

**PhD DISSERTATION PROJECT**

# DEVELOPMENT OF NEW MICROBIAL BIOSTIMULANTS FOR THE SUSTAINABLE PRODUCTION OF FOOD CROPS

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This PhD thesis research project is aimed to obtain new commercial formulations starting from newly isolated microorganisms, with plant growth-promoting activity capable of stimulating plant growth in water or saline stress conditions. The formulations obtained, developed with a view to eco-sustainable agriculture, could represent an innovative solution to increase the yield of plants of food interest and reduce the negative impact that climate change and drought have on our production.

## **Sviluppo di nuovi biostimolanti microbici per la produzione sostenibile di colture a interesse alimentare**

Questo progetto di tesi di dottorato mira ad ottenere nuove formulazioni commerciali a partire da microrganismi appena isolati, con attività fitopromotrice in grado di stimolare la crescita delle piante in condizioni di stress idrico o salino. I formulati ottenuti, sviluppati nell'ottica di un'agricoltura ecosostenibile, potrebbero rappresentare una soluzione innovativa per aumentare la resa delle piante di interesse alimentare e ridurre l'impatto negativo che il cambiamento climatico e la siccità stanno avendo sulle nostre produzioni.

### **1. State-of-the-Art**

The Green Revolution, initiated in the 1970s, played a pivotal role in increasing agricultural production through mechanization and chemicals. The utilization of synthetic fertilizers and pesticides boosted crop productivity and averted global famine. However, modern agriculture heavily reliant on the indiscriminate use of synthetic chemicals has had adverse effects on soil, the environment, and human health. Furthermore, abiotic stresses such as drought, heat, and pollution impact plant growth, development, and productivity (Naik et al. 2019). In recent years, there has been a pursuit of eco-friendly agricultural practices to promote plant growth and productivity. Among emerging strategies, biostimulants are emerging as a cornerstone for a new agricultural revolution towards sustainable food production (Nephali et al. 2020). Biostimulants are products that stimulate plant nutritional processes to enhance various plant or rhizosphere characteristics, such as nutrient use efficiency, abiotic stress tolerance, quality traits and nutrient availability in soil or rhizosphere. The recent literature demonstrates that the ability to promote plant growth is associated with two main groups: microbial biostimulants, with several bacteria such as Plant Growth-Promoting Rhizobacteria (PGPR) and fungi and non-microbial biostimulants, such as algae extracts, humic substances, protein hydrolysates, and other biopolymers. PGPR are microorganisms that promote plant growth and yield through direct and indirect mechanisms. Indirect mechanism affects plant health by protecting against pathogens through the production of siderophores, antibiotics, cell wall degrading enzymes, and soluble and volatile metabolites. Direct plant growth-promoting mechanisms positively affect the availability of essential nutrients (e.g., nitrogen, phosphorus, iron) and produce and regulate compounds involved in plant growth (e.g., phytohormones) (Luziatelli et al. 2023). Among plant regulators, indole-3-acetic acid (IAA) is the most abundant member of the auxin family. The effects of biostimulants are partially regulated by hormonal changes in plants (Ruzzi et al. 2015). Phytohormones, such as auxins, are involved in growth promotion by PGPR. Biostimulants can also emit volatile organic compounds that support plant development and induce pathogen resistance.

The utilization of PGPR microorganisms is a promising strategy to improve agricultural productivity. These microorganisms can be used as biocontrol agents, biofertilizers, and biostimulants, and they can also contribute to the bioremediation of contaminated environments. The combination of different microorganisms in a formulated product can promote synergistic interactions and enhance plant growth.

### **2. PhD Thesis Objectives and Milestones**

In detail, the objectives of this research activity aim to:

- Isolate microorganisms (bacteria and fungi) from different natural habitats with plant-promoting activity.
- Taxonomically characterize the strains with innovative phenotypic and sequencing approaches.
- Characterize the metabolome of the isolated strains with the use of chromatographic techniques to fully understand the spectrum of action and obtain formulations with different combinations of secreted metabolites.
- Develop an optimized protocol for the industrial production of different prebiotics and postbiotics.
- Drafting and planning a brochure that highlights the methods of use and conservation of the formulation

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1.

**Table 1:** Gantt diagram for this PhD thesis project.

Activity \ Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36		
<b>Isolation of microorganisms with PGPR action and under conditions of stress</b>	█	█	█	█	█	█	█	█	█	█	█	█																										
<b>Molecular and physiological characterization of the new isolates:</b>																																						
characterization of the spectrum of the substrates																																						
optimum growth temperature and pH																																						
for spore-forming strains evaluation of the sporification capacity and spore germination efficiency.																																						
<b>Evaluation of biostimulus and biocontrol activities</b>																																						
enzyme assays																																						
chromatographic analysis to evaluate the profile of metabolites produced by individual isolates																																						
ability of the strains to solubilize phosphate, fix nitrogen and produce phytohormones.																																						
Inhibition test to evaluate the ability of the strains to counteract the development of plant pathogenic microorganisms.																																						
<b>Characterization of the metabolome n CG-MS o LC-MS</b>																																						
<b>Evaluation of PG activity in controlled environments and in greenhouses.</b>																																						
<b>Production of specific formulations for the plants of interest</b>																																						
<b>Definition of large-scale production protocols for prebiotics and postbiotics</b>																																						
identification of cultivation parameters which allow to optimize the production of biomass/spores, the production of specific metabolites and the obtainment of bio-inoculants with high stability (survival, efficiency of germination).																																						
<b>Shelf-life evaluation of the formulation</b>																																						
Determination of the stability of the product stored at different temperatures.																																						
Identification of any stabilizers to be used in the final product.																																						
<b>Definition of the protocol for using the new formulations</b>																																						
<b>Experience abroad</b>																																						
<b>Thesis and Paper Preparation</b>																																						

### 3. Selected References

Luziatelli F, Nobili A, Nardilli F, Ruzzi M (2023) *Importance of Microbial Exo-Metabolites as Postbiotics for Sustainable Agriculture*. DOI: 10.23880/oajmb-16000257

Naik K, Mishra S, Srichandan H, Singh PK, Sarangi PK (2019). *Plant growth promoting microbes: Potential link to sustainable agriculture and environment*. Biocatalysis and Agricultural Biotechnology. DOI: <https://doi.org/10.1016/j.bcab.2019.101326>.

Nephali L, Piater LA, Dubery IA, Patterson V, Huysen J, Burgess K and Tugizimana F (2020). *Biostimulants for Plant Growth and Mitigation of Abiotic Stresses: A Metabolomics Perspective*. DOI: 10.3390/metabo10120505.

Ruzzi M, Aroca R (2015). *Plant growth-promoting rhizobacteria act as biostimulants in horticulture*. Scientia Horticulturae 124–134. DOI: <http://dx.doi.org/10.1016/j.scienta.2015.08.042>.