
ECOMONDO 8-10 GIUGNO - Difesa del Suolo e della Biodiversità

# IL MONDO DEI MICRORGANISMI DEL SUOLO

## Una risorsa da valorizzare per proteggere e salvaguardare la salute e la qualità dei suoli agricoli, le produzioni agroalimentari e l'ambiente

Webinar 9 Giugno 2021 - 10:00 - 12:00

ENEA
Agenzia nazionale per le nuove tecnologie, l'energia e lo sviluppo economico sostenibile
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SIMBA SANTA CHIARA LAB EJP SOIL EXCALIBUR MIRRI

Italian National Agency for New Technologies, Energy and Sustainable Economic Development

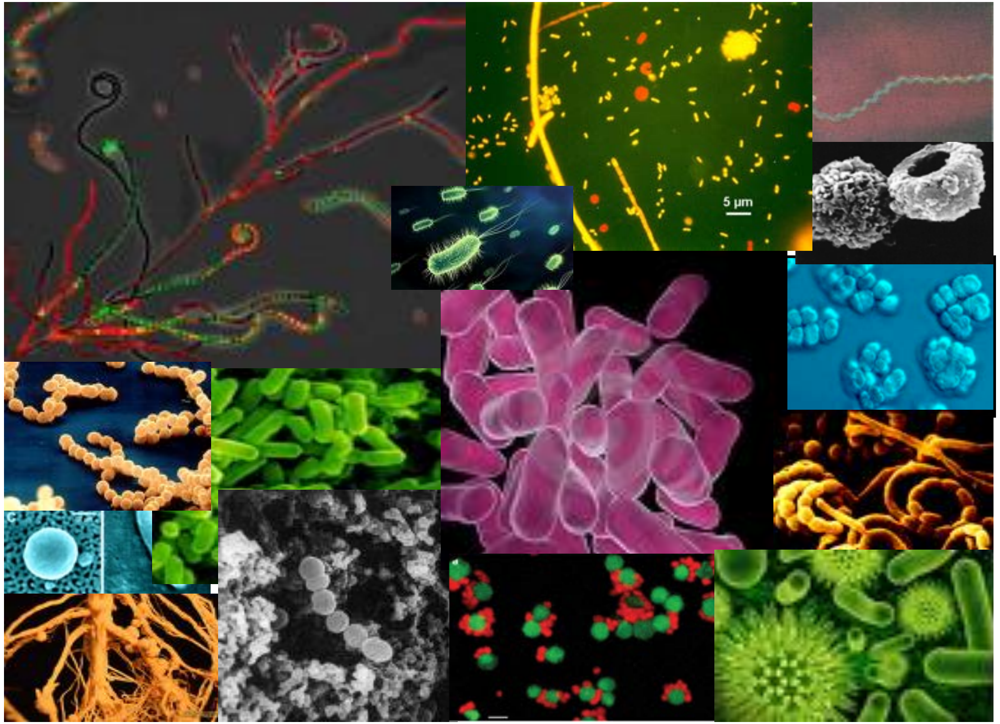
# Selezione e valorizzazione dei microorganismi benefici del suolo per un'agricoltura sostenibile: il progetto Horizon 2020 SIMBA

*ECOMONDO DIGITAL GREEN WEEKS 8-10 giugno 2021*

**Annamaria Bevivino, ENEA**

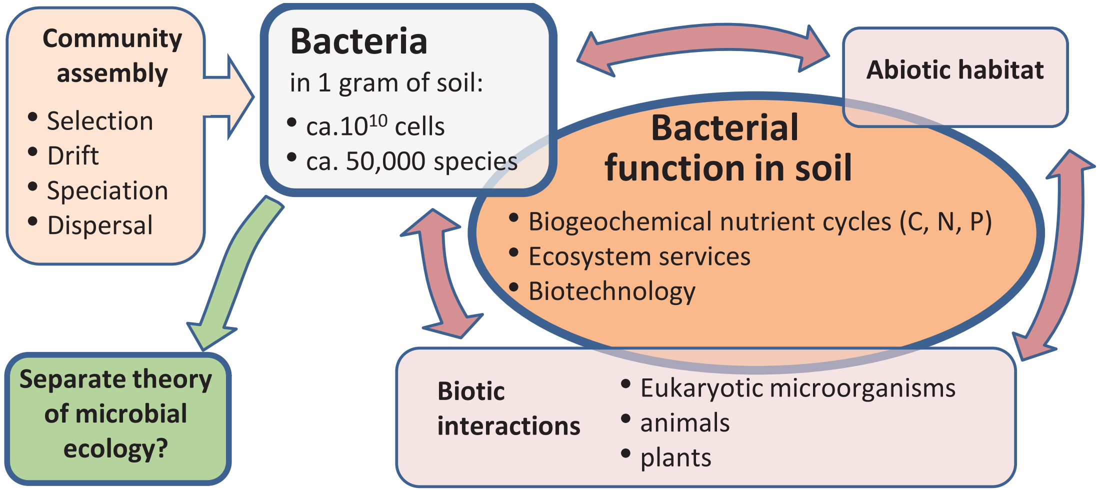
# Outline of my presentation

- The soil microorganisms: role and function
- Strategies to increase crop productivity: the microbiome-based solution
- The SIMBA Project: identification and selection of efficient inoculants
- Preliminary results from Work Package 2
- Ongoing activities



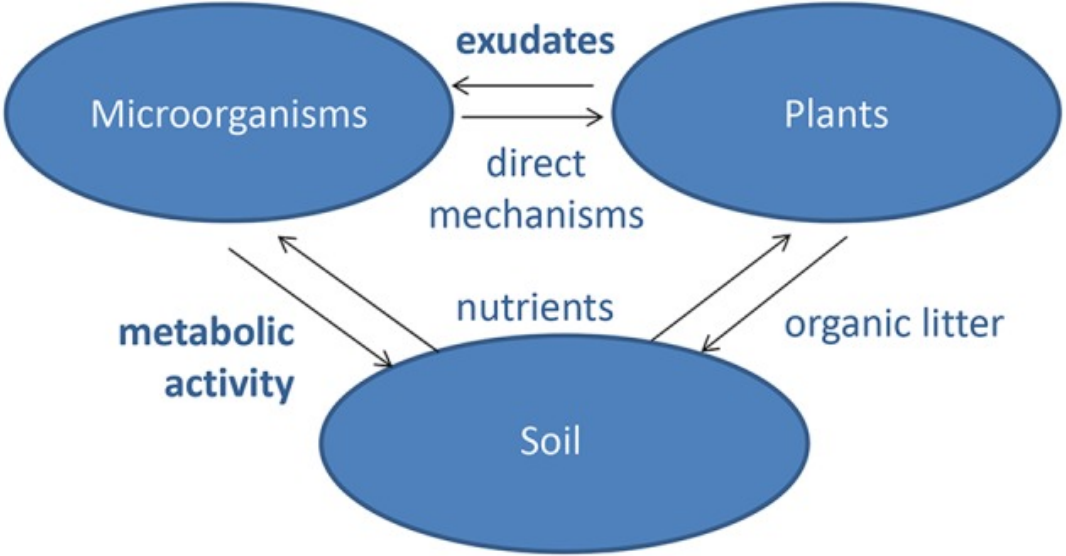
# The world beneath our feet

- Millions of microorganisms live and reproduce in a few grams of soil
- Microorganisms directly contribute to the biological fertility of soil
- They also play a central role in decomposing organic matter, in determining the release of mineral nutrients, and in nutrient cycling
- Changes in soil microbial community may directly affect soil ecosystem function, particularly carbon and nitrogen cycling

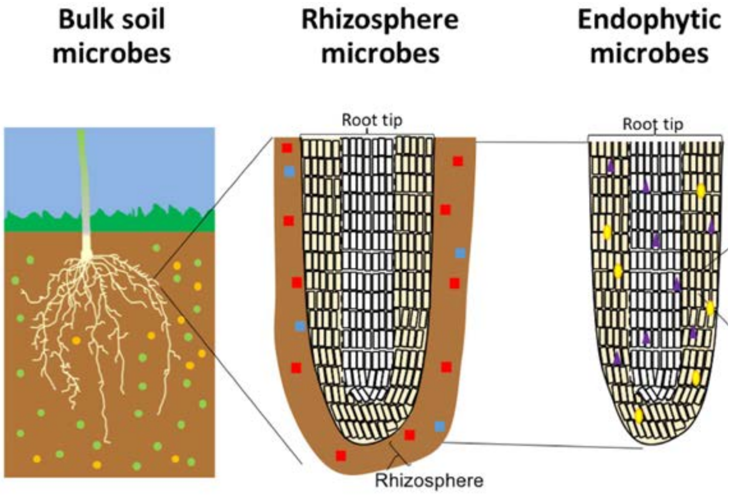



Modified from Johannes Sikorski 2015, The Prokaryotic Biology of Soil, Soil Organisms 87, 1-28

# The link between soil, microorganisms and plants

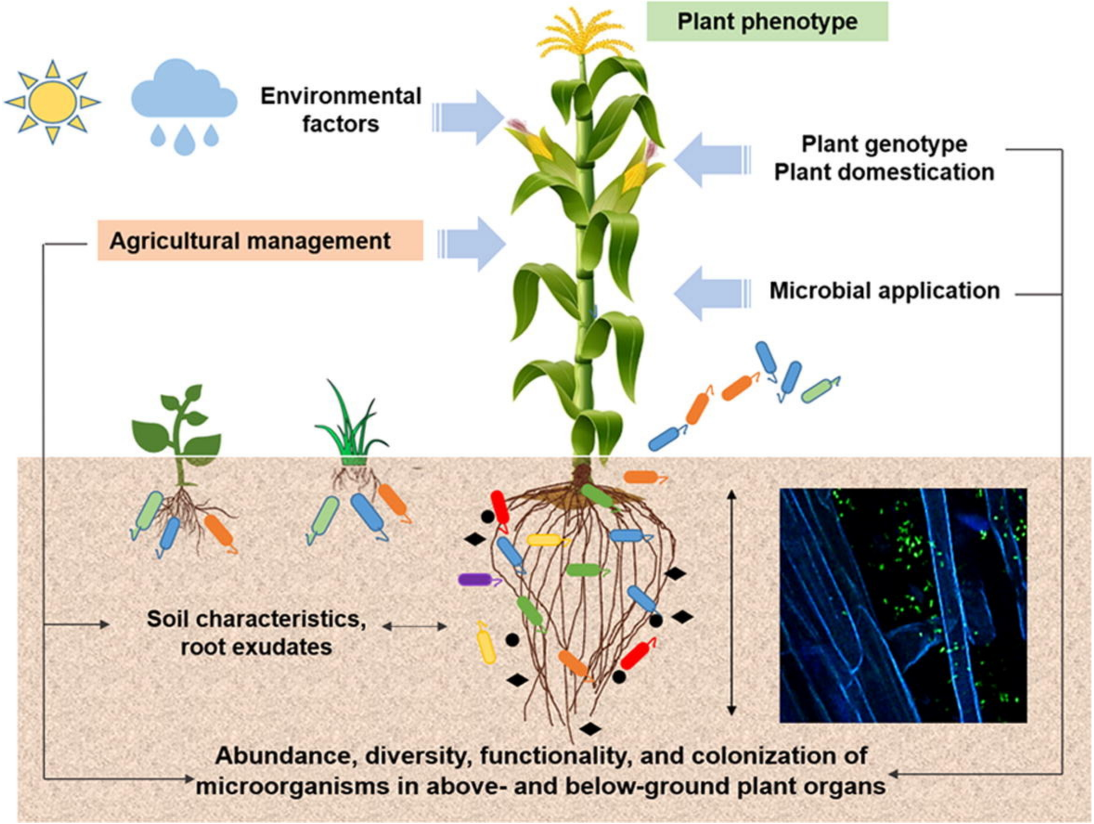



Jacoby et al. *Front Plant Sci*. 2017; 8:1617




Backer et al. (2018) *Front. Plant Sci.* 9:1473

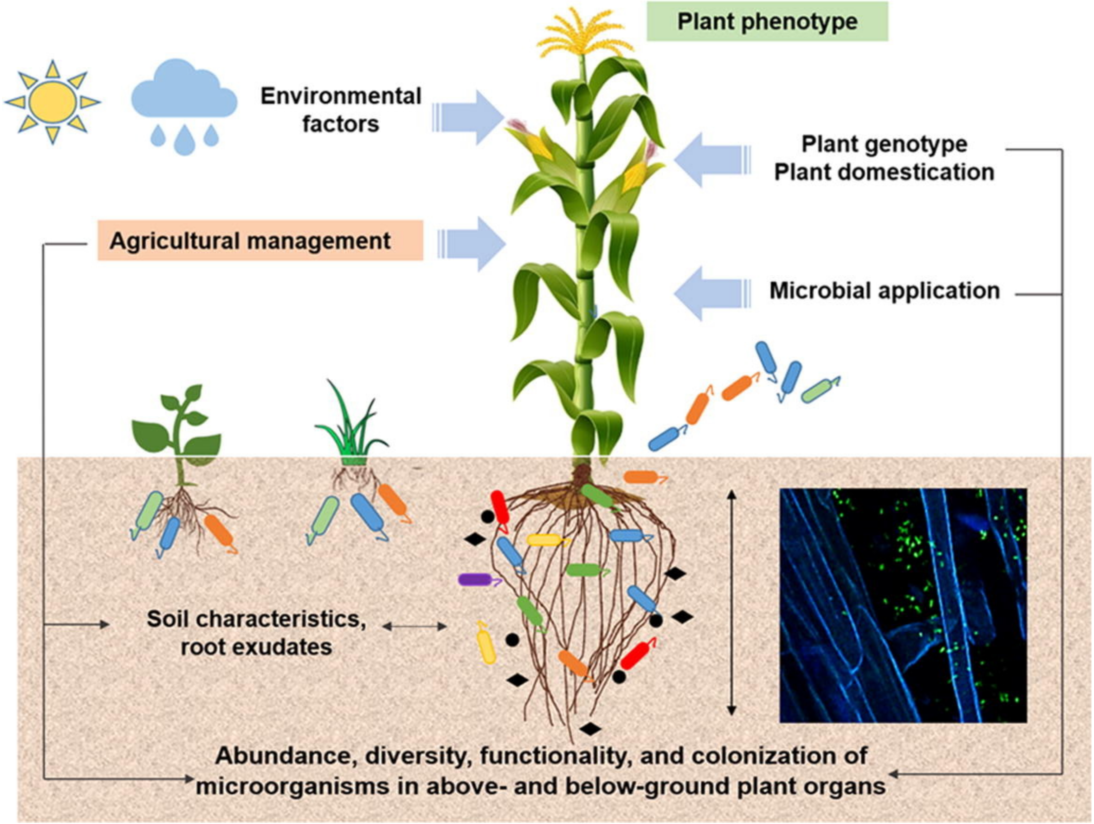
# How to increase crop productivity and soil fertility?




Compant *et al*., 2019 *J Adv Res 19:29-37*

- **Understanding soil-root microbiome diversity and function** to uncover novel microbes that can be used as biofertilisers and biopesticides
- **Promoting crop–microbe associations** through the development and optimisation of microbial inocula
- **Enhancing beneficial soil microbiome** diversity and function through optimising soil management methods

# How to increase crop productivity and soil fertility?

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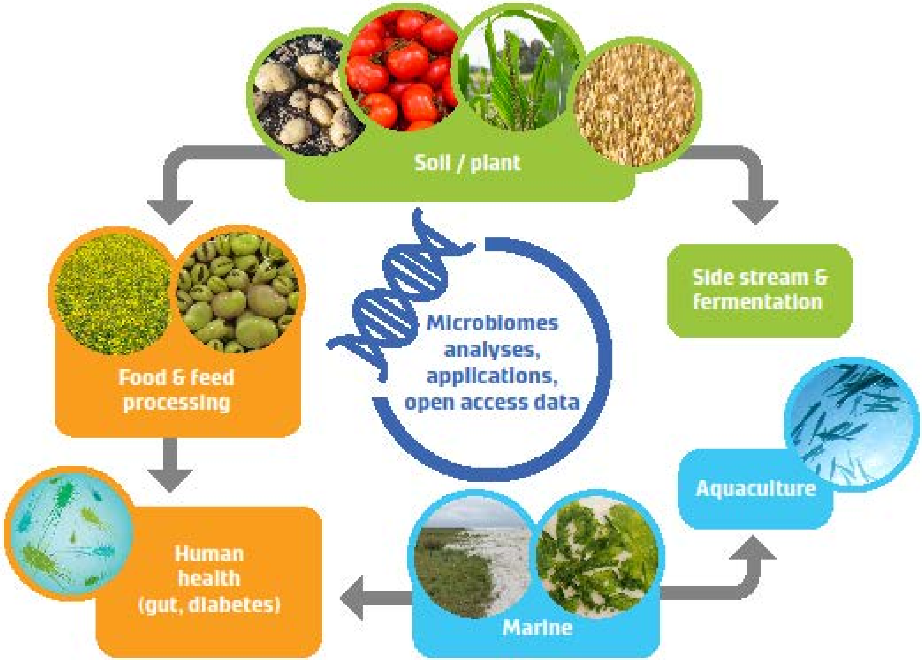
# THE SIMBA PROJECT

- Moving towards a more sustainable production system is one of the major challenges for the European Union food industry.
- The main goal of Horizon 2020 SIMBA project is to take advantage of microbiome support at all levels of the food chain.
- In this view, soil microbial applications appear a promising tool capable to foster a significant increase in crop yield and quality.
- https://vimeo.com/539607004




This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 818431 (SIMBA). This output reflects only the author's view and the Research Executive Agency (REA) cannot be held responsible for any use that may be made of the information contained therein.

# WP2 – Improvement of PGPMs field applications efficacy and reproducibility

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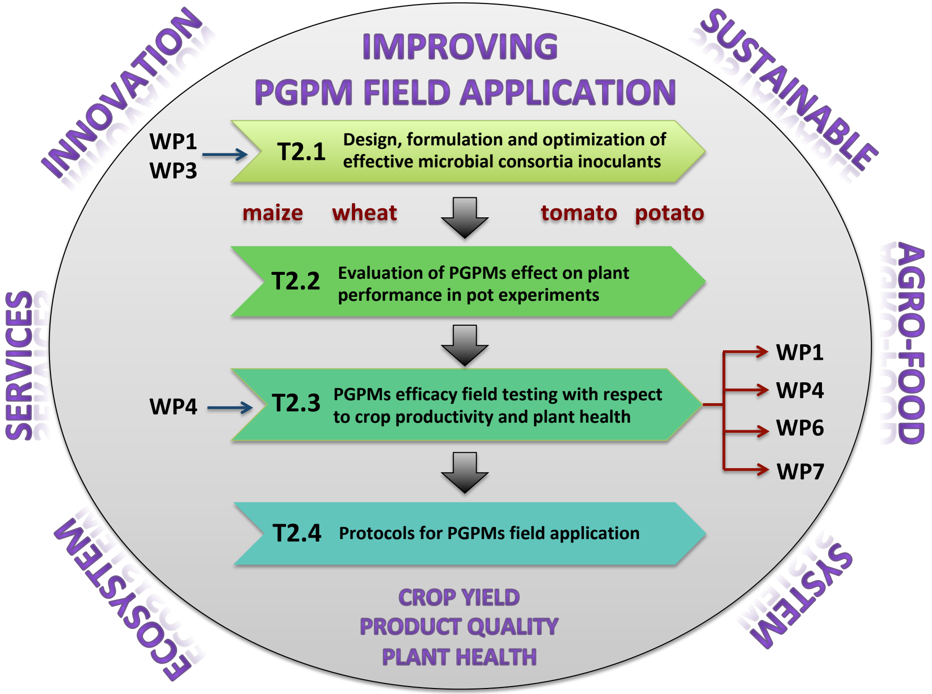


## Main Objective:

- to exploit the full potential of **Plant Growth-Promoting Microorganisms (PGPMs)** for sustainable crop production by optimising the efficacy and reproducibility of field applications.

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# 2.1 Design, formulation and optimization of Plant Growth-Promoting Microbes (PGPMs) for their use as microbial consortia inoculants

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## AIM OF THE PRESENT WORK:

Identify efficient microbial formulations to be applied as bioinoculants in arable crops in Italy and Germany, i.e. **WHEAT, MAIZE, POTATO and TOMATO**

- ✓ To improve soil fertility and functionality
- ✓ To enhance plant resistance to abiotic and biotic stresses
- ✓ To improve plant productivity for the the sustainable use of soil in different European farming system

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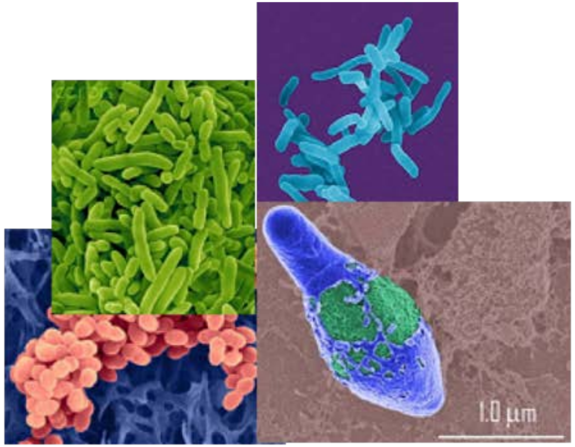
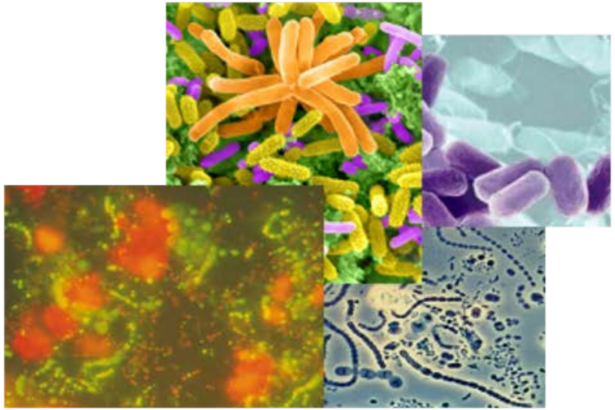
# How to choose efficient inoculants?

- The use of efficient inoculants is considered an important strategy for increasing crop productivity and reducing chemical inputs in agriculture
- When considering inoculation with PGPM, the first objective is to find the best bacteria available and to identify the best delivery method which determines the potential success of the inoculant




*Single inoculants*

?



*Microbial consortia*

# Microbial consortia for sustainable agriculture

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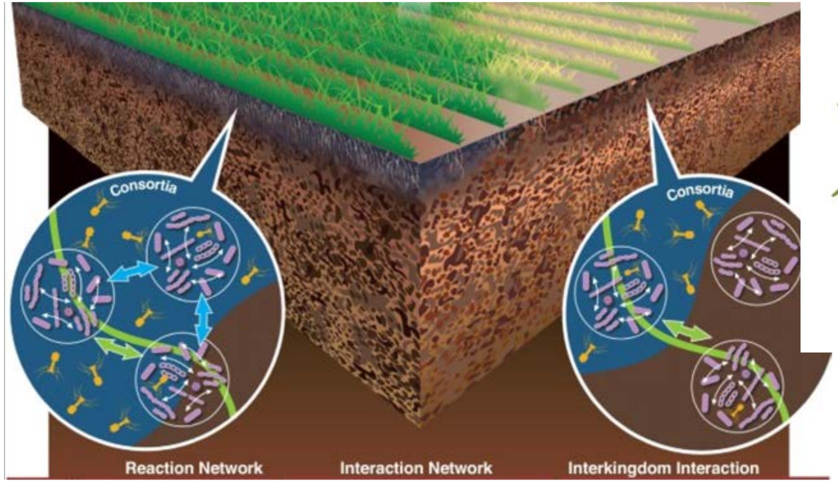
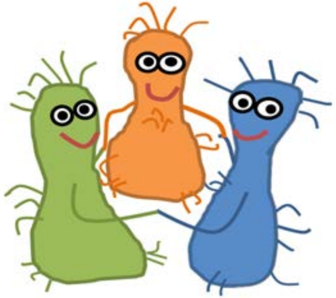
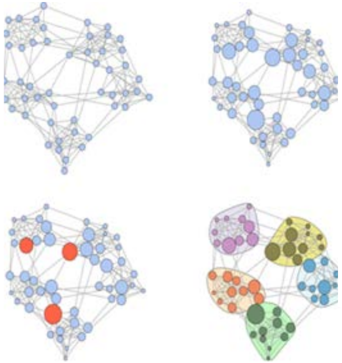
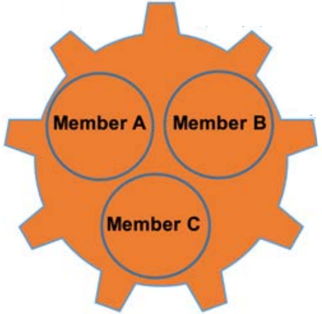
Microbial consortia have a higher potential to increase plant growth-promoting (PGP) effects compared to single inoculants








P solubilisation, $N_2$ fixation, PGP, Biocontrol activity

«microbial consortia” means not only living together but also labour division, leading to increased community efficiency and productivity.

microorganisms

MDPI

*Article*

# Identification of Beneficial Microbial Consortia and Bioactive Compounds with Potential as Plant Biostimulants for a Sustainable Agriculture

Silvia Tabacchioni [1,†], Stefania Passato [2], Patrizia Ambrosino [2], Liren Huang [3], Marina Caldara [4], Cristina Cantale [1,†], Jonas Hett [5], Antonella Del Fiore [1], Alessia Fiore [1], Andreas Schlüter [3], Alexander Sczyrba [3], Elena Maestri [4], Nelson Marmiroli [4], Daniel Neuhoff [5], Joseph Nesme [6], Søren Johannes Sørensen [6], Giuseppe Aprea [1], Chiara Nobili [1], Ombretta Presenti [1], Giusto Giovannetti [7], Caterina Giovannetti [7], Anne Pihlanto [8], Andrea Brunori [1] and Annamaria Bevivino [1,*]

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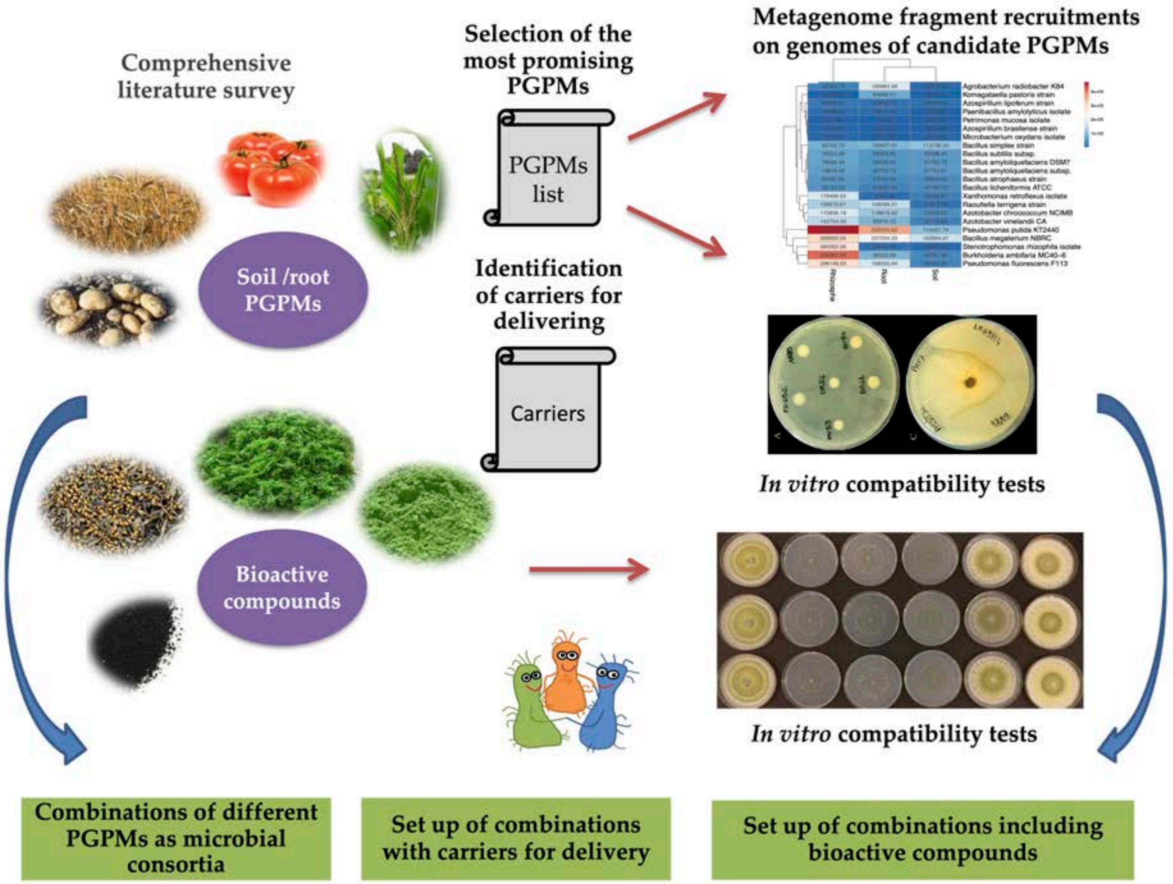
# Experimental design



# BOTTOM-UP APPROACH

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- Comprehensive literature survey
- Selection of the most promising microorganisms
- In vitro compatibility tests
- Set up of microbial consortia
- Set up of combinations with carriers for delivery
- Set up of combinations including bioactive compounds
- Greenhouse and growth-chamber experiments
- In field tests with abiotic stress
- Evaluation of performance

| Microorganism | Strain | Origin | Country of isolation | Properties |
|---|---|---|---|---|
| *Acaulospora morrowiae* | CL290 | Rhizosphere | STATI UNITI | PGP |
| *Agrobacterium radiobacter* | AR 39 | soil near peach tree | Ascoli Piceno, IT | biocontrol / PGP |
| *Azospirillum brasiliense* | CD/ATTC 29710 | *Cynodon dactylon* rhizosphere | USA | N-fixation |
| *Azospirillum brasiliense* | NCCB 78036 | soil under soy field | India | N-fixation |
| *Azospirillum lipoferum* | CRT1 | field grown maize | FR | N-fixation |
| *Azotobacter chroococcum* | 76A | soil | South IT | Nitrogen fixation |
| *Azotobacter chroococcum* | DSM 2286 | unknown | unknown | Nitrogen fixation |
| *Azotobacter chroococcum* | LS132 | Rhizosphere | South IT | N-fixation |
| *Azotobacter chroococcum* | LS163 | Rhizosphere | South IT | N-fixation |
| *Azotobacter chroococcum* | S-5 | unknown | Iran | N-fixation |
| *Azotobacter vinelandii* | DSM 2289 | unknown | unknown | N-fixation |
| *Bacillus* sp. | BV84 | Grape leafs | Ascoli Piceno, IT | biocontrol/PGP |
| *Bacillus amyloquefaciens* | BA41 | Wheat rhizosphere | Ascoli Piceno, IT | biocontrol/PGP |
| *Bacillus amyloquefaciens* | FZB42 | plant pathogen infested soil | DE | biocontrol/PGP |
| *Bacillus amyloquefaciens* | LMG 9814 | soil | UK | alpha-amylase , alpha-glucosidase, iso-amylase production |
| *Bacillus atrophaeus* | ABI02A | | Berlin, DE | PGP |
| *Bacillus licheniformis* | PS141 | Rhizosphere | South IT | Indole acetic acid (IAA) production |
| *Bacillus megaterium* | M3 | rice | unknown | P-solubilisation |
| *Bacillus megaterium* | PMC 1855 | unknown | unknown | P-solubilisation |
| *Bacillus pumilus* | LMG 24415 | soil | Ecuador | PGP |
| *Bacillus simplex* | R49538 | unknown | Ecuador | PGP/IAA production |
| *Bacillus subtilis* | FZB24 WG | | Berlin, DE | PGP |
| *Bacillus subtilis* | LMG 23370 | Forest soil | India | PGP/ biocontrol against *Rhizoctonia solani* |
| *Bacillus subtilis* | LMG 24418 | soil | Ecuador | PGP |
| *Bacillus subtilis* | OSU-142 | pepper | unknown | N-fixation, biocontrol |
| *Burkholderia ambifaria* | MCI7 | Maize rhizosphere | Lazio, IT | PGP |
| *Burkholderia ambifaria* | PHP7/LMG 11351 | Maize rhizosphere | FR | PGP |
| *Gigaspora gigantea* | PA125 | Rhizosphere | STATI UNITI | PGP |
| *Gigaspora rosea* | NY328A | Rhizosphere | STATI UNITI | PGP |
| *Komagataella pastoris* | PP59 | Grape rhizosphere | Ascoli Piceno, IT | PGP |
| *Paenibacillus sp* | R47065 | unknown | Ecuador | PGP/IAA production |
| *Paraburkholderia tropica* | MDIIIAzo225 | Maize rhizosphere | Caserta, IT | Nitrogen fixation |
| *Pseudomonas granadensis* | A23/T3c | soil | Lazio, IT | PGP |
| *Pseudomonas fluorescens* | DR54 | Sugar beet rhizosphere | Holeby, DK | biocontrol |
| *Pseudomonas putida* | P1-20/08 | soil | Ecuador | PGP |
| *Pseudomonas* sp. | PN53 | Grass rhizosphere | Ascoli Piceno, IT | PGP |
| *Rahnella aquatilis* | BB23/T4d | soil | Lazio, IT | PGP |
| *Raoultella terrigena* | FS152 | Rhizosphere | South, IT | Phytase activity, siderophore production |
| *Septoglomus constrictum* | FL328 | Rhizosphere | STATI UNITI | PGP |
| *Streptomyces* sp. | SA 51 | Rhizosphere | Liguria, IT | biocontrol |
| *Trichoderma gamsii* | 6085 | uncultivated soil | Crimea, UA | biocontrol |
| *Trichoderma harzianum* | OmG-08 | Orchid roots | Bernburg, DE | P- solubilisation |
| *Trichoderma harzianum* | OmG-16 | | Bernburg, DE | P-solubilisation |
| *Trichoderma harzianum* | T6776 | soil | Pisa, IT | biocontrol/PGP |
| *Trichoderma harzianum* | TH01 | Grass soil and rhizosphere | Ascoli Piceno, IT | PGP |
| *Trichoderma harzianum* | CBS 354.33/ATCC 48131 | soil | USA | chitinase production, biocontrol |

# List of PGPMs

BIOFECTOR PROJECT (FP7-KBBE/2012-2017)

VALORAM PROJECT ( FP7-KBBE/ 2007-2013)

- A total of 46 microbial strains were selected: **bacteria, mychorrizae and fungi**
- **26 out of 46** microbial strains **resulted available**
- available strains were **cross-checked** to evaluate their compatibility

# Bacteria-bacteria compatibility assays

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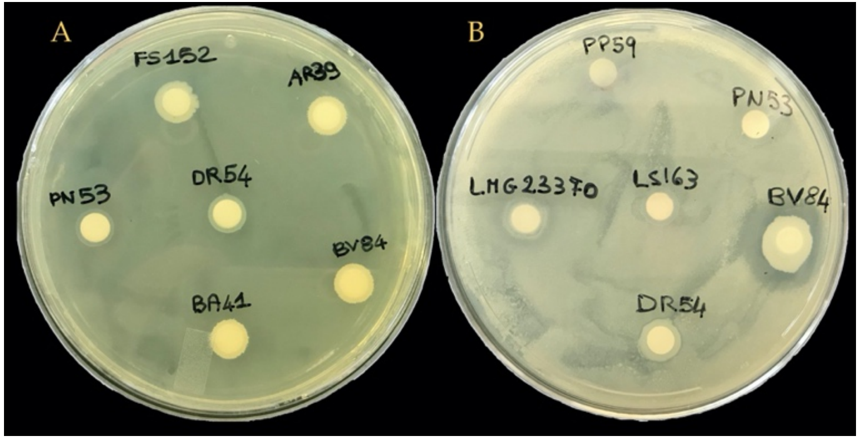
| Strain | *A. radiobacter* AR39 | *A. brasilense* ATCC 29710 | *A. brasilense* NCCB 78036 | *A. chroococcum* DSM 2286 | *A. chroococcum* LS132 | *A. chroococcum* LS136 | *A. vinelandii* DSM 2289 | *Bacillus* spp. BV84 | *B. amyloliquefaciens* BA41 | *B. amyloliquefaciens* LMG 9814 | *B. licheniformis* PS141 | *B. pumilus* LMG 24415 | *B. subtilis* LMG 23370 | *B. subtilis* LMG 24418 | *B. ambifaria* LMG11351 | *B. ambifaria* MCI7 | *B. ambifaria* LMG11351 | *K. pastoris* PP59 | *P. tropica* MDIIIAzo225 | *Pseudomonas* sp. PN53 | *P. fluorescens* DR54 | *P. granadensis* A23/T3c | *R. aquatilis* BB23/T4d | *R. terrigena* FS152 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. radiobacter* AR39 | | | | | | | | | | | | | | | | | | | | | | | | |
| *A. brasilense* ATCC 29710 | + | | | | | | | | | | | | | | | | | | | | | | | |
| *A. brasilense* NCCB 78036 | + | + | | | | | | | | | | | | | | | | | | | | | | |
| *A. chroococcum* DSM 2286 | - | - | - | | | | | | | | | | | | | | | | | | | | | |
| *A. chroococcum* LS132 | + | + | + | - | | | | | | | | | | | | | | | | | | | | |
| *A. chroococcum* LS136 | + | + | + | - | + | | | | | | | | | | | | | | | | | | | |
| *A. vinelandii* DSM 2289 | + | + | + | - | + | + | | | | | | | | | | | | | | | | | | |
| *Bacillus* spp. BV84 | + | - | + | + | + | + | + | | | | | | | | | | | | | | | | | |
| *B. amyloliquefaciens* BA41 | + | - | + | + | + | + | + | + | | | | | | | | | | | | | | | | |
| *B. amyloliquefaciens* LMG 9814 | + | nc | + | - | + | + | + | + | + | | | | | | | | | | | | | | | |
| *B. licheniformis* PS141 | + | + | + | - | + | + | + | - | - | - | | | | | | | | | | | | | | |
| *B. pumilus* LMG 24415 | + | + | + | + | + | + | + | - | - | - | - | | | | | | | | | | | | | |
| *B. subtilis* LMG 23370 | + | + | + | + | + | + | + | - | - | - | + | - | | | | | | | | | | | | |
| *B. subtilis* LMG 24418 | + | + | + | + | + | + | + | - | - | - | + | - | - | | | | | | | | | | | |
| *B. ambifaria* LMG11351 | + | + | + | - | + | + | + | + | + | + | + | + | + | - | | | | | | | | | | |
| *B. ambifaria* MCI7 | + | - | + | - | + | + | + | + | + | + | + | + | + | + | + | | | | | | | | | |
| *K. pastoris* PP59 | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | nc | + | | | | | | | |
| *P. tropica* MDIIIAzo225 | + | + | + | - | + | + | + | + | + | nc | + | + | nc | + | + | + | + | + | | | | | | |
| *Pseudomonas* sp. PN53 | + | + | + | + | + | + | - | - | + | + | + | + | + | + | + | + | + | + | + | | | | | |
| *P. fluorescens* DR54 | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | nc | | | | |
| *P. granadensis* A23/T3c | + | + | + | - | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | | | |
| *R. aquatilis* BB23/T4d | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | | |
| *R. terrigena* FS152 | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |

+: compatible; -: incompatible; nc: not clear.

Pairwise compatibility among microorganisms using the modified agar diffusion method on NA plates.



# Dual compatibility test among bacteria and fungi

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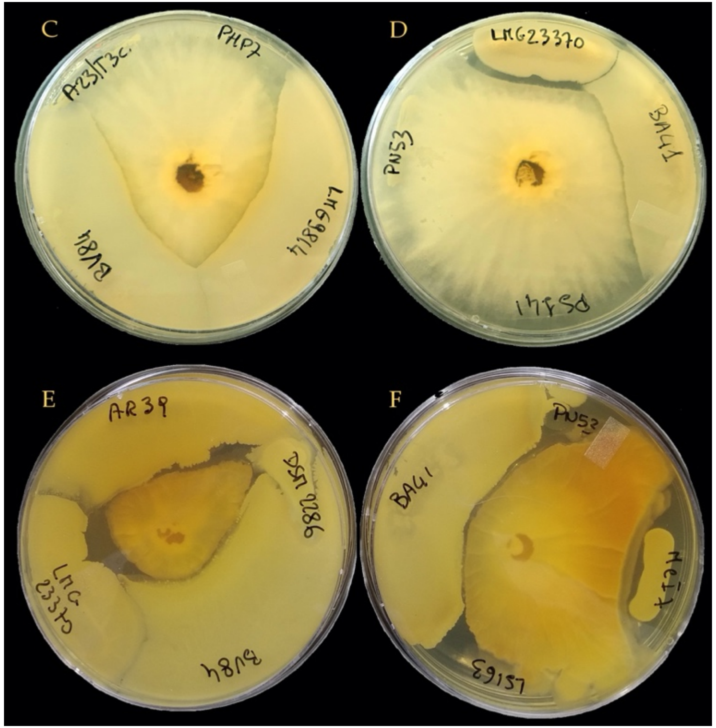


| Bacteria | *T. harzianum* ATCC 48131 | *T. harzianum* TH01 |
|---|---|---|
| *Azotobacter brasilense* ATCC 29710 | nc | + |
| *Azospirillum brasilense* NCCB 78036 | - | + |
| *Azotobacter chroococcum* DSM 2286 | - | nc |
| *Azotobacter chroococcum* LS132 | + | + |
| *Agrobacterium radiobacter* AR39 | - | - |
| *Azotobacter chroococcum* LS163 | - | + |
| *Azotobacter vinelandii* DSM 2289 | + | + |
| *Bacillus sp.* BV84 | - | + |
| *Bacillus amyloliquefaciens* BA41 | - | - |
| *Bacillus amyloliquefaciens* LMG 9814 | - | - |
| *Bacillus licheniformis* PS141 | + | + |
| *Bacillus pumilus* LMG 24415 | - | - |
| *Bacillus subtilis* LMG 23370 | - | - |
| *Bacillus subtilis* LMG 24418 | - | - |
| *Burkholderia ambifaria* LMG 11351 | - | - |
| *Burkholderia ambifaria* MCI7 | - | - |
| *Komagataella pastoris* PP59 | + | + |
| *Paraburkholderia tropica* MDIIIAzo225 | + | nc |
| *Pseudomonas* sp. PN53 | - | + |
| *Pseudomonas granadensis* A23/T3c | + | + |
| *Pseudomonas fluorescens* DR54 | + | nc |
| *Ranhella aquatilis* BB23/T4d | - | + |

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# Multifunctional synthetic microbial consortia

| MC | Microorganisms |
|---|---|
| A | *Azotobacter chrococcum* LS132<br>*Bacillus licheniformis* PS141<br>*Komagataella pastoris* PP59<br>*Pseudomonas granadensis* A23/T3c<br>*Paraburkholderia tropica* MDIIIAzo2225<br>*Trichoderma harzianum* TH01 |
| B | *Azotobacter vinelandii* DSM 2289<br>*Bacillus amyloliquefaciens* LMG 9814<br>*Bacillus* sp. BV84<br>*Pseudomonas fluorescens* DR54<br>*Rahnella aquatilis* BB23/T4d |
| C | *Azotobacter chrococcum* LS132<br>*Bacillus amyloliquefaciens* LMG 9814<br>*Burkholderia ambifaria* MCI7<br>*Rahnella aquatilis* BB23/T4d<br>*Pseudomonas fluorescens* DR54 |

Three microbial consortia inoculants named MC A, MC_B, and MC_C were developed in which a specific function was represented by at least one member

- nitrogen fixation
- P-solubilisation
- biocontrol
- amylolytic activity
- auxin or auxin-like compounds production

# The use of bioactive compounds

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- To reinforce the inoculum and favour plant growth under greenhouse and field conditions, **bioactive compounds** were considered



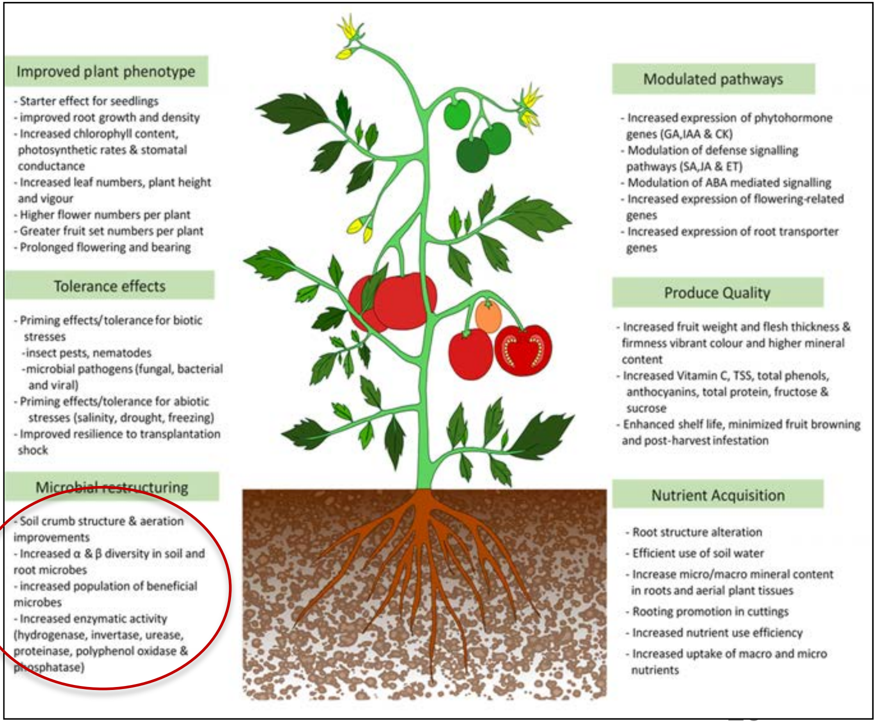

**Seaweed extracts, Humic substances, Agro-industrial residues, Plant-derived protein hydrolysate**

*plants* MDPI

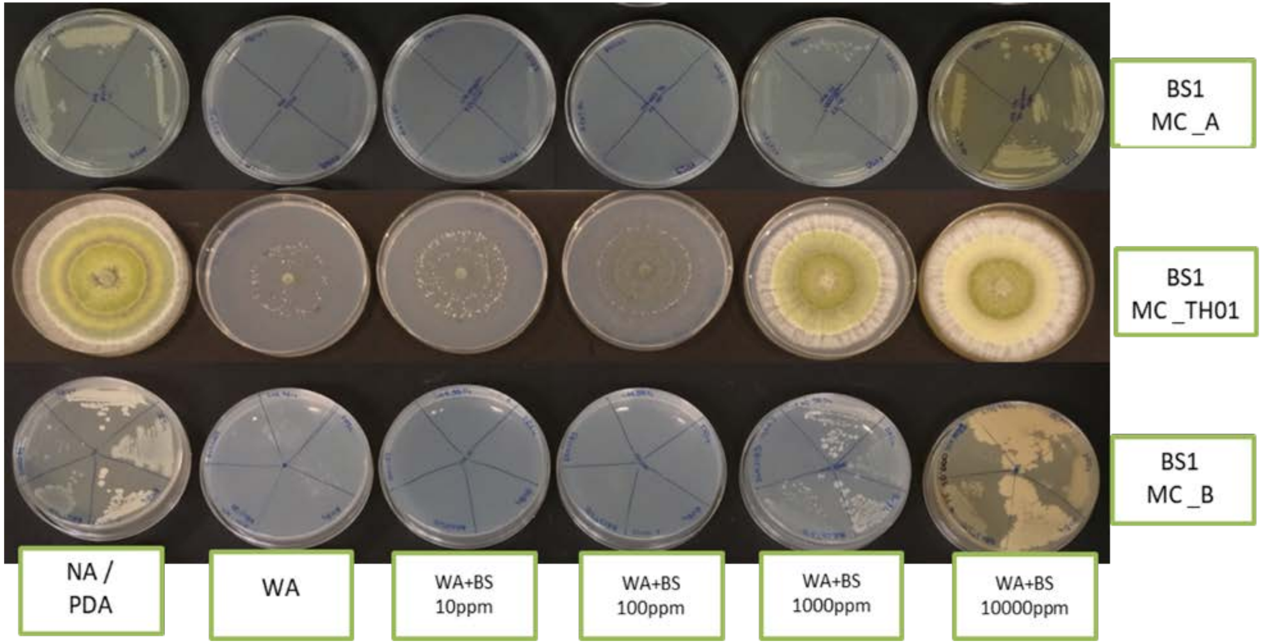
*Review*

**Biostimulant Properties of Seaweed Extracts in Plants: Implications towards Sustainable Crop Production**

*Plants* **2021**, *10*, 531. https://doi.org/10.3390/plants10030531



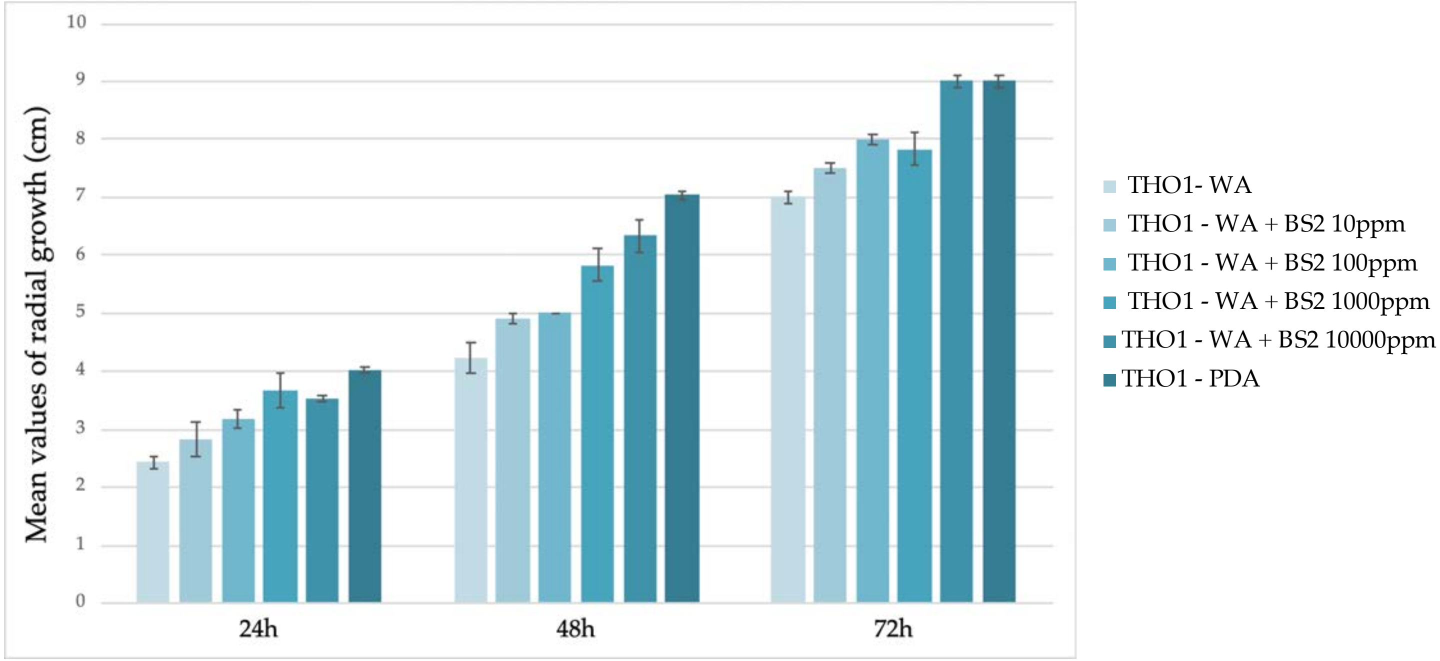
# In vitro test single microorganism- bioactive compounds




- **Agro-industrial residues**
- **Plant-derived protein hydrolysate**

- **bioactive compounds can promote the growth of beneficial microorganisms**

# Prebiotic effect of BS2 (cell-wall protein hydrolysate) on *T. harzianum* TH01 growth in starvation conditions



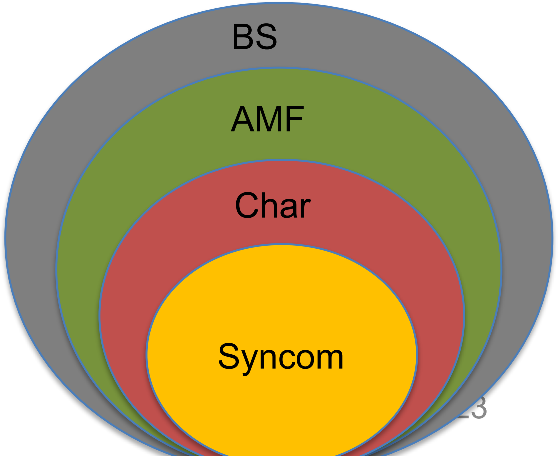

Overall, *in vitro* results showed that the presence of bioactive compound BS2 fostered a rapid increase of the number of beneficial microorganisms in starvation conditions, favouring the colonization and survival of strains under greenhouse and field conditions.

# Conclusions

SIMBA
SUSTAINABLE INNOVATION OF MICROBIOME APPLICATIONS IN THE FOOD SYSTEM

- Three main synthetic microbial consortia, common to all four crops, composed by bacteria, yeast and fungi with different functionalities, were identified
- Bioactive compounds are promising in favouring the colonization and survival of the microbial inoculants under greenhouse and field conditions
- Preliminary results of ongoing activities suggest that MC, applied alone or in combination with char and/or Arbuscolar Mycorrhizal Fungi, can improve yield and quality of crop plants

Exploiting the efficacy in greenhouse and field trials of the combined MC-bioactive compounds, as well as their overall ecological impact on the native soil microbiome, will permit to define new plant biostimulants for a more sustainable and resilient agriculture.



# Work in progress

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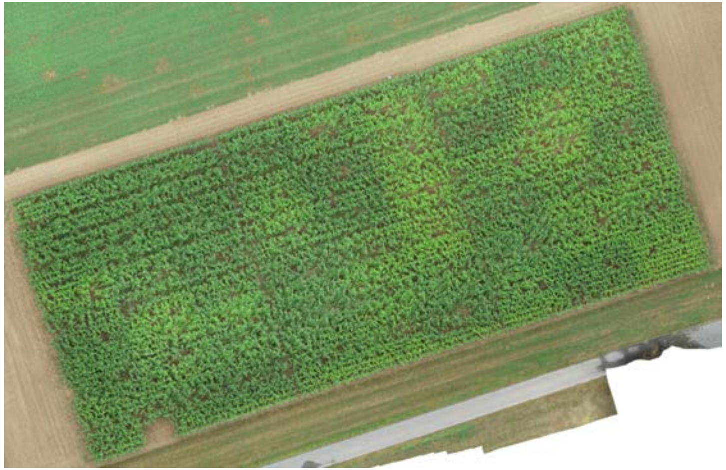
- Greenhouse experiments and field trials in Italy and Germany
- Evaluation of the effect of PGPMs application on the soil/rhizosphere microbiome
- The identification of a spectrum of metagenomic biomarkers (taxa, SNPs and metabolic modules) to be exploited as bioindicators of soil quality and fertility


Maize in Italy



Drone pictures from the experimental farm Wiesengut (Germany.

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# Thank you!

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