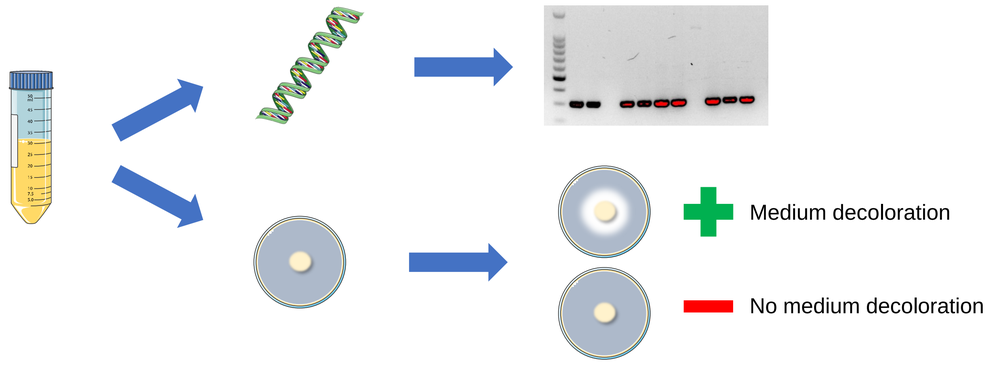
Antagonistic activity of *Bacillus mycoides* against potato late blight

Vivien Le Vourch[1], Louis Morandini[2], Jacques Mahillon[2] and Anne Legrève[1]

[1] Laboratory of Plant Health and [2]Laboratory of Food and Environmental Microbiology, Earth and Life Institute, Université catholique de Louvain, Croix du Sud 2 - box L7.05.03, 1348 Louvain-la-Neuve, Belgium

Introduction

Potato is a major crop in Belgium (100,000 ha in 2020) and still requires the use of huge amounts of pesticides. Today, the development of new environmental-friendly approaches is greatly encouraged. While bacteria from *Bacillus subtilis* group are well studied for biocontrol application, other *Bacillus* are left out. *Bacillus mycoides* is a rhizoid growing species of the *Bacillus cereus* group. Its potential to control plant diseases and the underlying mechanisms have been rarely reported in the literature [1,2] and remain largely unknown. This work investigates the activity of three *B. mycoides* strains against *Phytophthora infestans,* the causal agent of potato late blight.

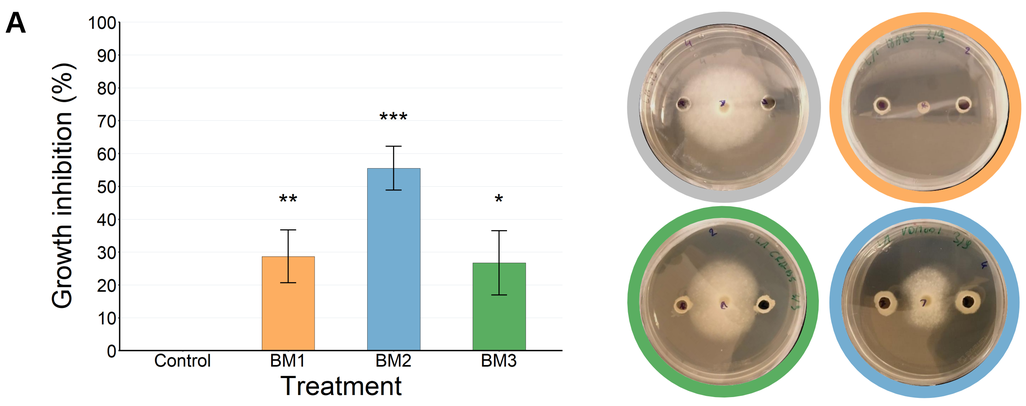


The three *B. mycoides* strains (BM1-3) were analysed by PCR for the detection of genetic determinants linked to antagonistic metabolites using previously described primers [3,4]. *In vitro* analyses for the production of bioactive enzymes (chitinase, cellulase or protease) were also performed on specific media. Our strains displayed the same profiles of cellulolytic and proteolytic activities. No genetic determinants related to virulence factors of *B. cereus s.l.* were detected by PCR.

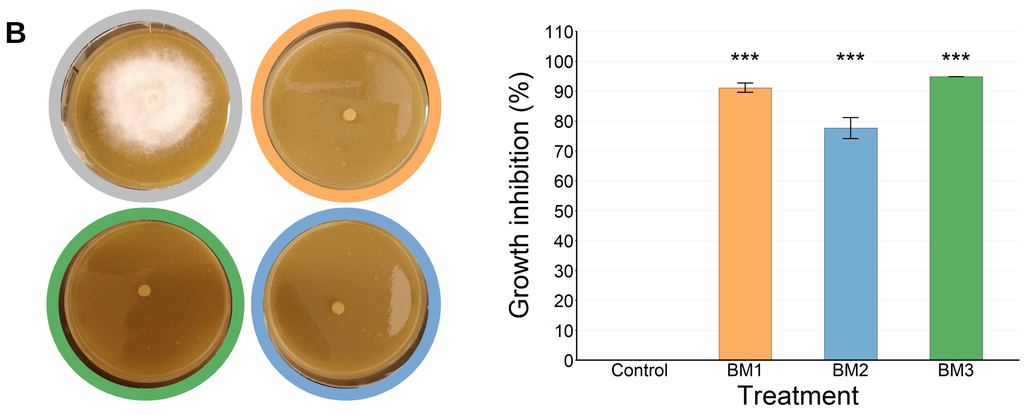
Molecular and PCR characterization of the three *B. mycoides* strains

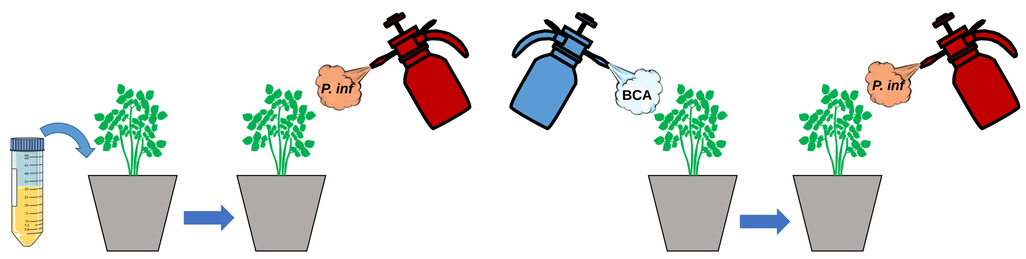
	Detection of the genetic determinants of antagonistic activity														
	Enzymes					**Hybrids PKs/NRPs**	**Lipopeptids**	**Potential virulence factors**				**Degrading enzymes**		
Strains	Exochitinase		AHL-lactonases	Malonyl-CoA ACP transacylase	Glucanase	Zwittermicin A	Kurstakin	Cereulide		Cytotoxin K1	Cytotoxin K2	Chitinase	Protease	Cellulase
BM1	-	-	-	-	-	-	+	-	-	-	-	-	+	+
BM2	-	-	-	-	-	-	+	-	-	-	-	-	+	+
BM3	-	-	-	-	-	-	+	-	-	-	-	-	+	+
	chi1 / chi3	chi 36 -F /-R	Lac -F /-R	Malacptransac -F /-R	Glucanase -F /-R	A0677 / A0678	AH -F/-R	CesF1/CesR2	EM1 -F /-R	cytK1 -F /-R	cytK2 -F /-R			
	Primers [3,4]													

Growth inhibition of *P. infestans* caused by the three *B. mycoides* strains BM1, BM2 and BM3.



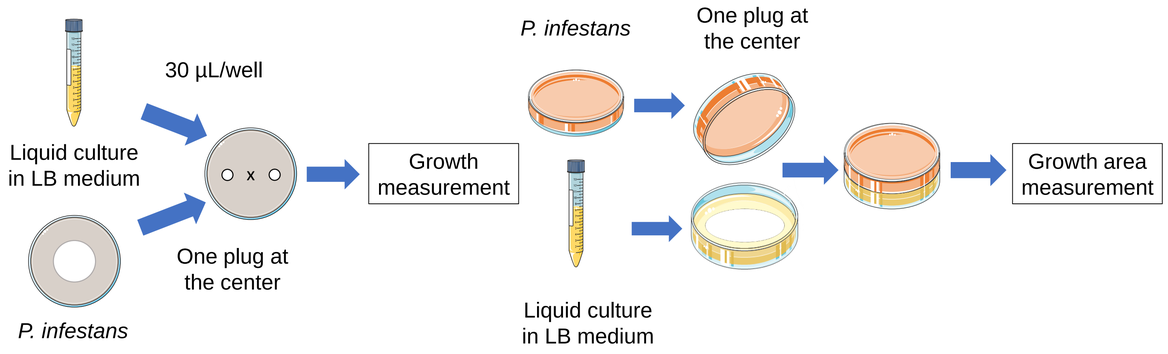
Growth inhibition of *P. infestans* caused by VOCs the three *B. mycoides* strains BM1, BM2 and BM3.





In vivo experiments were conducted in order to assess the antagonistic effect of the three tested strains against *P. infestans* on the sensitive « Bintje » potato variety. Bacterial suspension were either applied on roots or on foliage, six hours before *P. infestans* inoculation. Each treatment was applied on five plants. Late blight symptoms were monitored on three leaves per plant during four days after the onset of the disease. Mean values of Area Under the Disease Progress Curve (AUDPC) were calculated to compare treatments to the non treated control. None of the strains were able to control late blight through root application, but they all reduced significantly the disease progress when applied on leaves.

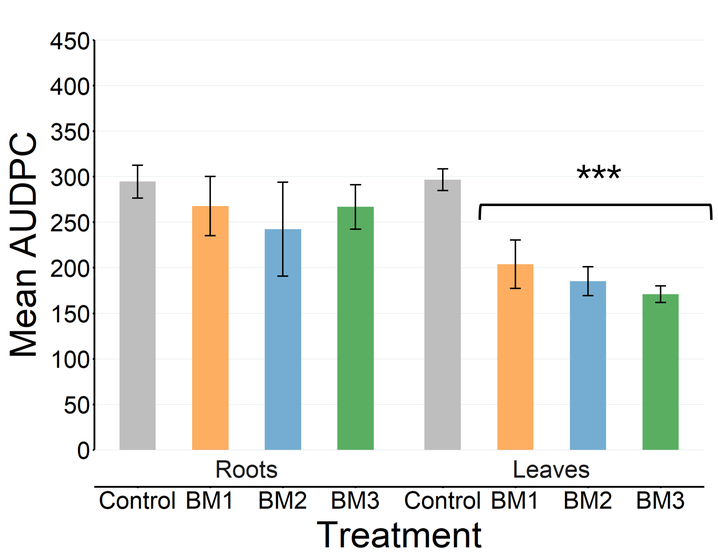
BIOACTIVITY



Antagonism through diffusion of metabolites (**A**) was tested by direct confrontation with the pathogen on PDA medium. At the same time, antagonism by emission of Volatile Organic Compounds (VOCs) (**B**) was tested through a “double dish” experiment on LBA *(B. mycoides*) and V8 (*P. infestans*) media. Both experiments were conducted three times with five replicates. Plates were scanned and growth inhibition percentages were calculated through image analysis. The three strains were able to inhibit the growth of *P. infestans* through metabolites (BM1: 28,7%, BM2: 55,5% and BM3: 26,7%) and by VOC emission (BM1: 91,1%, BM2: 77,6% and BM3: 94,8%)

IN PLANTA EFFECTS

Effect of foliar and root treatments of BM1, BM2 and BM3 on late blight severity



Conclusion and Perspectives

This work shed a light on the potential of *B. mycoides* strains to control potato late blight. The three tested strains were able to inhibit the growth of *P. infestans in vitro* and to limit potato late blight severity. The study of *B. mycoides* could lead to the discovery of new biocontrol mechanisms.

[1] Bargabus, R. L., Zidack, N. K., Sherwood, J. E., & Jacobsen, B. J. (**2002**). Mol Plant Pathol 61(5), 289-298.
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[3] Caulier, S. *et al*. (**2018**). Frontiers in Microbiology. 9(143)
[4] Abdellaziz, L., Chollet, M., Abderrahmani, A. *et al.* (**2018**). Arch Microbiol 200, 1205-1216

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