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
Can biopesticides replace chemical fungicides?

- Food safety
- Efficacy
- Cost
- “Easy use”
- Effects on non-targets
- Environmental threats
- Human health
- Resistances appearance
- Survival during storage
- Homologation process
- Efficacy in field
- Toxicity
- Diversity of modes of action
- Knowledges
- Less resistances
- Environmentally friendly
- Delay chemical pesticide use
- Less dangerous for humans

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)

- Direct antagonistic effects
- Production of antimicrobial compounds

In vitro

In vivo

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.
Characteristics of a good biocontrol agent (BCA)

- **In vitro**
  - Direct antagonistic effects
  - Production of antimicrobial compounds

- **In vivo**
  - Plant defences stimulation

- **Indigenous**
  - BCA
  - 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

\[
\text{Growth Inhibition Percentage} = \left(1 - \frac{S_{\text{strain}}}{S_{\text{control}}} \right) \times 100
\]

Caulier et al., 2018, Frontiers in Microbiology, 9, 143-158
Characteristics of the strain 30B-B6

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**Direct Antagonism**

- Bacteria
  - *Pseudomonas campestris*
  - *Pseudomonas putida*
- Chromista
  - *Alteromonas sp.
- Fungi
  - *Fusarium solani*

**Antagonism Gene Detection**

- Enzymes
  - Exochitinase
  - Chitinase
- Lipopeptides
  - Bacilysin
  - Surfactin
- Peptides
  - Zwitterlicnine A
  - Difidocilin
- Polymyxins
  - Macrolactin

**Virulence Factors**

- Bio-active compound production
- Enzymatic activities
- Translocators
- Bio-surfactants

**ID by 16S rRNA sequencing**

- B. subtilis 30B-B6 MF062631

Caulier et al., 2018, Frontiers in Microbiology, 9, 143-158
Characteristics of the strain 30B-B6

**In vitro**

- **Direct Antagonism**

- **Antagonism Gene Detection**
  - Enzymes
  - Lipopeptides
  - Peptides
  - Polypeptides

- **Virulence Factors**
  - Bio-active compound production

**In vivo**

- **Direct Antagonism**
  - Greenhouse trials
  - Field trial

Caulier et al., 2018, Frontiers in Microbiology, 9, 143-158
Pilot field trial (2016)

![Graph showing late blight severity and precipitation over days, with treatments indicated.]

July, 8th

Caulier et al., 2018, Frontiers in Microbiology, 9, 143-158
Versatile Antagonistic Activities of Soil-Borne *Bacillus* spp. and *Pseudomonas* spp. against *Phytophthora infestans* and Other Potato Pathogens

Simon Caulier¹,², Annika Gillis², Gil Colau¹, Florent Licciardi², Maxime Liépin¹, Nicolas Desognières¹, Pauline Modrie², Anne LeGrève¹, Jacques Mahillon² and Claude Bragard¹*

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?

**SAR:** Systemic Acquired Resistance

** ISR:** Induced Systemic Resistance

*Salicylic Acid (SA)*

*Jasmonic Acid (JA)*

*Ethylene (ET)*

**BCA**

**Plant defences stimulation**

**Indigenous, present in the environment**

Study on a model plant: *Solanum lycopersicum*

**Non-pathogenic rhizobacteria or fungi (PGPF/PGPR)**

**Crosstalk**

**Induction of defence mechanisms**
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

A. solani
Conidia suspension
15 000 c/mL

6 h
Symptoms quantification
Stimulation of systemic plant resistance

Evolution of the symptoms

Area Under the Disease Progress Curve (% x day)

AUDPC

N=10
## Stimulation of systemic plant resistance

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<th>Treatments</th>
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<th>On leaves</th>
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<td>30B-B6_SR</td>
<td>Bacterial culture supernatant</td>
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### Legend:

- Alternaria solani

- No contact between bacteria and pathogen!

- Symptom quantification

**Inoculation of A. solani 6h post bacteria**
Stimulation of systemic plant resistance

- Bacterial suspension
Stimulation of systemic plant resistance

- Bacterial suspension
Stimulation of systemic plant resistance

- Bacterial culture supernatant

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

![Graph showing the evolution of disease severity over time](image)
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

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<th>Year</th>
<th>Period of year</th>
<th>Mean RH (%)</th>
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**Tukey test (p-value)**

- *** < 0.001
- ** < 0.01
- * < 0.05

**Evolution of the protection of tomato plants**

- **30B-B6_preventive**
- **30B-B6_curative**
- **30B-B6_supernatant**
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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