Antagonistic soil-borne *Bacillus* spp. and *Pseudomonas* spp. bacteria against late blight and other potato pathogens: From the laboratory to the field

Caulier[1,2], A. Gillis[2], G. Colau[1], F. Licciardi[2], M. Liépin[1], N. Desoignies[1], P. Modrie[2], A. Legrève[1], J. Mahillon[2]* and C. Bragard[1]*

Université catholique de Louvain, [1]Phytopathology and [2]Food and Environmental Microbiology Laboratories,
Earth and Life Institute, Louvain-la-Neuve, Belgium
simon.caulier@uclouvain.be

Introduction and Objectives

All over developed countries the potato crop has become a major consumer of chemicals turning it into an unsustainable production. Indeed, large amount of inputs are used to reach yield going over 40 T.ha^{-1} when the average production in developing countries barely reach 8 to 9 T.ha^{-1}. The main part of these inputs are fungicides used to control *Phytophthora infestans*, causing the destructive late blight disease. Its worldwide management has been estimated to cost over four billion dollars each year (Hijmans *et al.*,2000). In the perspective of developing a more sustainable approach of potato crop management, there is an increasing interest on the microbial alternative offered by plant growth promoting rhizobacteria able to improve crop yield and control pests and diseases.

The aim of this study was to investigate the potential of soil-borne bacteria to effectively control potato pathogens in field with a particular focus on potato late blight disease (Fig 2).

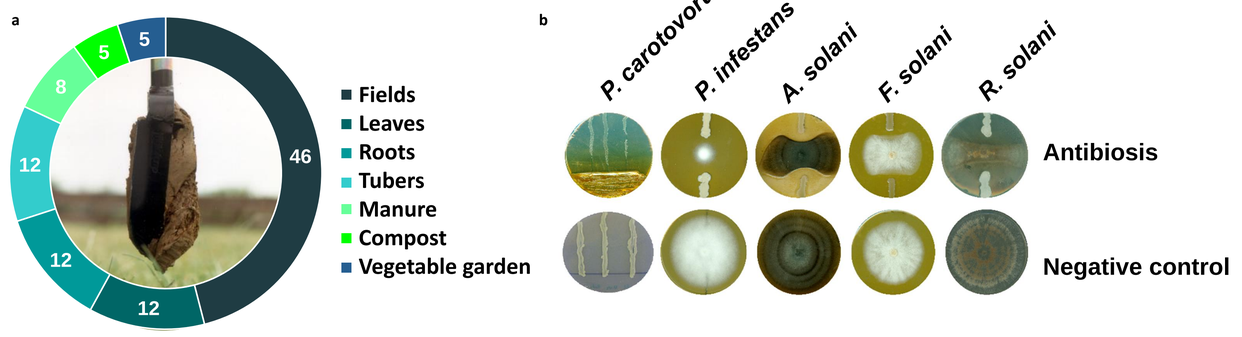


Figure 1: a) Samples distribution according the origins and **b)** *in vitro* confrontation allowing the determination of growth inhibition percentage (GIP) performed through image analysis by comparing the area covered by tested pathogen with negative control. Each test was done three times, in triplicate.

Isolation and characterization

A collection of 2.826 bacterial strains was obtained from 157 samples taken mainly from potato field soils but also in potato plant tissues, manure and composts (Fig 1a). Isolates were screened for their *in vitro* antagonistic activities toward at least one of the five pathogens tested : *A. solani*, *F. solani*, *R. solani*, *P. carotovorum*, *P. infestans* (Fig 1b). The screening consists in an *in vitro* confrontation with the pathogen in triplicate. Plates were then scanned and a growth inhibition percentage (GIP) was calculated through image analysis.

Fifty-two *Bacillus* spp. and eight *Pseudomonas* spp. strains showing GIP ≥ 50% were selected for further characterization (Table 1):

- Identification through 16S rRNA gene sequencing
- PCR screening for genetic determinants involved in the biosynthesis of known bio-active metabolites and virulence factors
- *In vitro* screening on specific media for the production of siderophores, cellulolytic, chitinolytic and proteolytic enzymes
- Drop-collapse screening for bio-surfactant production

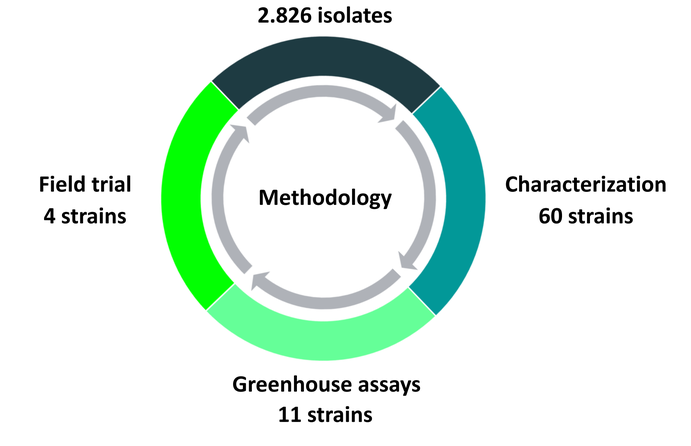


Figure 2: Methodology applied in this work starting with bacterial isolation in field and ending in a pilot field assay.

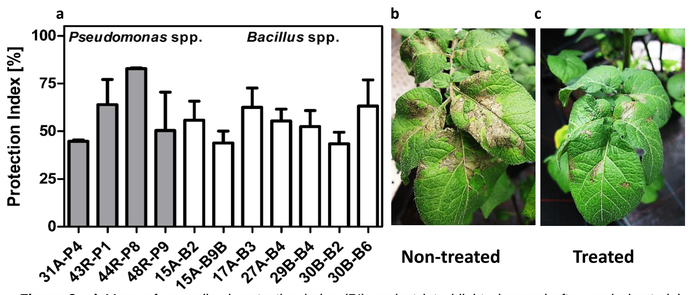


Figure 2: a) Mean of normalized protection index (PI) against late blight observed after crude bacterial suspension foliar sprayed on potato plants (standard deviation based on four assays). Late blight progression observed on: **b)** sensitive 'Bintje' variety of potato plant and **c)** potato plants previously treated with *P. protegens* 44R-P8 suspension.

Greenhouse assays

In vivo experiments were performed in order to evaluate the antagonistic effect of seven *Bacillus* spp. and four *Pseudomonas* spp. showing high *in vitro* antagonistic activities (GIP ≥ 70%) against *P. infestans* on the sensitive "Bintje" potato variety. These assays conducted under controlled conditions were designed and tested beforehand to ensure a well-established and reliable pathosystem (Fig 3). Results coming from 72 replicas for each tested strain, distributed among four assays, confirmed their strong antagonistic activities. Indeed as shown in Figures 3a,b and c three *Bacillus* spp. (30B-B6, PI: 67%; 15A-B2, PI: 65%; 17A-B3, PI: 62%) and two *Pseudomonas* spp. (44R-P8, PI: 83%; 43R-P1, PI: 64%) gave significant protection index (PI > 60 %) against late blight, as calculated on the basis of normalized area under disease progression curve.

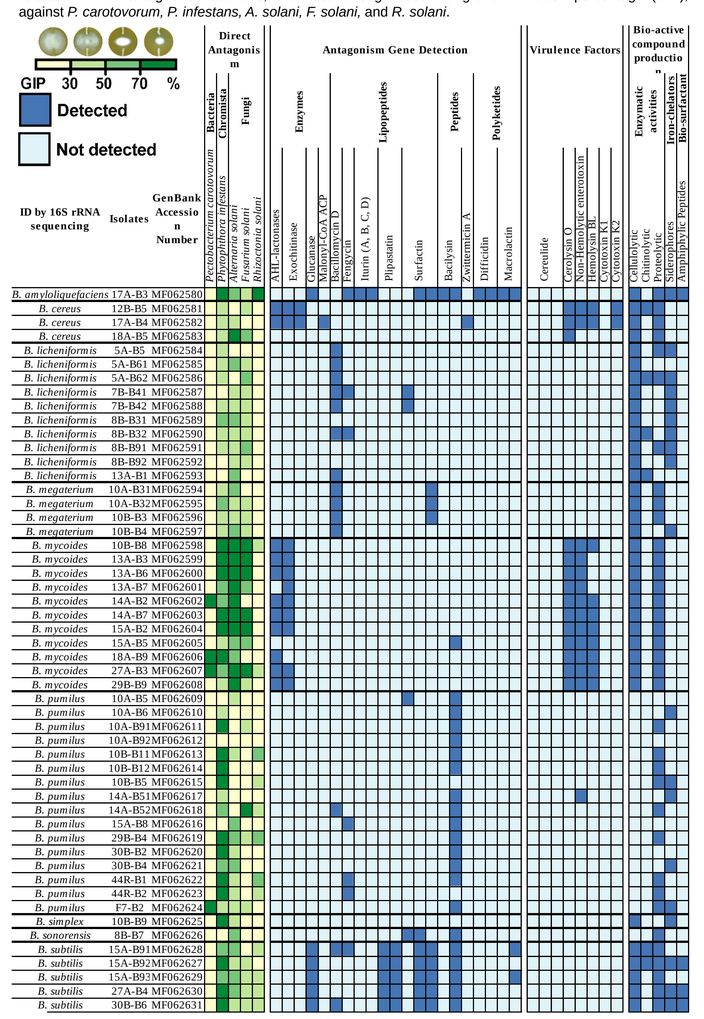
Pilot field trial

A field assay designed in randomized complete block aimed at assessing four selected bacteria effectiveness as bio-control agents of *P. infestans*. Crude bacterial cultures suspensions were foliar-sprayed once a week for a total of 12 applications along the potato crop growing season. Fungicides were applied six times following Belgian warning prediction system (CARAH, PCA). The protection conferred by bacterial treatments was less effective than fungicide treatments at any time in the assay. However, the bacterial treatments with *P. protegens* 44R-P8 strain provided a significant protection for 16 days after the first signs of symptoms (Fig. 3b-d) and up to 45 days with *B. subtilis* 30B-B6 (Fig. 3b-f). Moreover, treatment with 30B-B6 strain allowed shifts in time of five days to reach 25% and 50% of infection compared to controls. Interestingly, protection conferred by the treatment with *B. subtilis* 30B-B6 stabilized around 20% for nearly 30 days from day 14 to day 42 (Fig. 3g). At the end of the trial, the treatment with *B. subtilis* 30B-B6 allowed a statistically significant reduction of late blight severity (PI: 22%; p-value < 0.001).

Conclusions

- In this study the **prevalence of bacilysin** related genes was highlighted in *B. pumilus* and *B. subtilis* strains showing strong *in vitro* inhibition against *P. infestans*.
- *B. amyloliquefaciens*, *B. subtilis* and *Pseudomonas* spp. strains producing **both bio-surfactants and siderophores** were shown to be the most effective against *P. infestans* (GIP ≥ 70%).
- The interest in *B. mycoides* strains with a strong *in vitro* antagonistic activity against *P. infestans*, has been raised and is thought to be **mediated by competition for surface colonization**.
- Taken together, this work highlights the benefit to **combine molecular screening** for bio-active metabolites with ***in vitro*** and **greenhouse assays to successfully select field effective biocontrol agents.**

Table 1: Full characterization of *Bacillus* spp. strains isolated in this study and selected for their statistically relevant *in vitro* antagonistic activities, evaluated through observed growth inhibition percentage (GIP), against *P. carotovorum*, *P. infestans*, *A. solani*, *F. solani*, and *R. solani*.



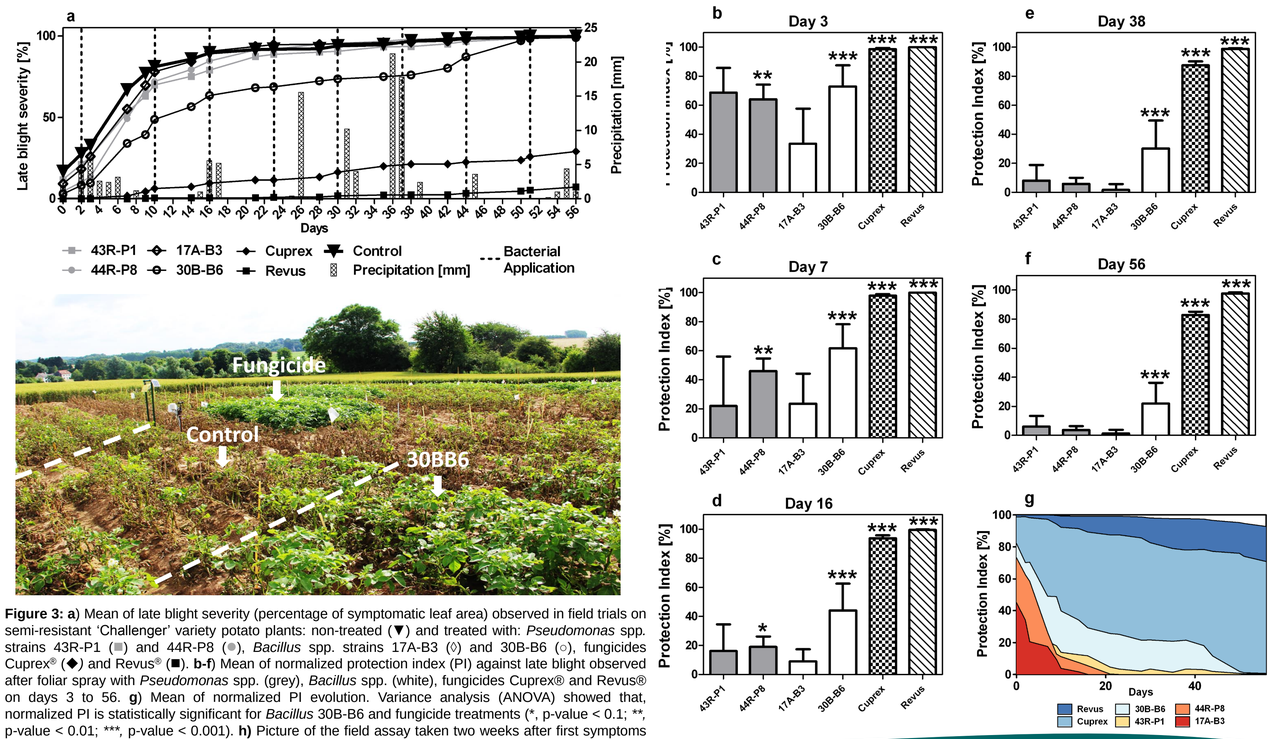


Figure 3: a) Mean of late blight severity (percentage of symptomatic leaf area) observed in field trials on semi-resistant 'Challenger' variety potato plants: non-treated (▼) and treated with: *Pseudomonas* spp. strains 43R-P1 (■) and 44R-P8 (●), *Bacillus* spp. strains 17A-B3 (◊) and 30B-B6 (○), fungicides Cuprex® (◆) and Revus® (■). **b-f**) Mean of normalized protection index (PI) against late blight observed after foliar spray with *Pseudomonas* spp. (grey), *Bacillus* spp. (white), fungicides Cuprex® and Revus® on days 3 to 56. **g**) Mean of normalized PI evolution. Variance analysis (ANOVA) showed that, normalized PI is statistically significant for *Bacillus* 30B-B6 and fungicide treatments (*, p-value < 0.1; **, p-value < 0.01; ***, p-value < 0.001). **h)** Picture of the field assay taken two weeks after first symptoms appearance.

*References:

Arguelles-Arias, Anthony, et al. "*Bacillus amyloliquefaciens* GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens." *Microb Cell Fact* 8.63 (2009): 1-12.

Caulier, S., Gillis, A., Colau, G., Licciardi, F., Liépin, M., Desoignies, N., Modrie, P., Legrève, A., Mahillon, J. & Bragard, C. (2018). Versatile Antagonistic Activities of Soil-Borne *Bacillus* spp. and *Pseudomonas* spp. against *Phytophthora infestans* and Other Potato Pathogens. Frontiers in microbiology, 9, 143.

Hijmans, R. J., Forbes, G. A., & Walker, T. S. (2000). Estimating the global severity of potato late blight with GIS-linked disease forecast models. Plant Pathology, 49(6), 697-705.

Raaijmakers, J. M., de Bruijn, I., & de Kock, M. J. (2006). Cyclic lipopeptide production by plant-associated Pseudomonas spp.: diversity, activity, biosynthesis, and regulation. Molecular Plant-Microbe Interactions, 19(7), 699-710.

Acknowledgments

S. Caulier is a fellow of the *Fonds de Formation à la Recherche en Industrie et Agriculture* (FRIA)