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Screening and modes of action of antagonistic bacteria to control two fungal pathogens, *Phaeomoniella chlamydospora* and *Neofusicoccum parvum*, involved in grapevine trunk diseases

Haidar Rana, Marc Fermaud

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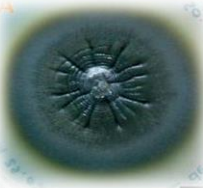



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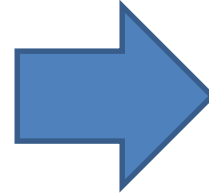
Screening and modes of action of
antagonistic bacteria to control two fungal
pathogens, *Phaeomoniella chlamydospora*
and *Neofusicoccum parvum*, involved in
grapevine trunk diseases

Haidar Rana

UMR Santé et Agroécologie du Vignoble (SAVE)
(INRA / Bordeaux Sciences Agro)

2001

sodium arsenite

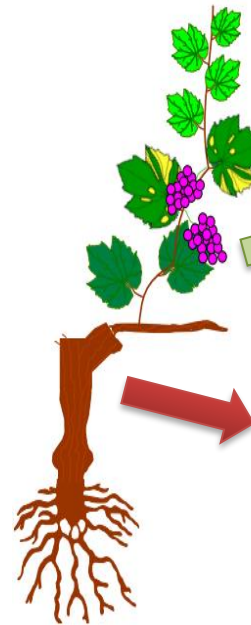


Control with biological control agents (BCAs)

No efficient strategies to control GTDs

**Bacteria
as BCAs against GTDs**

46 bacterial
strains



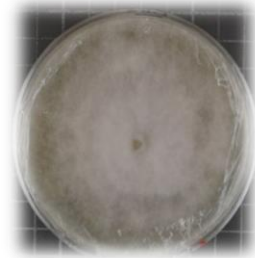
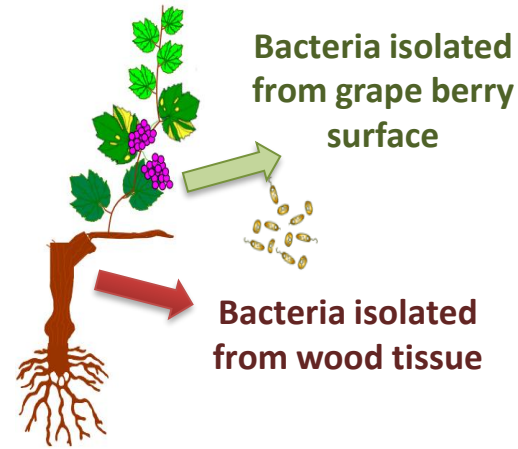
Bacteria isolated from
grape berry surface

Martins et al., 2012

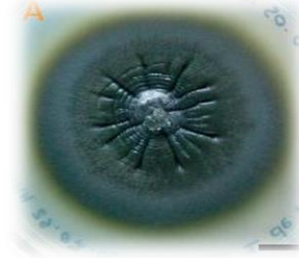
Bacteria isolated from
wood tissue

Bruez et al., 2015





N. parvum (Np)



P. chlamydospora (pch)

1

Evaluation, *in planta*, of the antagonistic activity of **46** bacterial strains against *P. chlamydospora* and *N. parvum*



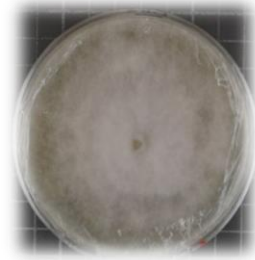
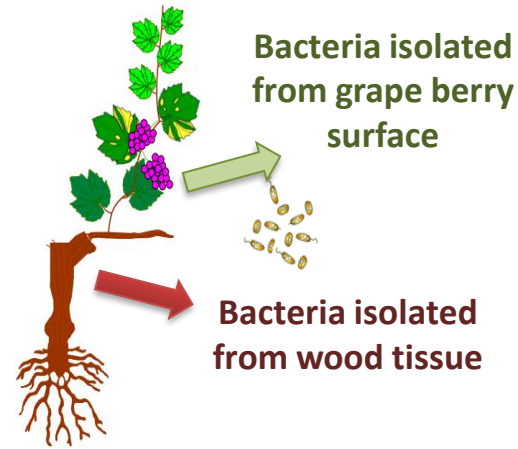
2

Evaluation of the effect of application method on biocontrol efficacy of **9** selected strains

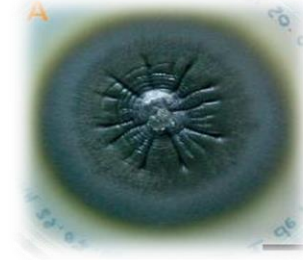


3

Identification of the modes of action for **3** selected strains



N. parvum (Np)



P. chlamydospora (pch)

1

Evaluation, *in planta*, of the antagonistic activity of 46 bacterial strains against *P. chlamydospora* and *N. parvum*



2

Evaluation of the effect of application method on biocontrol efficacy of 9 selected strains



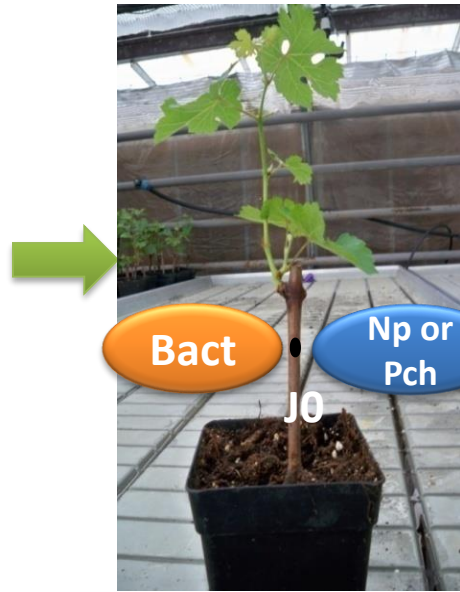
3

Identification of the modes of action for 3 selected strains

Experimental design :



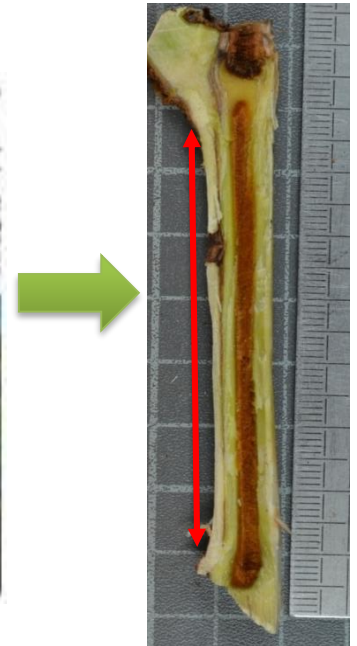
Cabernet Sauvignon cuttings



Bacteria/pathogen Co-inoculation

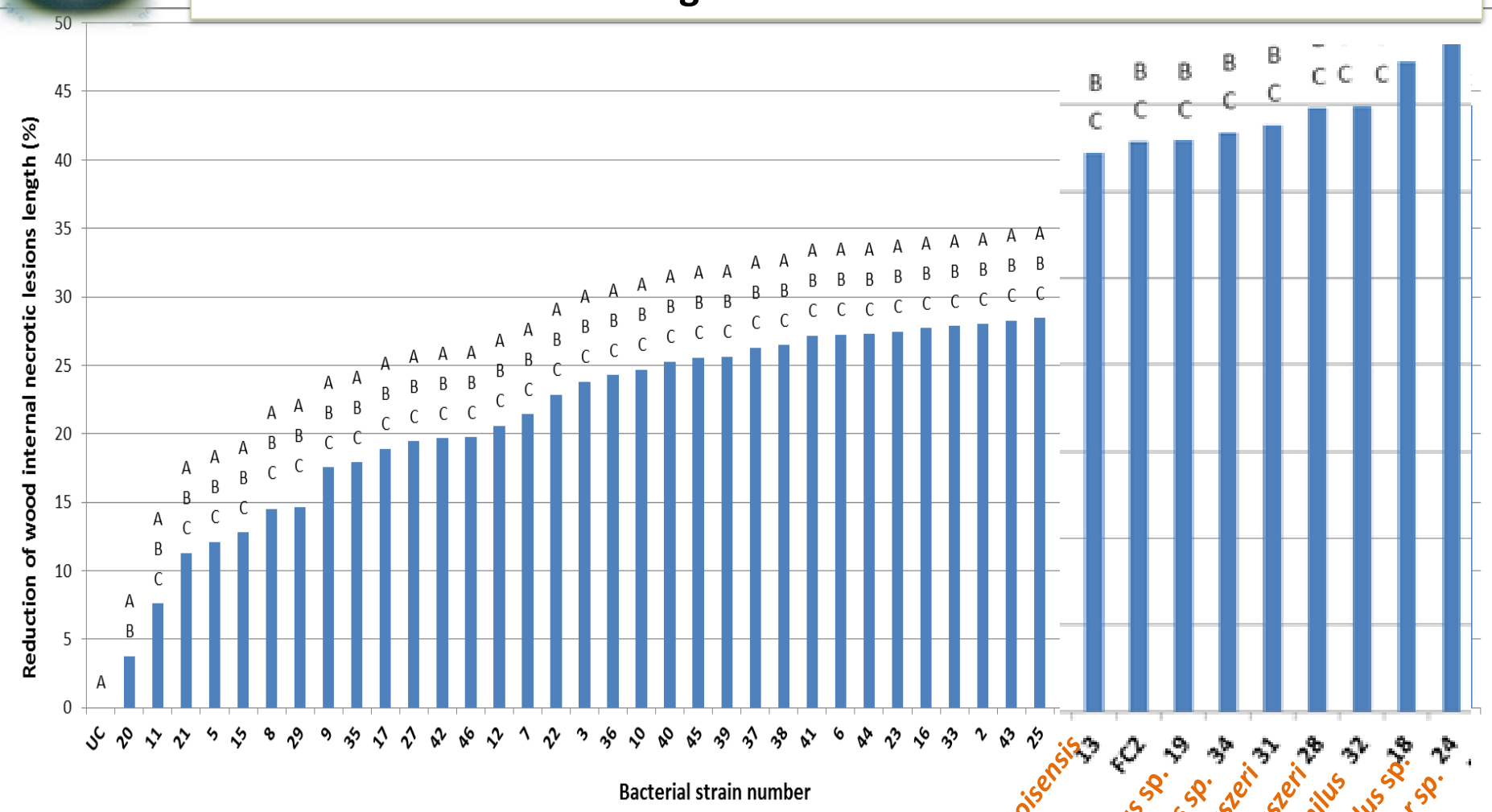


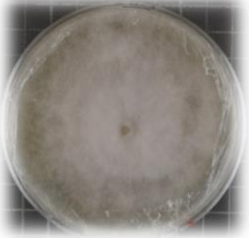
Incubation in open greenhouse



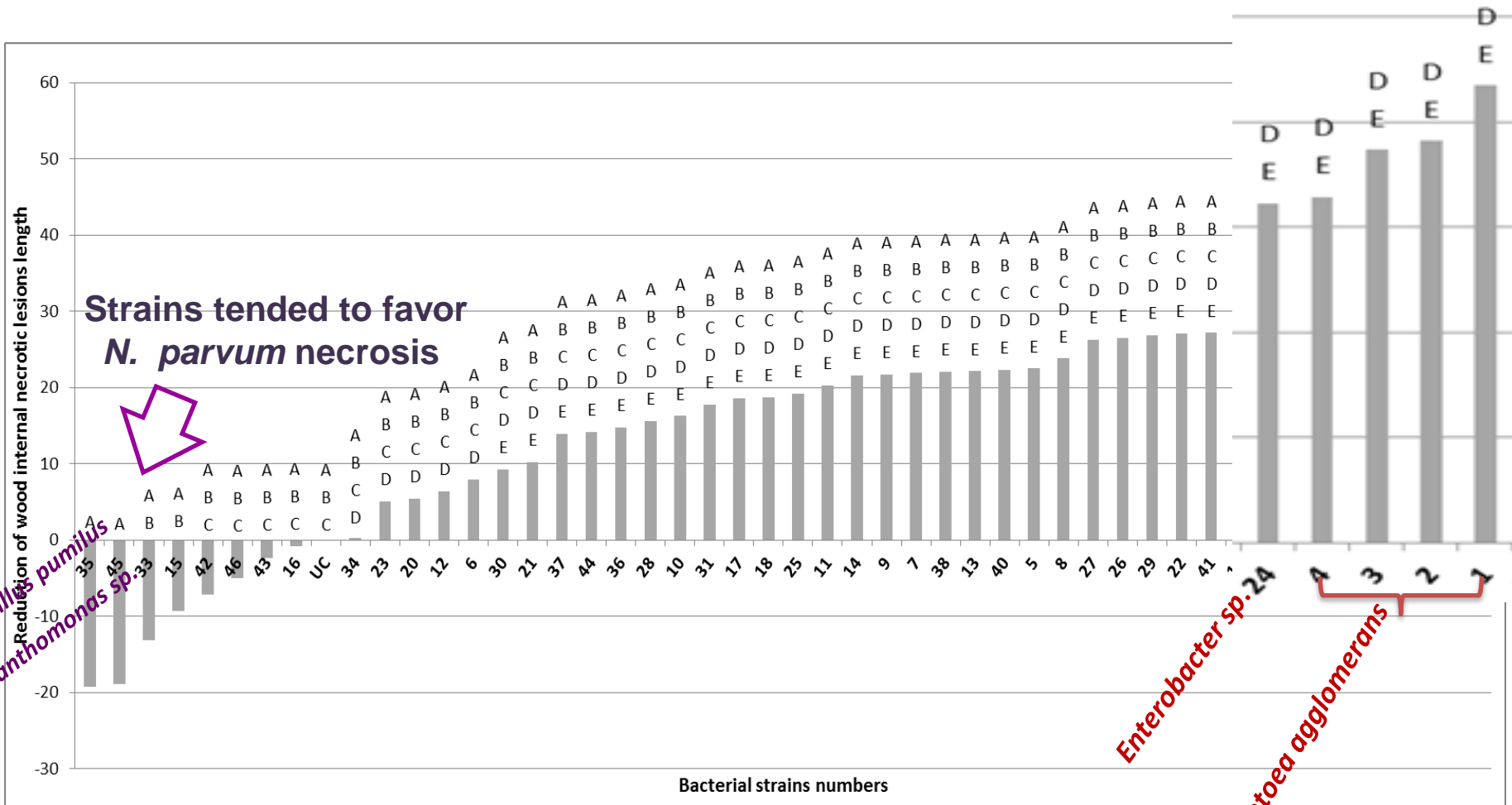
Measuring of necrotic lesions

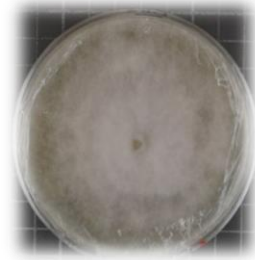
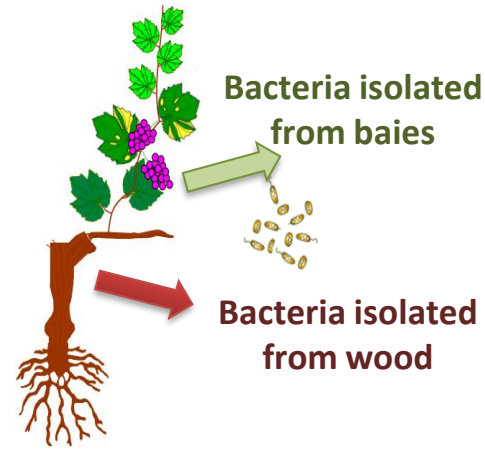
8 bacteria strains: significant reduction of necrosis length in stem cuttings between 32 and 39%



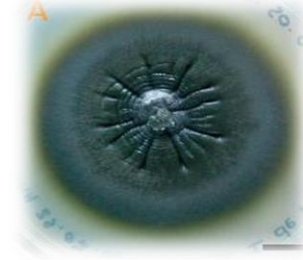


5 bacteria strains: significant reduction of necrosis length in stem cuttings between 33 and 44%





N. parvum (Np)



P. chlamydospora (pch)

1

Evaluation, *in planta*, of the antagonistic activity of 46 bacterial strains against *P. chlamydospora* and *N. parvum*

2

Evaluation of the effect of application method on biocontrol efficacy of 9 selected strains

3

Identification of the modes of action for 3 selected strains

1st *in planta* bioassay

9 strains

N. parvum

Brevibacillus reuszeri (S27)

Bacillus firmus (S41)

Pantoea agglomerans (S1, S3)

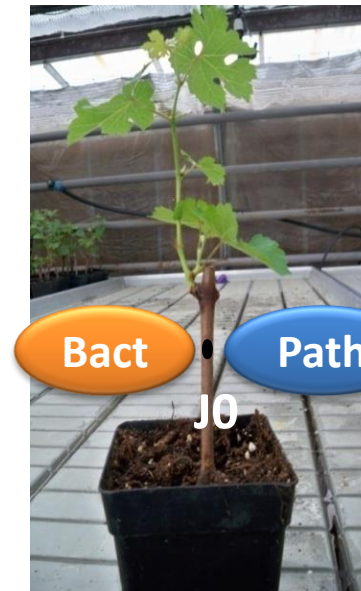
P. chlamydospora

Enterobacter sp. (S24)

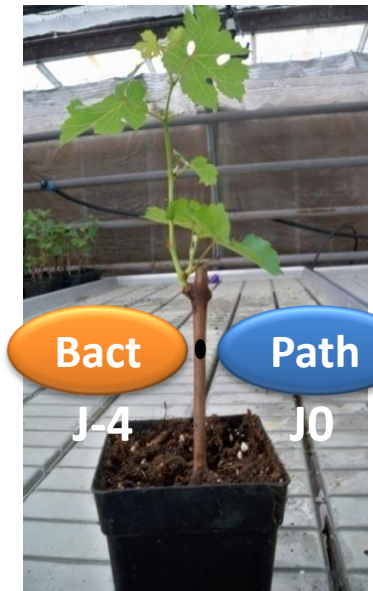
Paenibacillus sp. (S18, S19)

Bacillus pumilus (S32)

Brevibacillus reuszeri (S28)



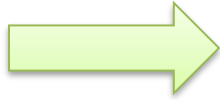
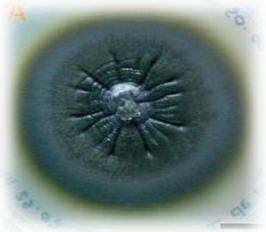
Co-inoculation



Preventive inoculation in the hole



Preventive soil inoculation



P. chlamydospora

Haidar et al., 2016; *Microbiological Research*

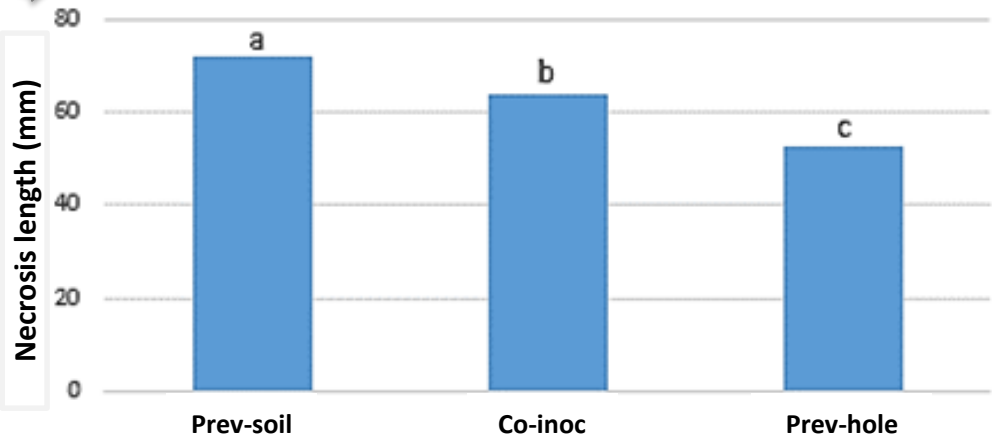
The effect of bacterial strain and the effect of application method was not significant



✓ bacterial efficiency was more strain dependent than inoculation method dependent

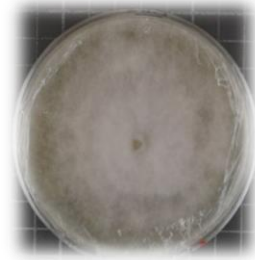
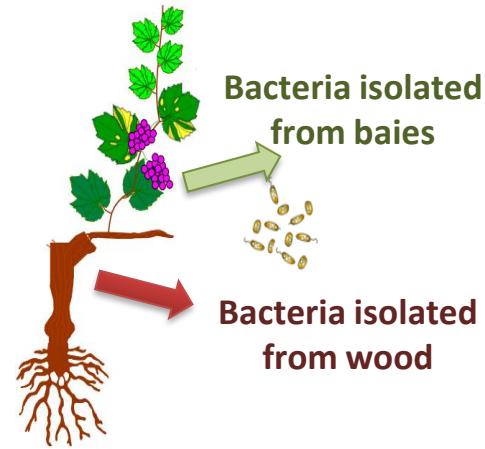


N. parvum

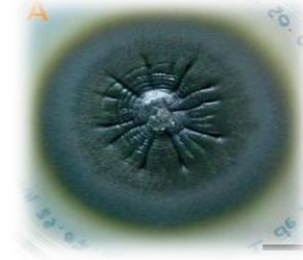


✓ bacterial efficiency dependent on the inoculation method

✓ Drenching the plant soil with the same bacterial strains was less efficient than the application in the hole



N. parvum (Np)



P. chlamydospora (pch)

1

Evaluation, *in planta*, of the antagonistic activity of 46 bacterial strains against *P. chlamydospora* and *N. parvum*

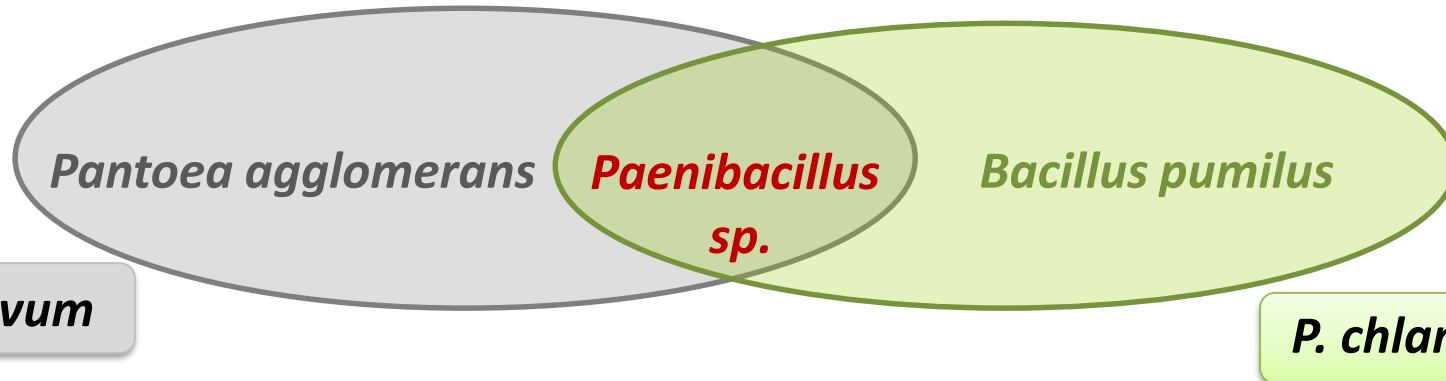
2

Evaluation of the effect of application method on biocontrol efficacy of 9 selected strains

3

Identification of the modes of action for 3 selected strains

3 selected strains:



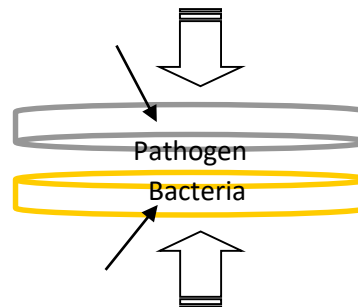
Modes of action of selected bacteria

Induction of grapevine defense



qPCR

Production of volatile compounds

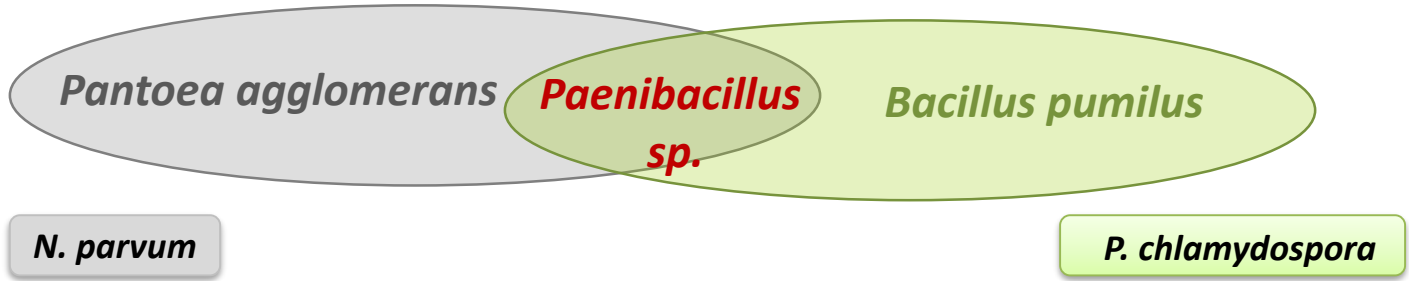


Production of diffusible compounds



confrontation

3 selected strains:

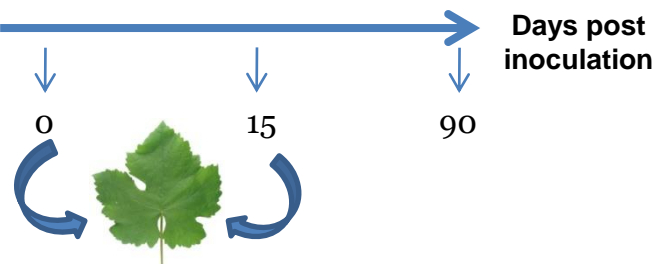


Modes of action of selected bacteria

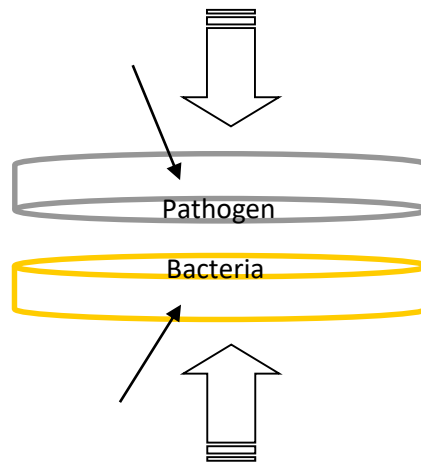
Induction of grapevine defense



qPCR



Production of volatile compounds



Production of diffusible compounds



confrontation

Bact/Path
Prev hole



Bact/Path
Co-inoc

Results

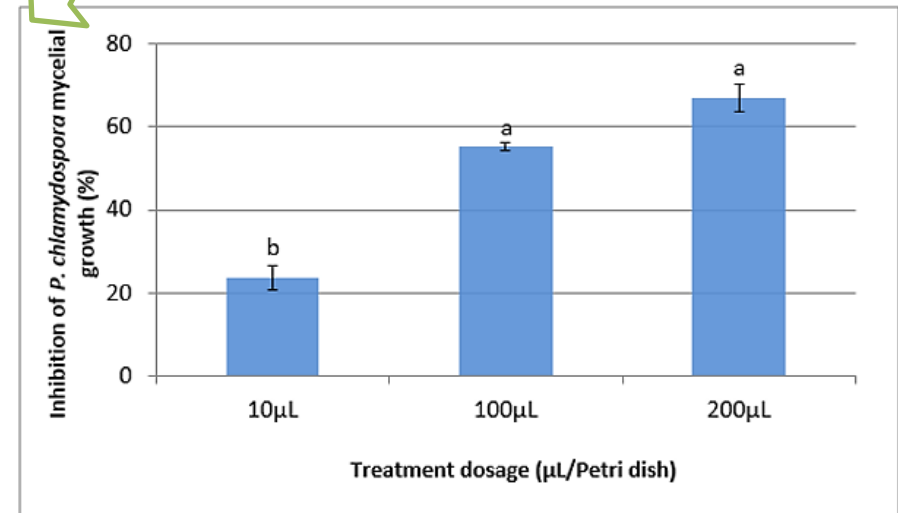
Production of volatile compounds

Bacterial strain	Volatile compound	Retention time (minute)	Molecular weight (g/mol)
S19 (<i>Paenibacillus</i> sp.)	Compound of pyrazine type	12.8	-
	2,6-Bis (2-methylpropyl) pyrazine	12.4	192.3
	1-Octen-3-ol	6.9	128.22
	2,5-dimethyl Pyrazine	5.4	108.14
S32 (<i>Bacillus pumilus</i>)	3-octanone	6.6	128.21
	trimethyl-pyrazine	6.8	122.17
	2-ethyl-3,5-dimethyl pyrazine	8.1	136.19
	S1 (<i>P. agglomerans</i>)	Phényl éthyl alcohol	8.6

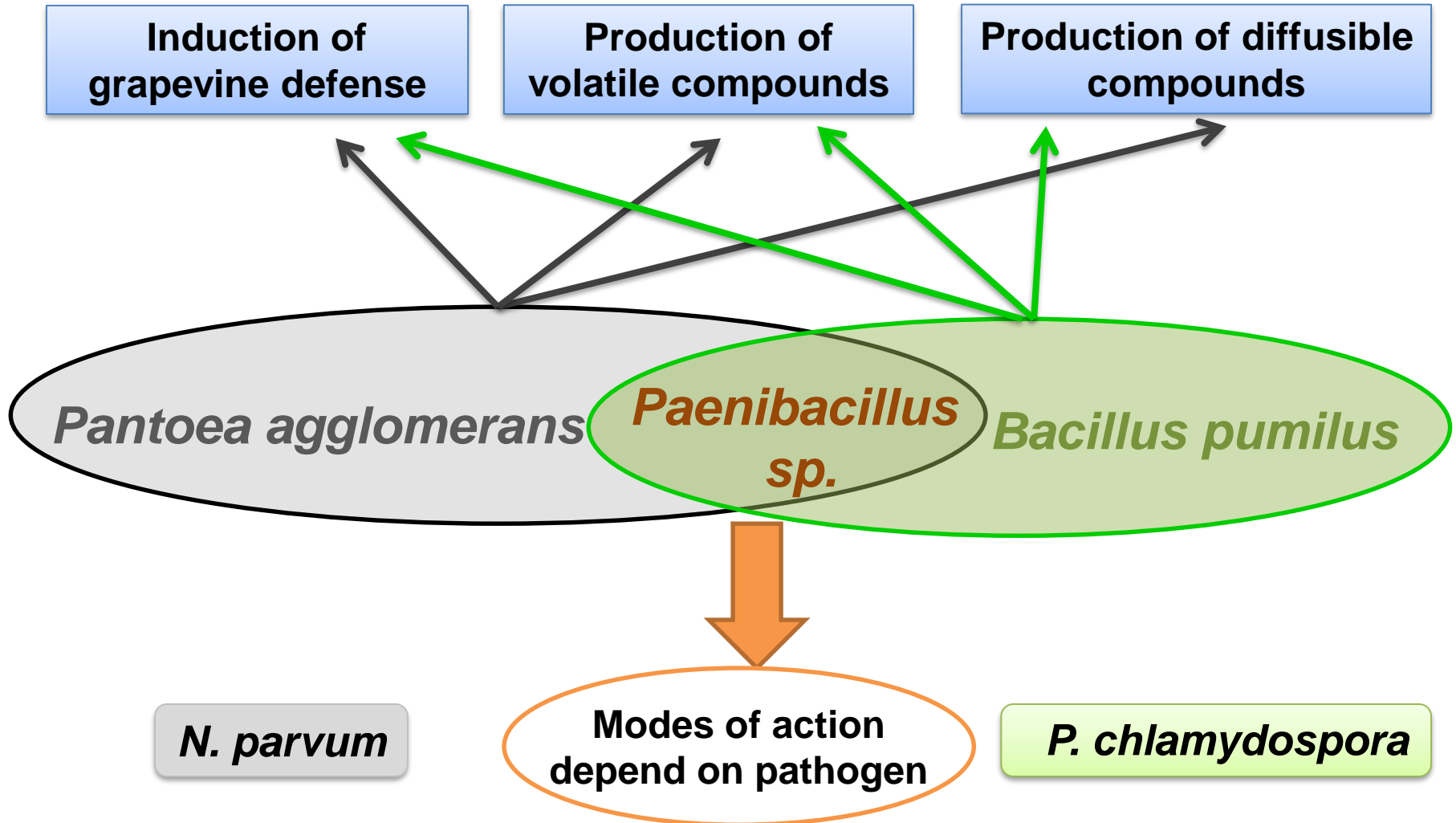


Gas Chromatography-Mass Spectrometry

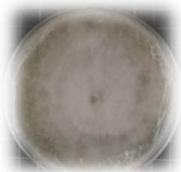
1-Octen-3-ol:
Inhibition of Pch
>96%



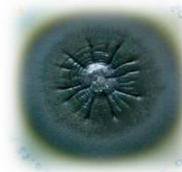
2,5-dimethyl pyrazine

3 selected strains:

Conclusions



N. parvum



P. chlamydospora

1

➤ The most efficient strains:
Enterobacterales

➤ Some bacterial strains increase *N. parvum* necrosis



➤ The most efficient strains:
Bacillales

No

2

➤ Bacterial efficiency dependent on the inoculation method



No

3

No

➤ *Paenibacillus* sp. inhibits *Np* by the induction of grapevine defense



➤ *Paenibacillus* sp. inhibits *Pch* by production of volatile compounds

No



**Marc Fermaud, Alain Deschamps
(Supervisors)
Patrice Rey
Jean Roudet
Emilie Bruez
Jessica Vallence**



Casdar V1302





Thanks for your attention



PR proteins	<i>VvPR1</i>	PR protein class1
	<i>VvPR10</i>	PRprotein class10
	<i>VvCHIT3</i>	ChitinaseclassIII
	<i>VvGLU</i>	β -1,3glucanase
cell wall reinforcement	<i>VvCALS</i>	Callosesynthase
Redox status	<i>VvGST</i>	GlutathioneS-transferase
Indole and phenylpropanoid pathways	<i>VvANTS</i>	Antranilatesynthase
	<i>VvSTS</i>	Stilbenesynthase
	<i>VvCHS</i>	Chalconesynthase
	<i>VvPAL</i>	Phenylalanineammonialyase

