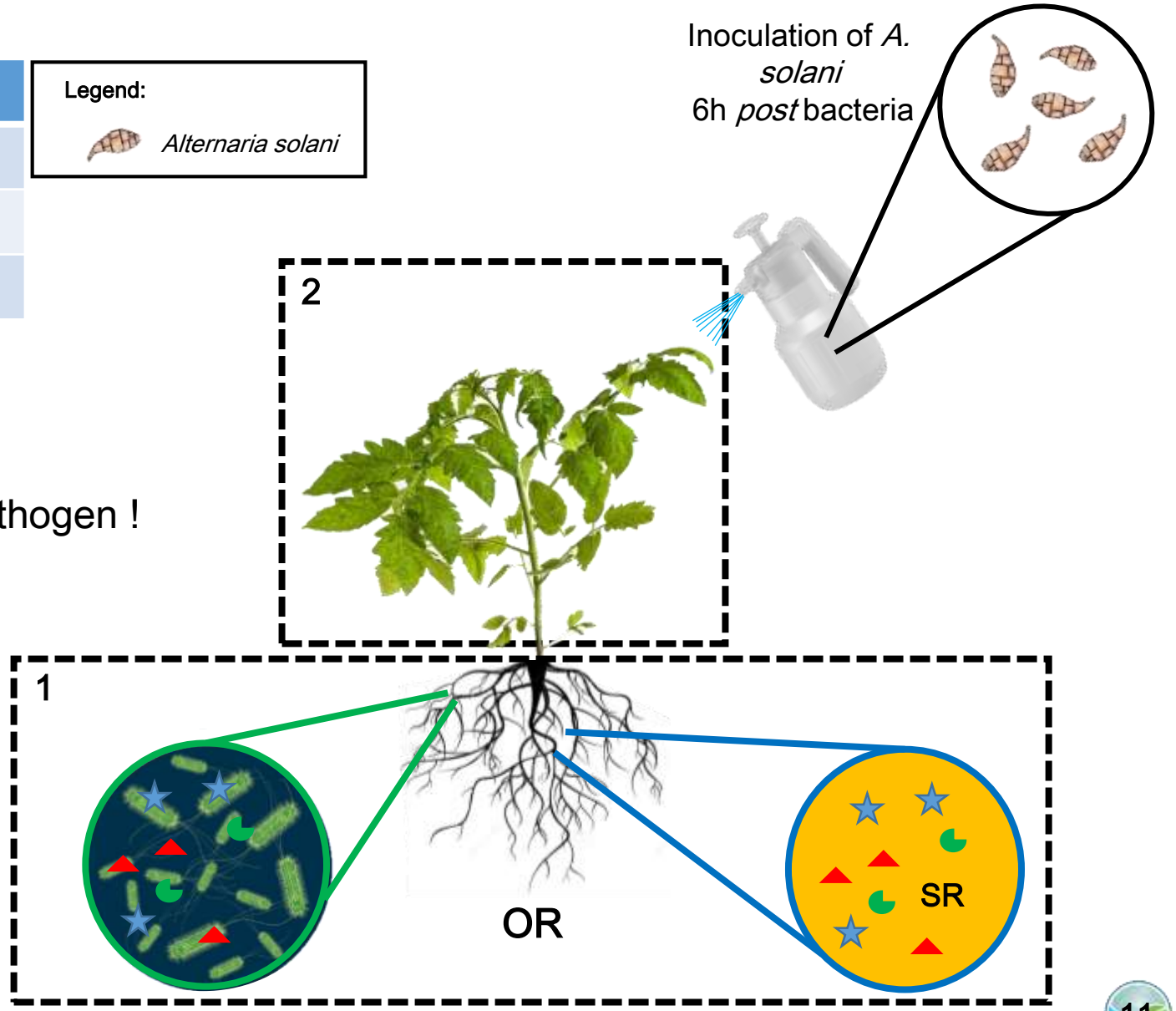


Stimulation of systemic plant resistance

Treatments	On roots	On leaves
Control	LB medium	
30B-B6	Bacterial suspension	
30B-B6_SR	Bacterial culture supernatant	

Legend:

Alternaria solani

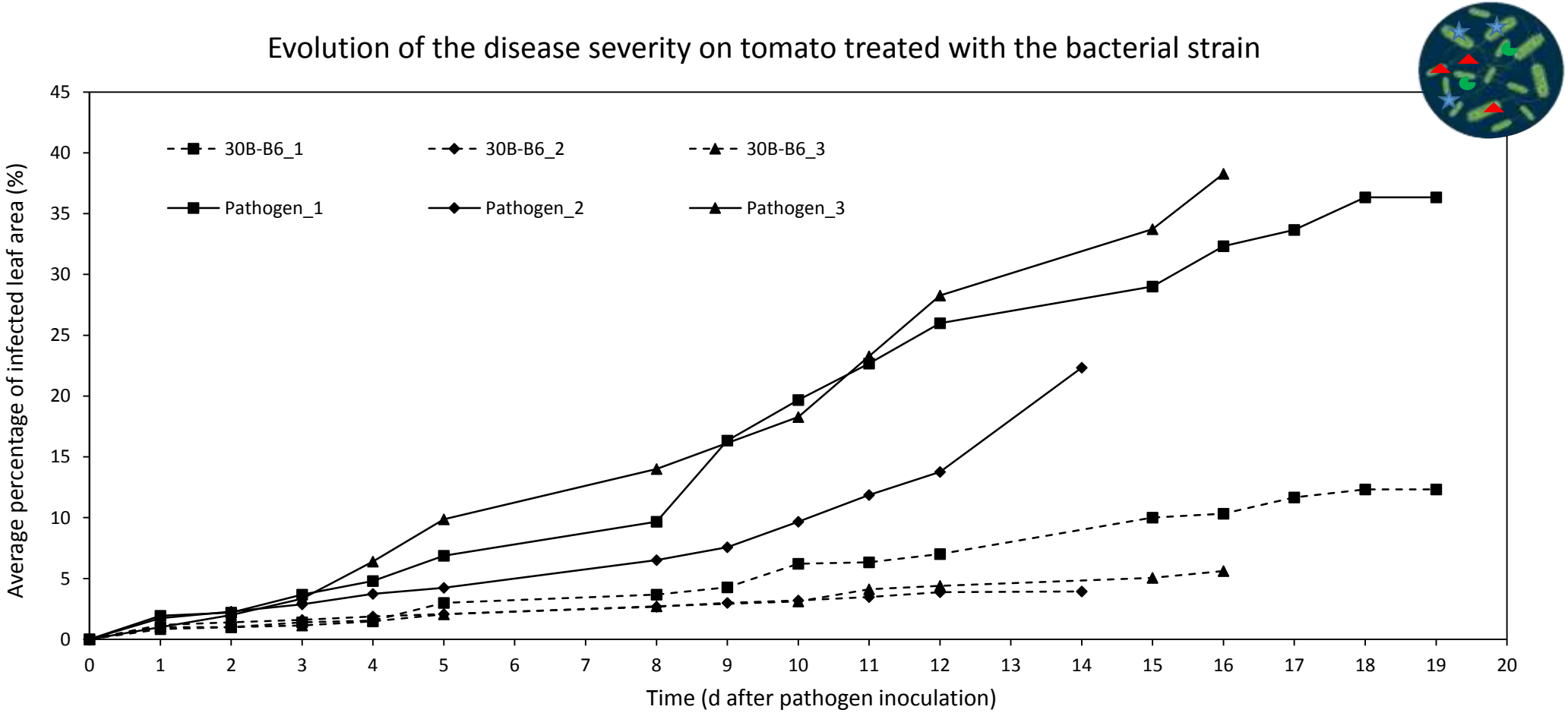


➡ No contact between bacteria and pathogen !

➡ Symptom quantification

Stimulation of systemic plant resistance

- Bacterial suspension



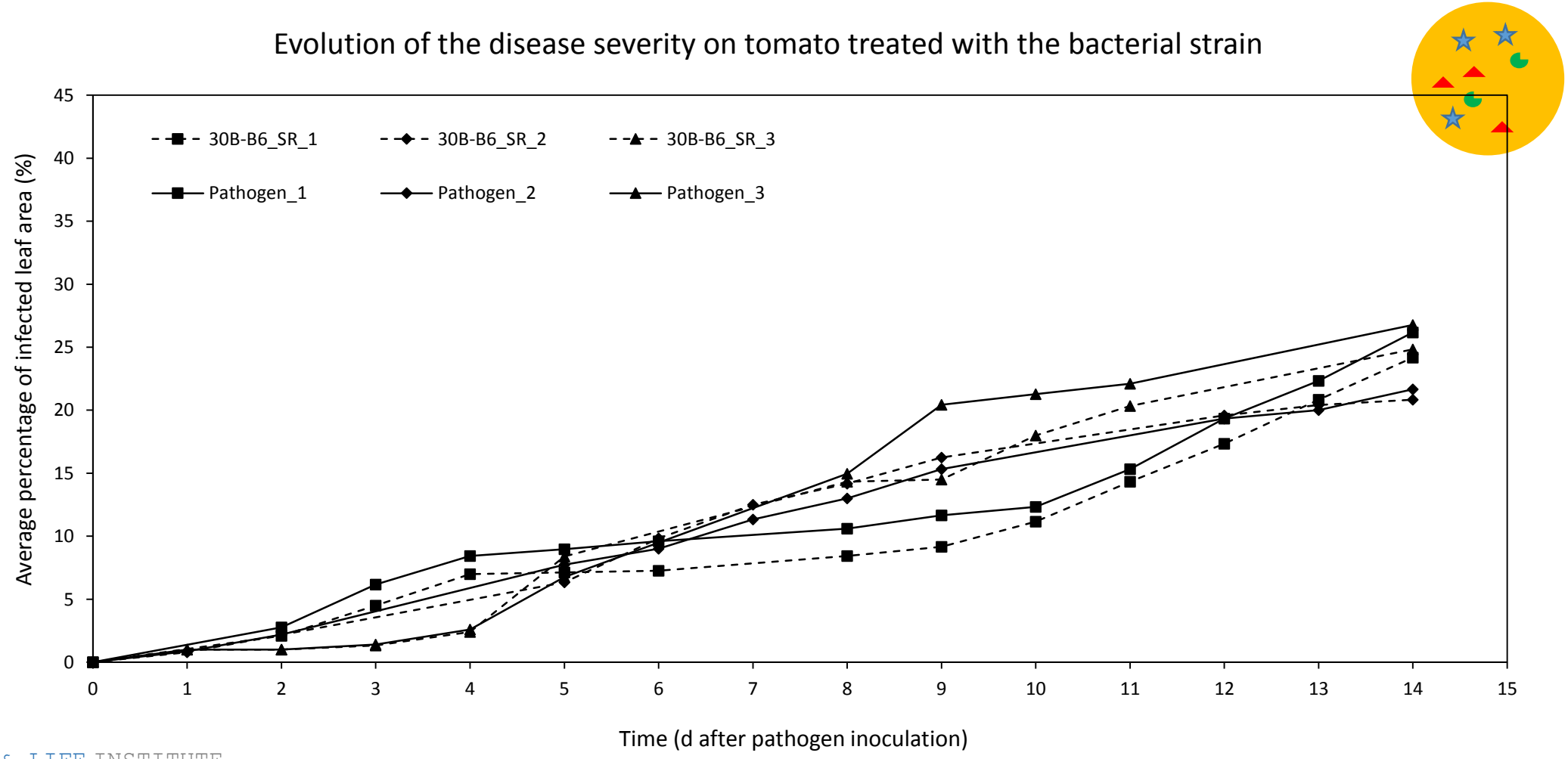
Stimulation of systemic plant resistance

- Bacterial suspension

12 days *pi*

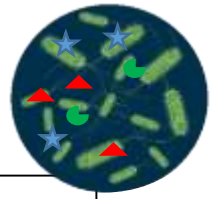
Stimulation of systemic plant resistance

- Bacterial culture supernatant

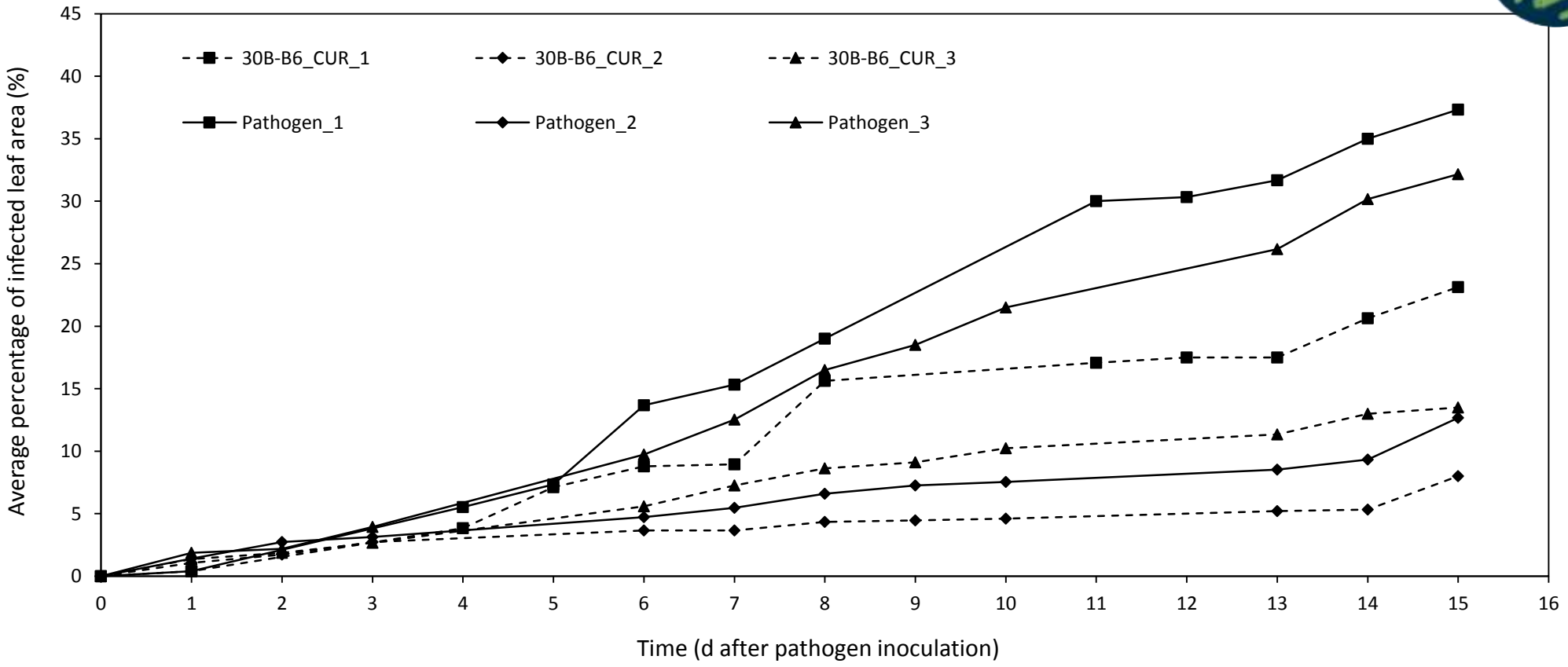


Curative effect of 30B-B6

- Application of the bacterial suspension 24 *hpi*



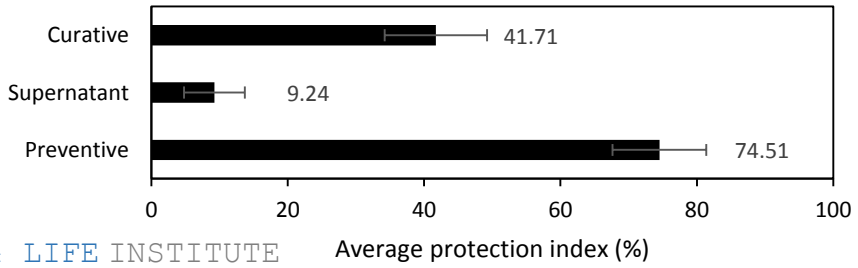
Evolution of the disease severity on tomato treated with the bacterial strain



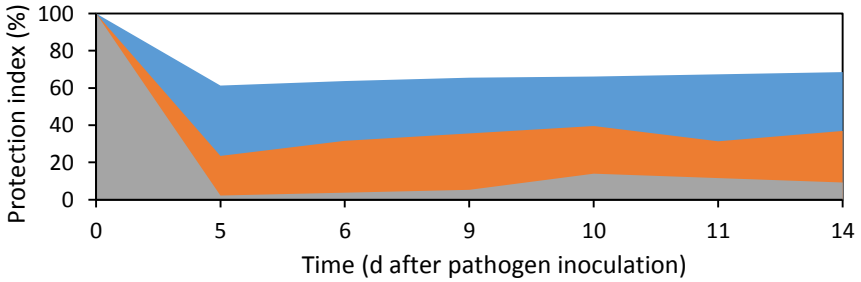
Summary: the preventive and curative effect of 30B-B6 against *A. solani* under controlled conditions

Exp		n	AUDPC 14d	SD AUDPC	PI 14d (%)	PI Mean (%)	SD PI	Year	Period of year	Mean RH (%)	Mean Td (°C)	Mean Tn (°C)	Duration (d)	STAT AUDPC
Preventive	Rep_1	5	46,35	10,28	73,00	74,51	6,89	2017	March/April	ND	ND	ND	19	***
	Rep_2	10	38,22	7,45	68,50			2017	May	90,85	16,92	13,91	14	***
	Rep_3	6	35,08	6,26	82,03			2017	October	73,01	20,98	17,56	16	***
	Cont_1	5	171,67	21,87	0,00	0,00	0,00	2017	March/April	ND	ND	ND	19	
	Cont_2	10	121,33	62,79	0,00			2017	May	90,85	16,92	13,91	14	
	Cont_3	6	195,22	24,80	0,00			2017	October	73,01	20,98	17,56	16	
SR	Rep_1	10	128,15	35,46	14,19	9,24	4,44	2017	June	77,66	19,37	15,72	14	–
	Rep_2	5	153,80	28,17	5,59			2018	July/Augustus	75,49	19,11	15,77	14	–
	Rep_3	10	161,12	33,71	7,94			2018	October	78,30	15,64	13,65	14	–
	Cont_1	10	149,33	75,35	0,00	0,00	0,00	2017	June	77,66	19,37	15,72	14	
	Cont_2	5	162,90	37,95	0,00			2018	July/Augustus	75,49	19,11	15,77	14	
	Cont_3	10	175,02	27,07	0,00			2018	October	78,30	15,64	13,65	14	
Curative	Rep_1	5	143,77	25,33	36,24	41,71	7,50	2018	March	84,60	15,79	14,01	15	**
	Rep_2	5	46,21	9,81	38,63			2018	June	77,58	19,41	15,51	15	**
	Rep_3	10	93,72	27,10	50,26			2018	July/Augustus	75,49	19,11	15,77	15	***
	Cont_1	5	225,50	34,37	0,00	0,00	0,00	2018	March	84,60	15,79	14,01	15	
	Cont_2	5	75,30	16,22	0,00			2018	June	77,58	19,41	15,51	15	
	Cont_3	10	188,42	52,44	0,00			2018	July/Augustus	75,49	19,11	15,77	15	

Protection index after 14 days of scoring



Evolution of the protection of tomato plants



Tukey test (p-*value*)
*** < 0,001
** < 0,01
* < 0,05

Take home messages

- The strain 30B-B6 has a direct antagonistic activity *in vitro* against *Phytophthora infestans, Rhizoctonia solani, Fusarium solani* and *Alternaria solani*

- PCR assays revealed:
  - the presence of several genes involved in the production of antimicrobial compounds (LPs, bacilysin, glucanase)
  - the absence of the potential virulence factors to humans tested

- The strain 30B-B6 is able to control *P. infestans* on potato plants under controlled conditions (PI: 70%) and under field conditions (PI:22%)

- The strain 30B-B6 does activate the systemic defences of tomato against *A. solani* by interacting with tomato roots. Preventive effect: PI:75%; curative effect: PI:42%.

Perspectives

- To identify the modes of action of the strain 30B-B6:
  - Impact of siderophores and/or lipopeptides (production, purification and application on tomato roots)
  - Dosage of hormones (ET, SA,…) related to plants defences
  - RNAseq analyses
- To characterize the environmental factors that influence the strain's ability to stimulate the plant's defences
- To optimize the bacteria formulation (UV, desiccation, leaching,…)

  → Improve the efficacy in the field
- To introduce bacteria applications in an integrated pest management strategy

  → Combination with chemical or organic fungicides
  - The strain 36B-B6 is not susceptible to copper oxychloride in co-culture (data not shown)

Thank you for your attention !

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