Unleashing the Power of *Pseudomonas chlororaphis* ST9:

Transforming Agriculture Sustainably with Bio-Stimulation and Biological Control

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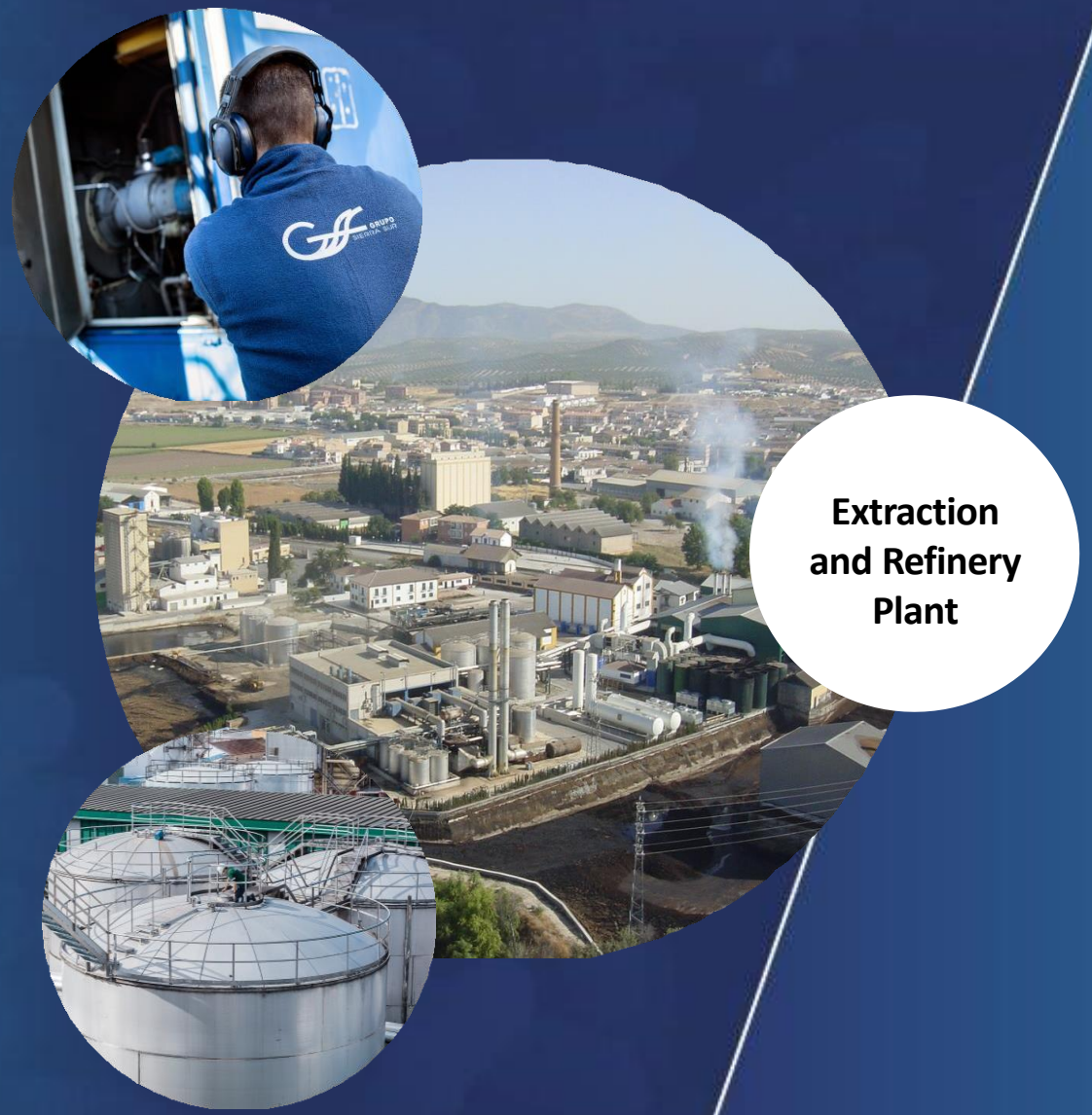
Sierra Sur Group

- The sectors encompass the following industries: agricultural and food, olive oil, wine, electrical, biotechnology, renewable energy, and plastic.
- With a total workforce exceeding 250 professionals, all of whom take great pride in our homeland.
- TOP TEN companies according to the ranking of the largest companies in Andalusia by Andalucía Económica.



Extraction and Refinery Plant

GRUPO SIERRA SUR

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- Formulation capacity of 20 metric tons per day.
- Microbiology Department.
- Bacterial Fermentation Plant: 300 liters.
- Fungal Fermentation Plant: 1,000 liters.

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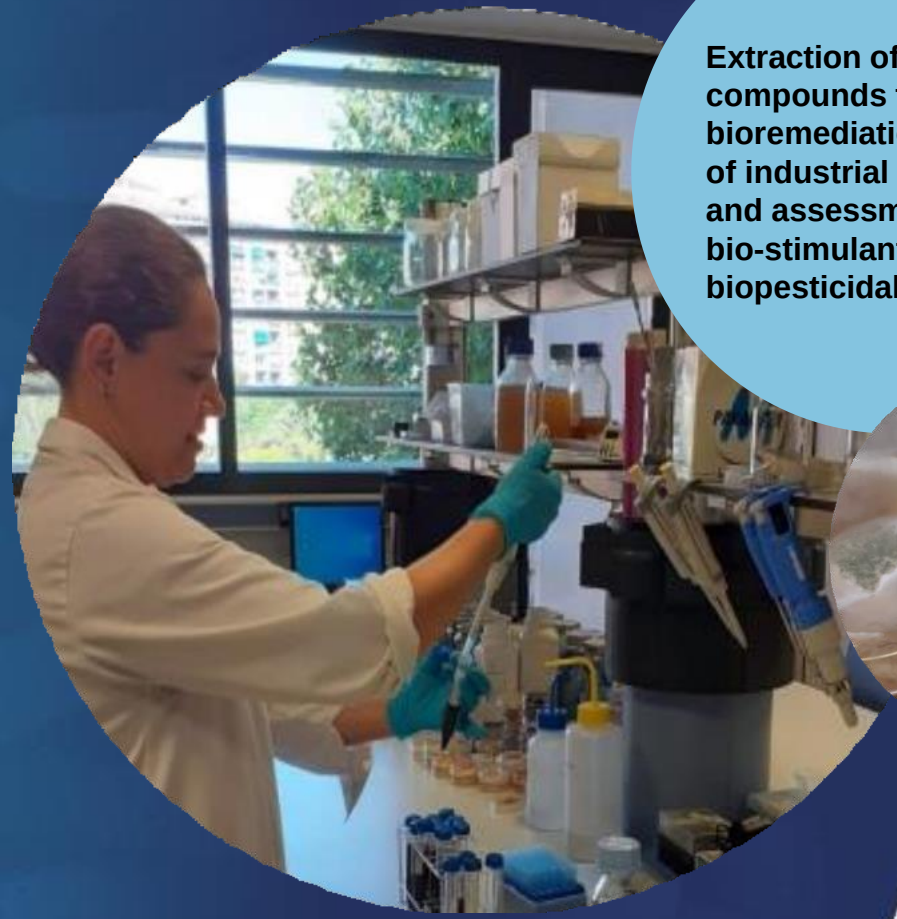
MYCO-BROW

- Microremediation of olive mill wastewater with saprotrophic fungi, eliminating its toxicity.
- Production of molecules with biopesticidal activity.
- Valorization of olive oil waste, yielding a bio-stimulant product.



Extraction of bioactive compounds through bioremediation processes of industrial olive oil waste and assessment of their bio-stimulant and biopesticidal activities.

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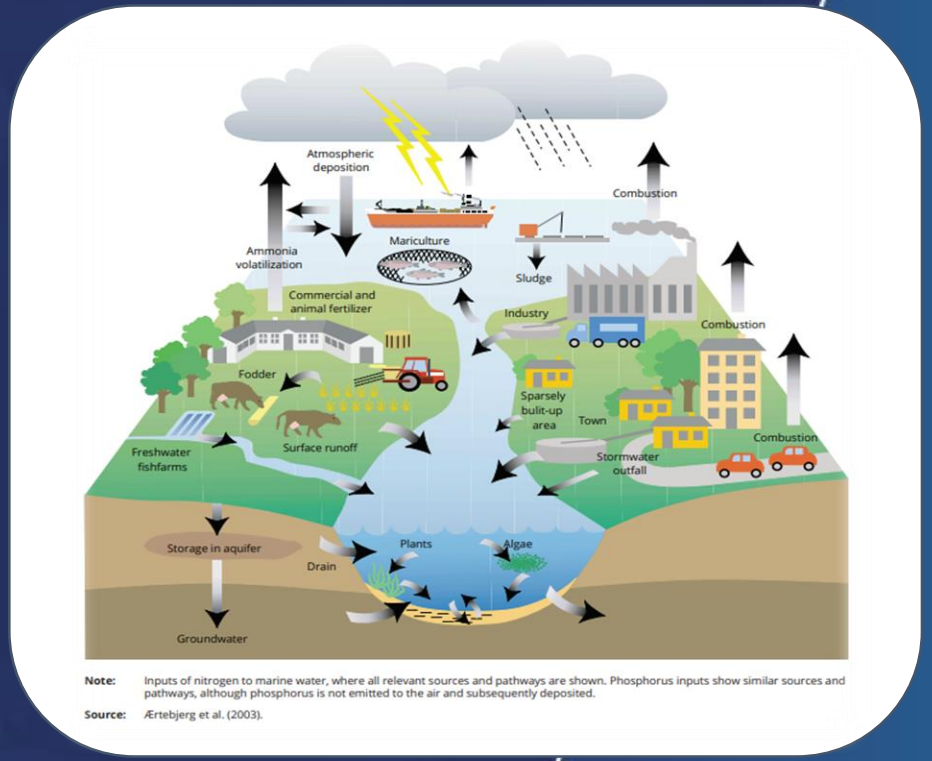
Traditional Fertilizers and Pesticides

- The excessive/misuse of traditional chemicals harms the environment.
- Eutrophication: Excessive nutrient input, often from agriculture or sewage, causing nutrient overabundance in water.
  - Effects:
  - Harmful algal blooms
  - Oxygen depletion
  - Ecological disruption

- Eutrophication is **a major water pollution concern** in the EU, emphasized by the EEA report.



European Environment Agency. (2019). Nutrient enrichment and eutrophication in Europe's seas. Recuperado de https://www.eea.europa.eu/publications/nutrient-enrichment-and-eutrophication-in

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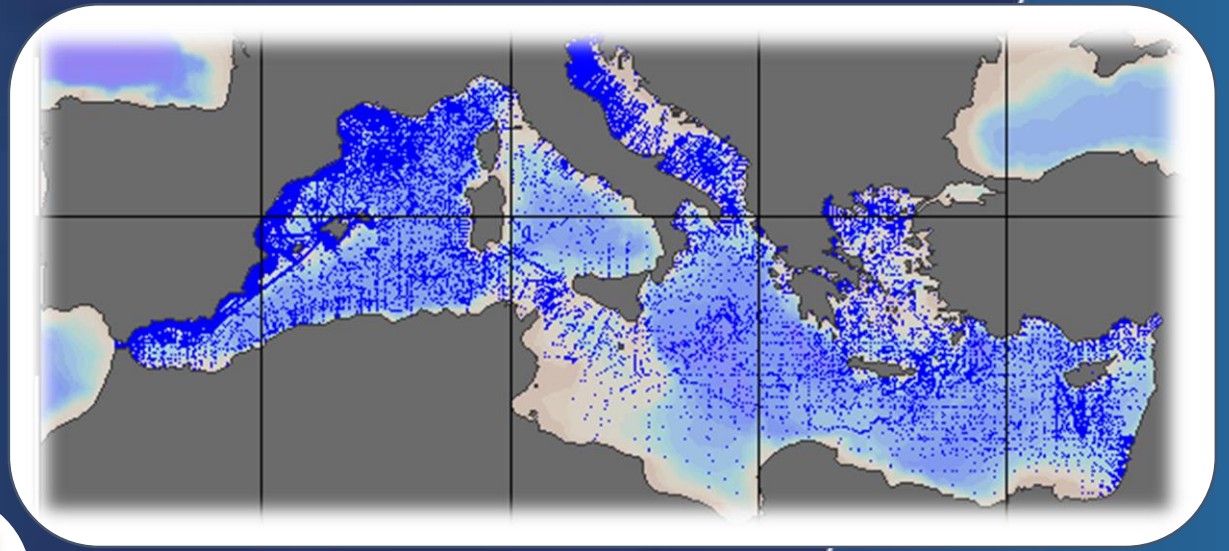
Eutrophication: Damages in EU

- Hypoxia in multiple locations of the Mediterranean and Baltic sea due to advanced eutrophication.
- Resulted in marine fauna mortality.
- Caused by nitrogen and phosphorus influx from intensive agriculture and other human activities.



Ministerio para la Transición Ecológica y el Reto Demográfico. (s.f.). Problemática actual - Planes y estrategias - Mar Menor. Retrieved September 20, 2023, from https://www.miteco.gob.es/es/ministerio/planes-estrategias/mar-menor/problematica-actual.html
European Marine Observation and Data Network (EMODnet). (2021). Eutrophication. Recuperado de https://emodnet.ec.europa.eu/en/eutrophication

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The Soil Secret: Unlocking Nature's Powerhouse

RIZOSPHERE

- Influenced by the roots.
- 5% of its matter comprises macro and microorganisms.
- Symbiosis PLANT-Microbiota.

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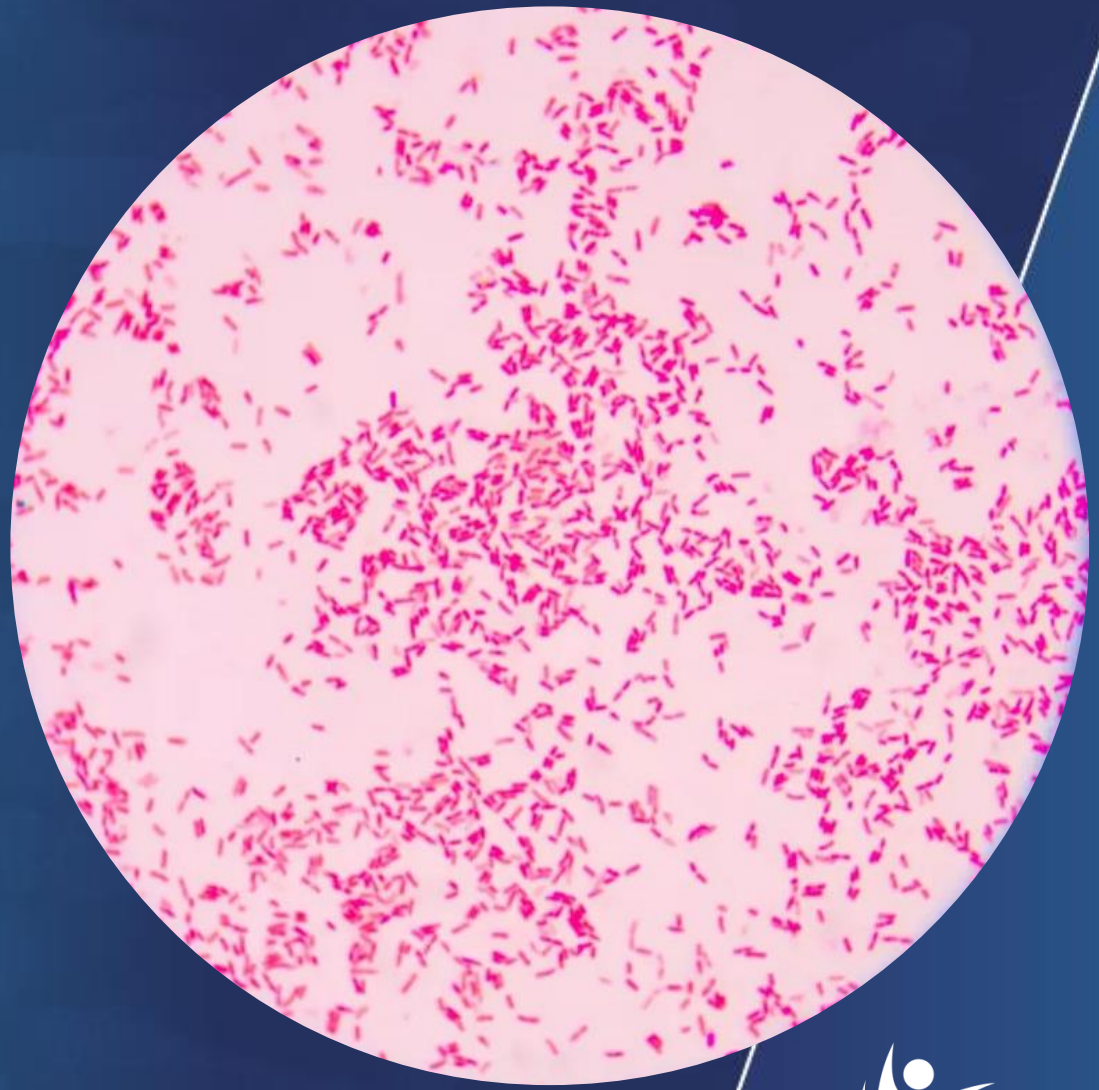
Pseudomonas chlororaphis
ST9

Plant Growth Promotion Rhizobacteria

- **Gram negative**
- **Rod shape** (2-4x0,5-1 µM)
- **Motile** (polar flagella)
- **Aerobic** (microaeróbica)
- **Genome:** 6,744,510 pb · G-C: 62,9%.



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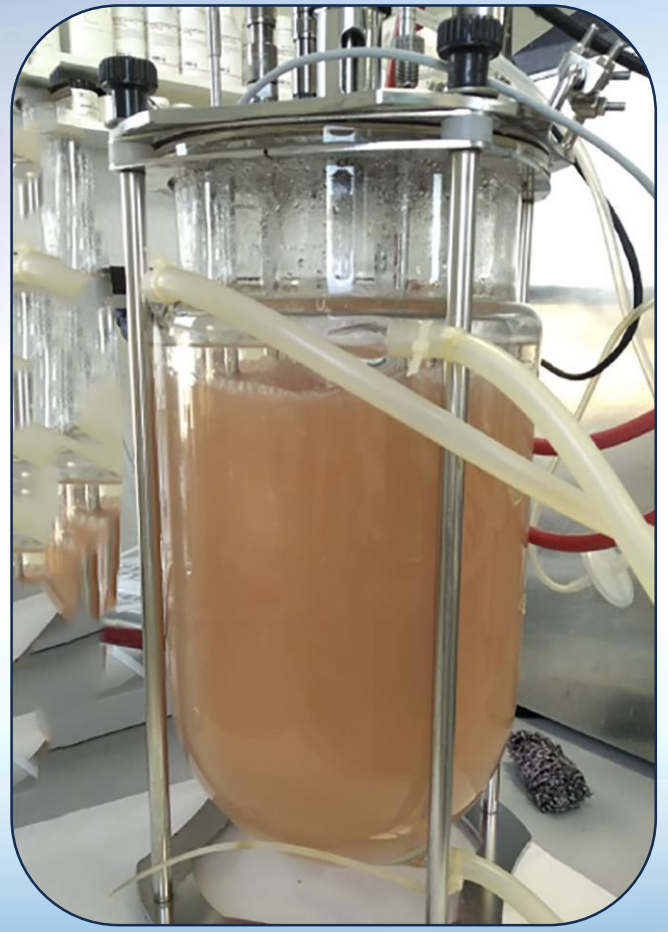
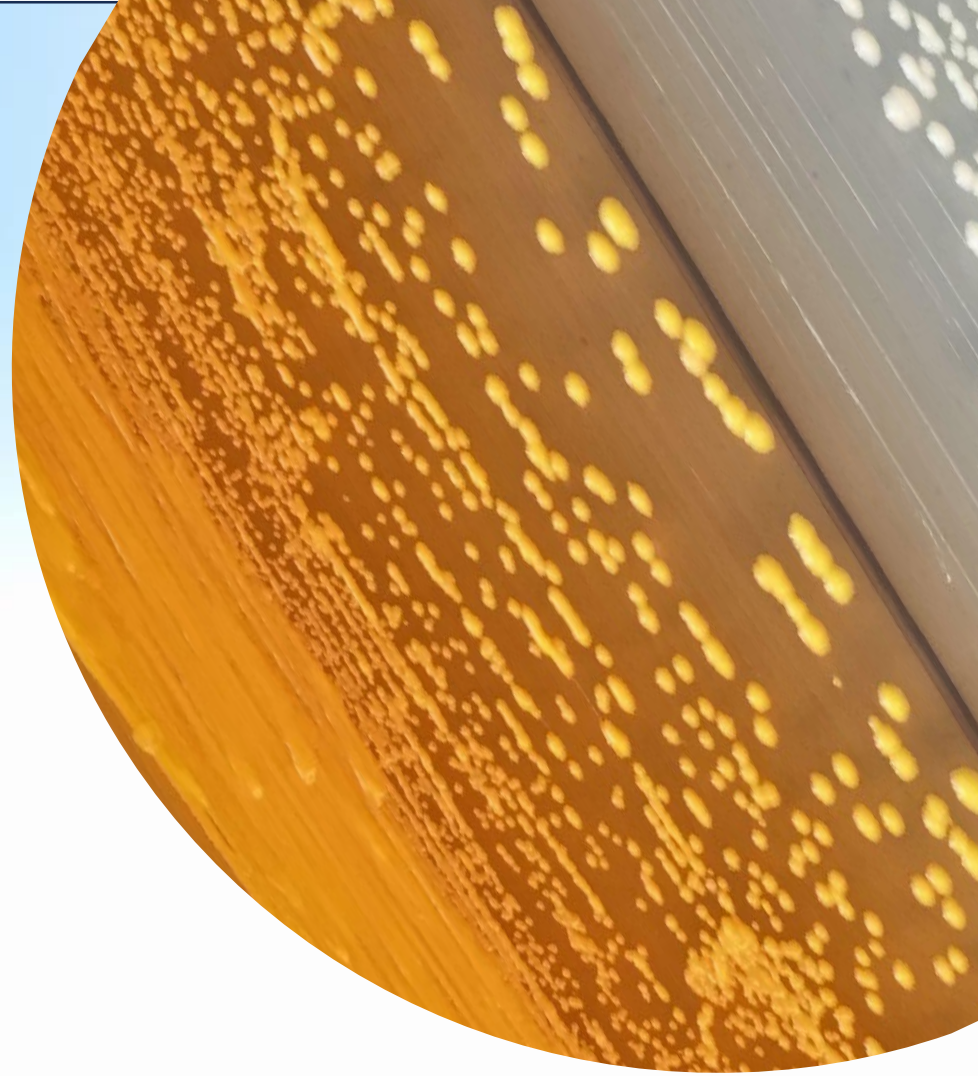
P.Chlororaphis -Benefits:

Plant Growth Promotion Rhizobacteria





- Improvement of nutrients absortion
- Biological control agent.
- Production of biologically significant substances:
  - Auxins (IAA)
  - Exopolysaccharides (EPS)

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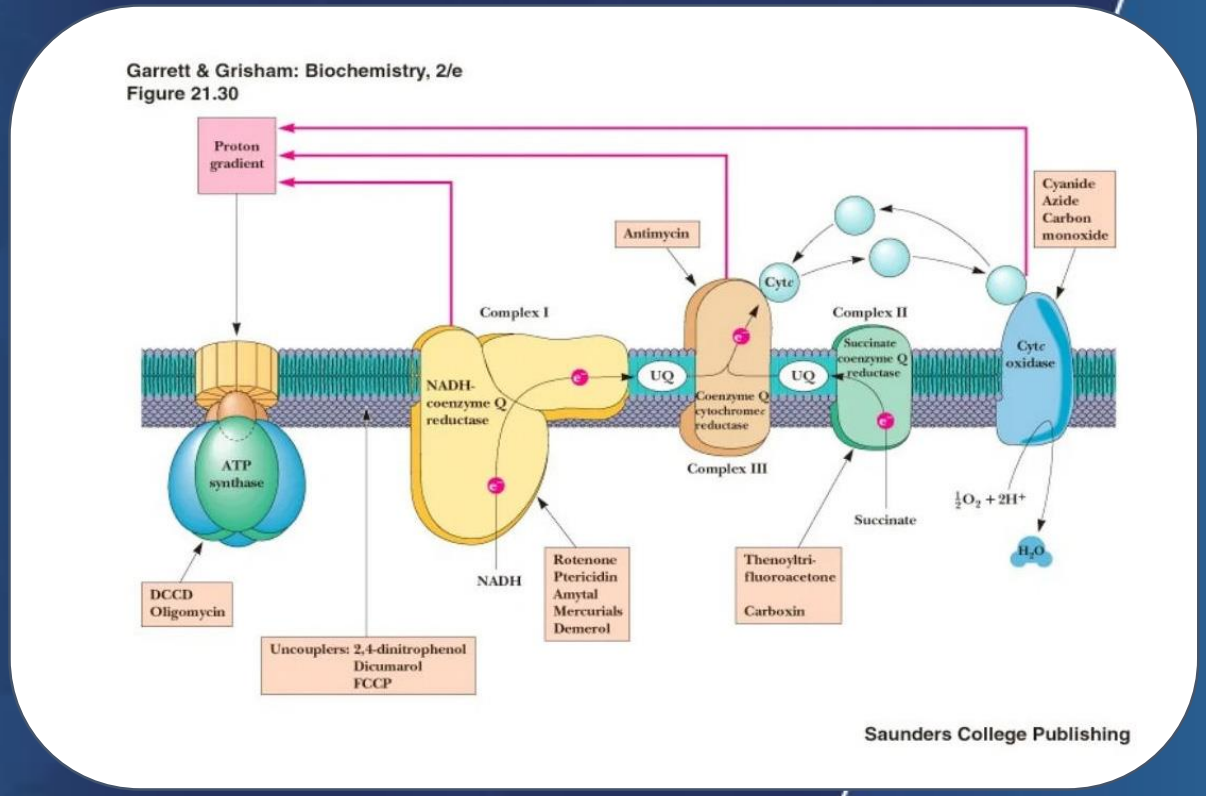
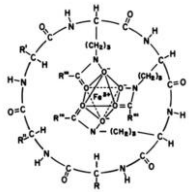
Ferrous Chelators (FE)

- Fe+ is a primordial ion
- Form complex structures (siderophores), which increase the Fe biodisponibility.
- Funcional Group: O2 o N2.
- ST9 produce pioverdin amongs other compounds.

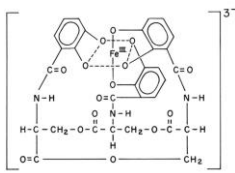


Functional Groups:

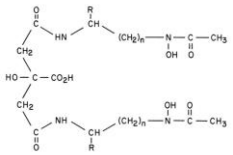
Ferricromo



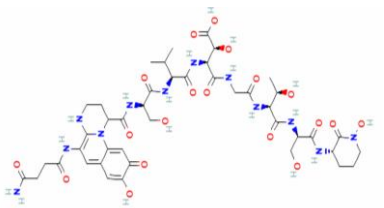
Enterobactina



Citrato monoxadato



Pioverdina



Ganz, T. (2018). Iron and infection. International Journal of Hematology, 107(5), 7-15. doi: 10.1007/s12185-018-2513-8-

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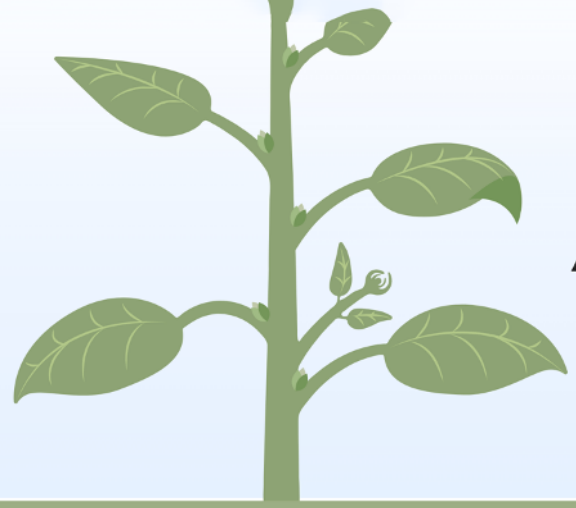
PIOVERDIN´s ACTION IN CROPS



Significant growth.
Vigorosity.

ST9

Pioverdin

Inhibition of pathogens

Biostimulation

Fe assimilation

Fe **Insoluble iron**

Fe Fe Fe Fe **Fe-Pioverdin Complex**

Pathogen suppression

+ Microbiote Biofilm induction

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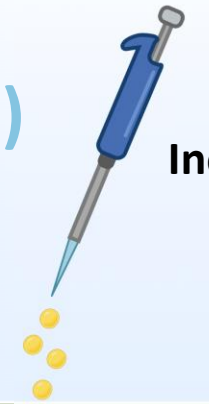
"Pioverdins, Siderophores with Antifungal Activity, from Pseudomonas fluorescens" de M. Miethke y M. A. Marahiel, publicado en el Journal of Bacteriology en 2007. "Iron acquisition by Pseudomonas aeruginosa in the lungs of patients with cystic fibrosis" de M. E. Meyer et al., publicado en Molecular Microbiology en 2016 "Pioverdina, el sideróforo estrella en el biocontrol de patógenos de plantas" de A. V. Sánchez-Sánchez, publicado en la revista Agrosfera en 2018.

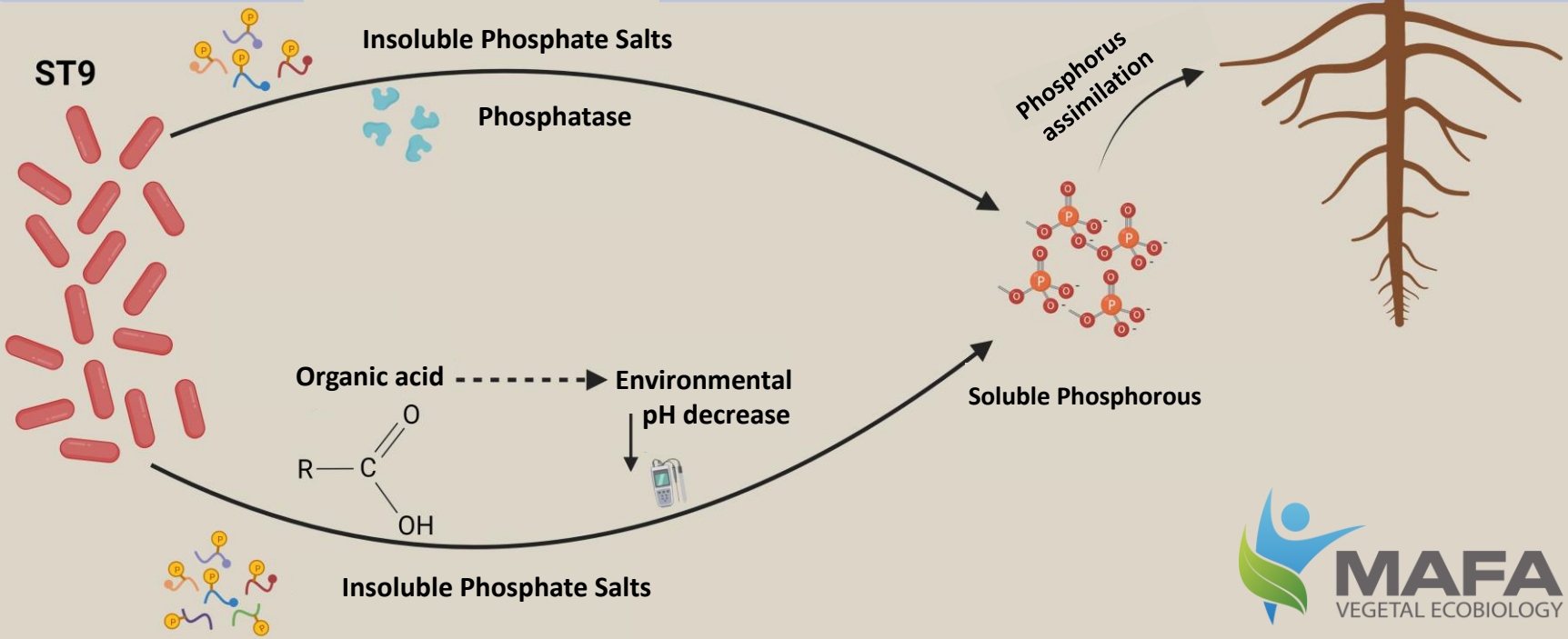
PHOSPHORUS SOLUBILIZATION (P)



Inoculation

Essential element:

- Nucleic Acids.
- Key energy molecule (ATP).
- Photosynthesis.
- Lipid bilayers.



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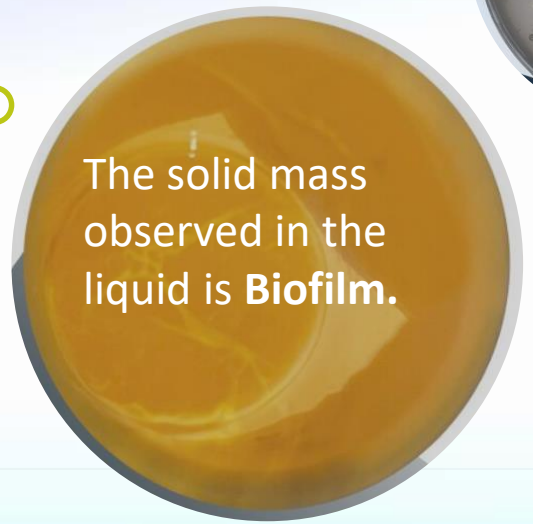
Results ST9

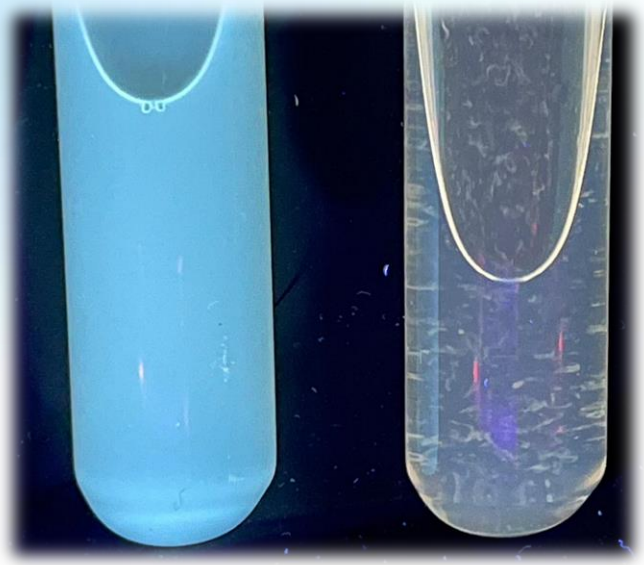
Plant Growth Promotion Rhizobacteria

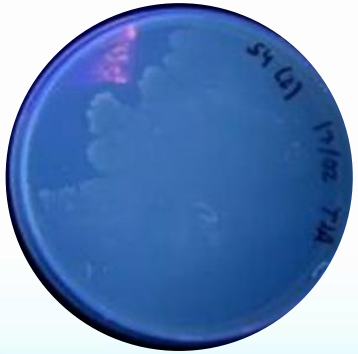
- The solubilization of P creates **phosphate clearing zones** in this specific medium.

- The solid mass observed in the liquid is **Biofilm.**



- **Pioverdin** imparts a fluorescent green coloration.





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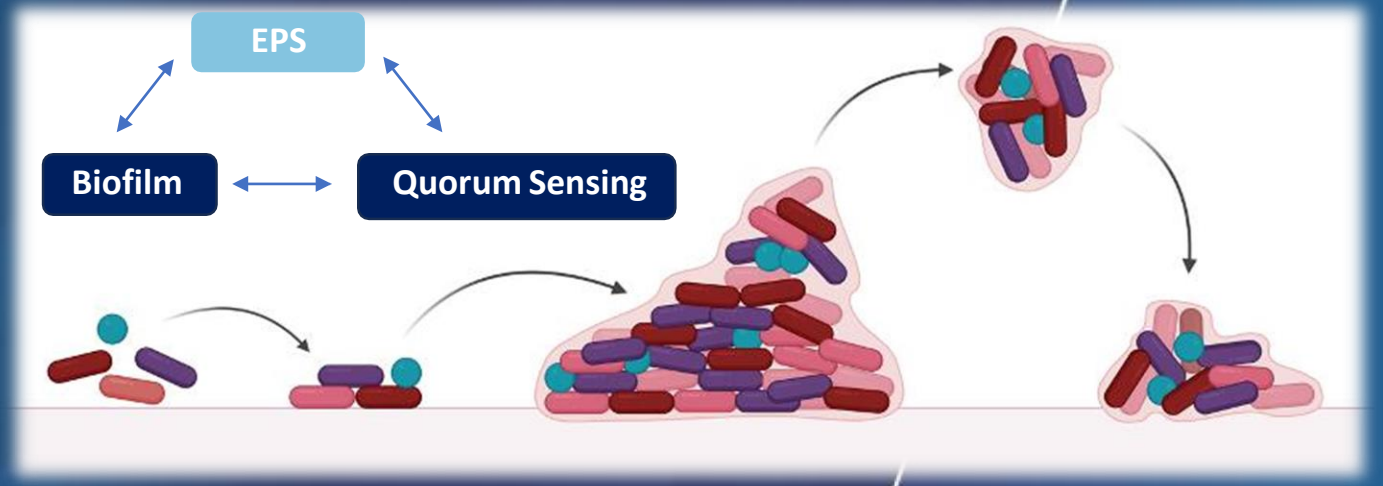
Exopolysaccharide biosynthesis (EPS), Biofilm y Quorum Sensing

- High molecular weight glucidic polymers
- **Functional groups:** amino/phosphate/ acetyl
- Constructs an **extracellular matrix.**

Facilitates functionality:

- **Biofilm**: Physical protective barrier, stimulates the solubilization of P and siderophores.
- **Quorum sensing:** Autoinducers (AHL)**.**
  Comunication:
  Microorganism-Microorganism.
  Microorganism- Envirovement.
  Coordinates:
  Microbial growth.
  Collective metabolic function.



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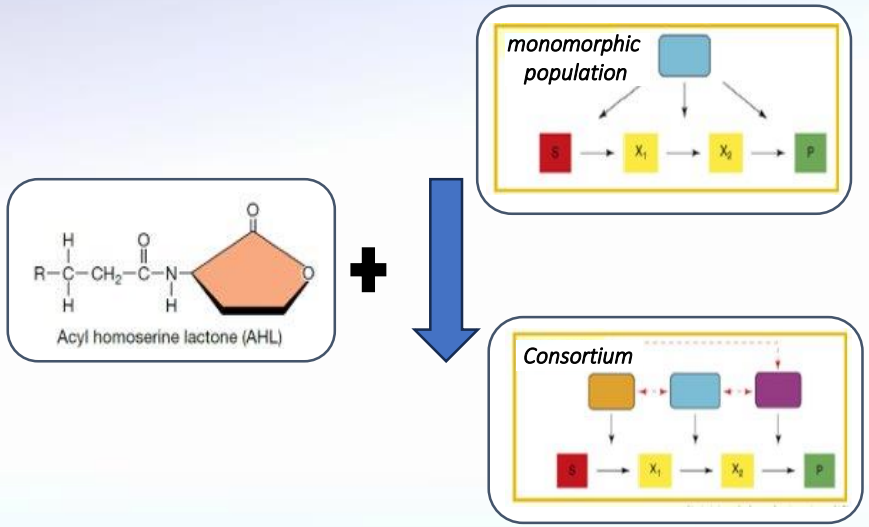
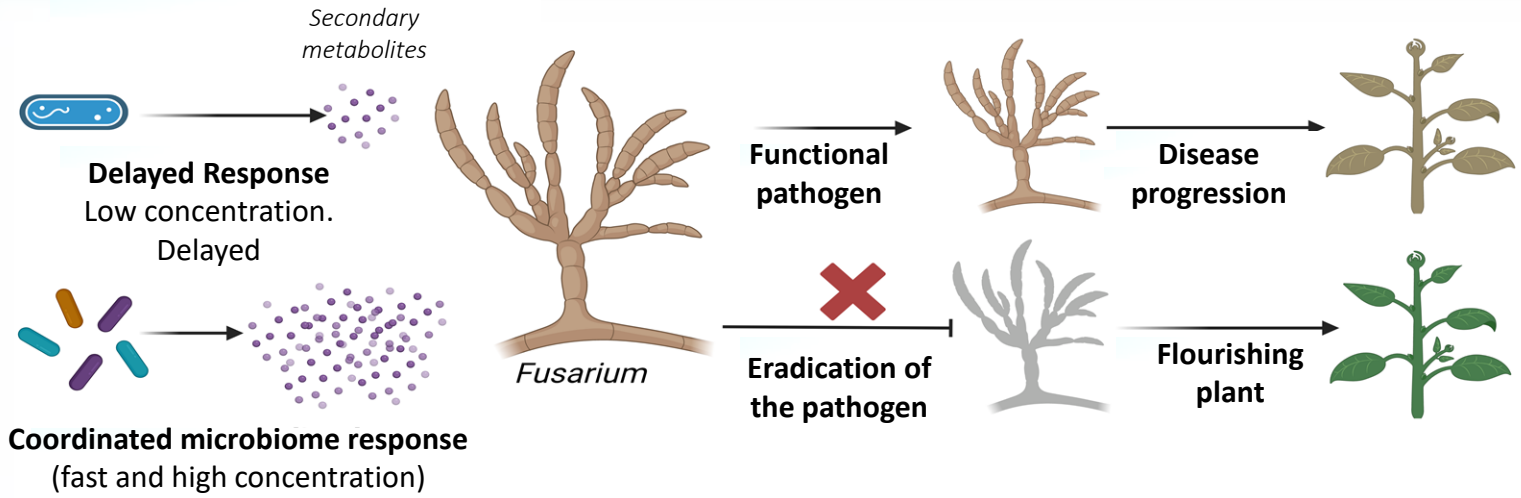
Application *EPS · BIOFILM · QUORUM SENSING*

A coordinated/joint response:

- Promotes growth of the microbiota.
- Effective biocontrol.





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Microbiological results of ST9 (Metagenomics)

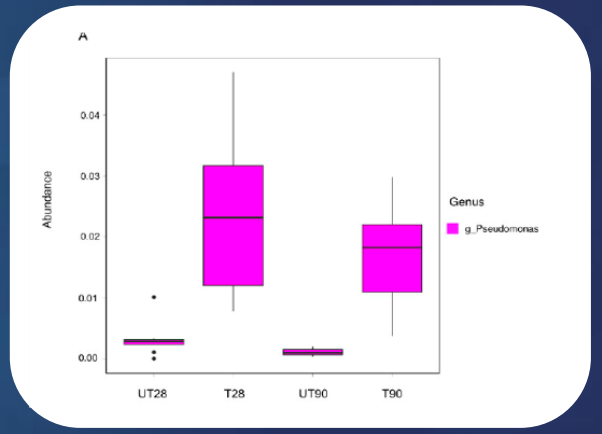
Statistically significant results:

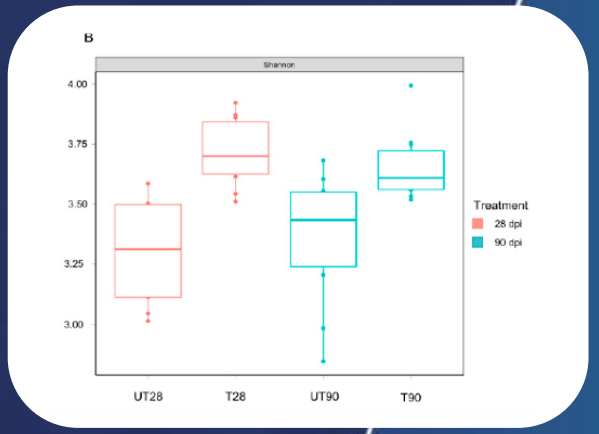
- (A) High Pseudomonas concentration is maintained up to 90 days post-inoculation
- (B) **Shannon test:** Higher alpha-diversity in treated microbial populations.
- (C) Bray Curtis:

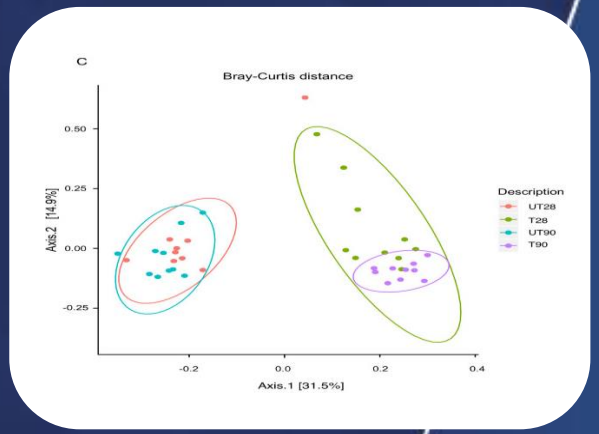
  Two distinct populations are observed.

  Enhanced diversity is observed in the **treated** ones

  Following that, **an optimization** takes place.







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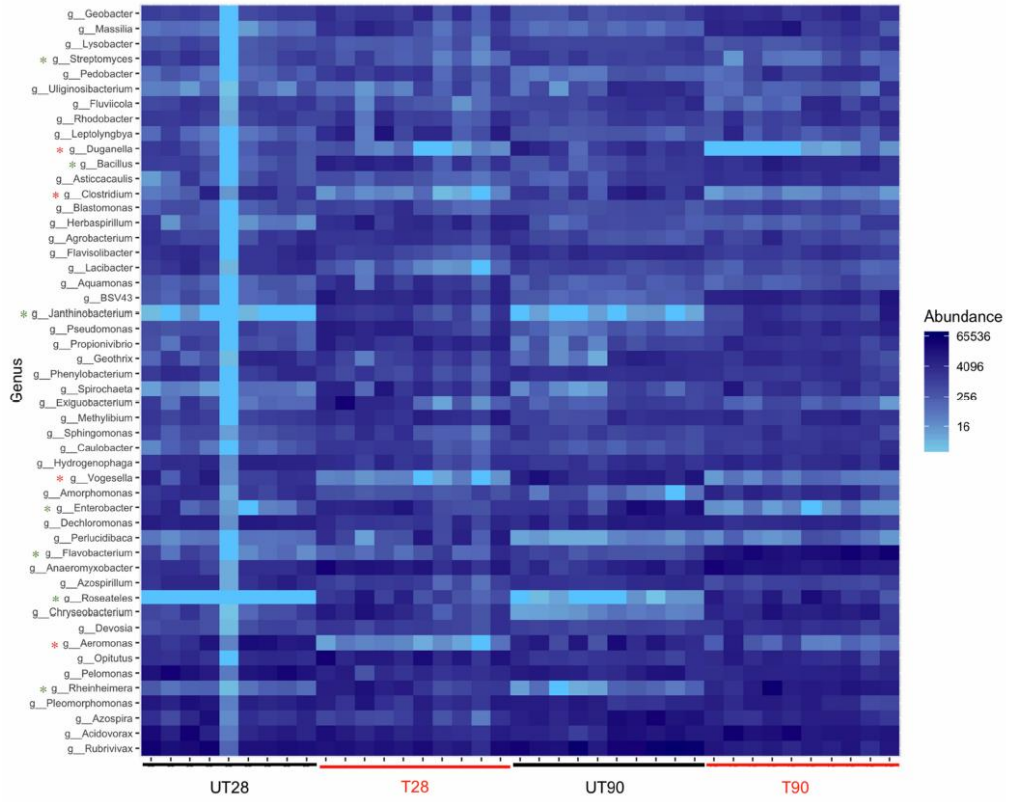
ST9 results in the microbiota (Metagenomics)



Higher presence of the microbial group

Lower presence of the microbial group

Heatmap Poblaciones microbianas en el suelo (% secuencias leídas).
UT: sin tratar T:tratado a 28 y 90 días de la inoculación

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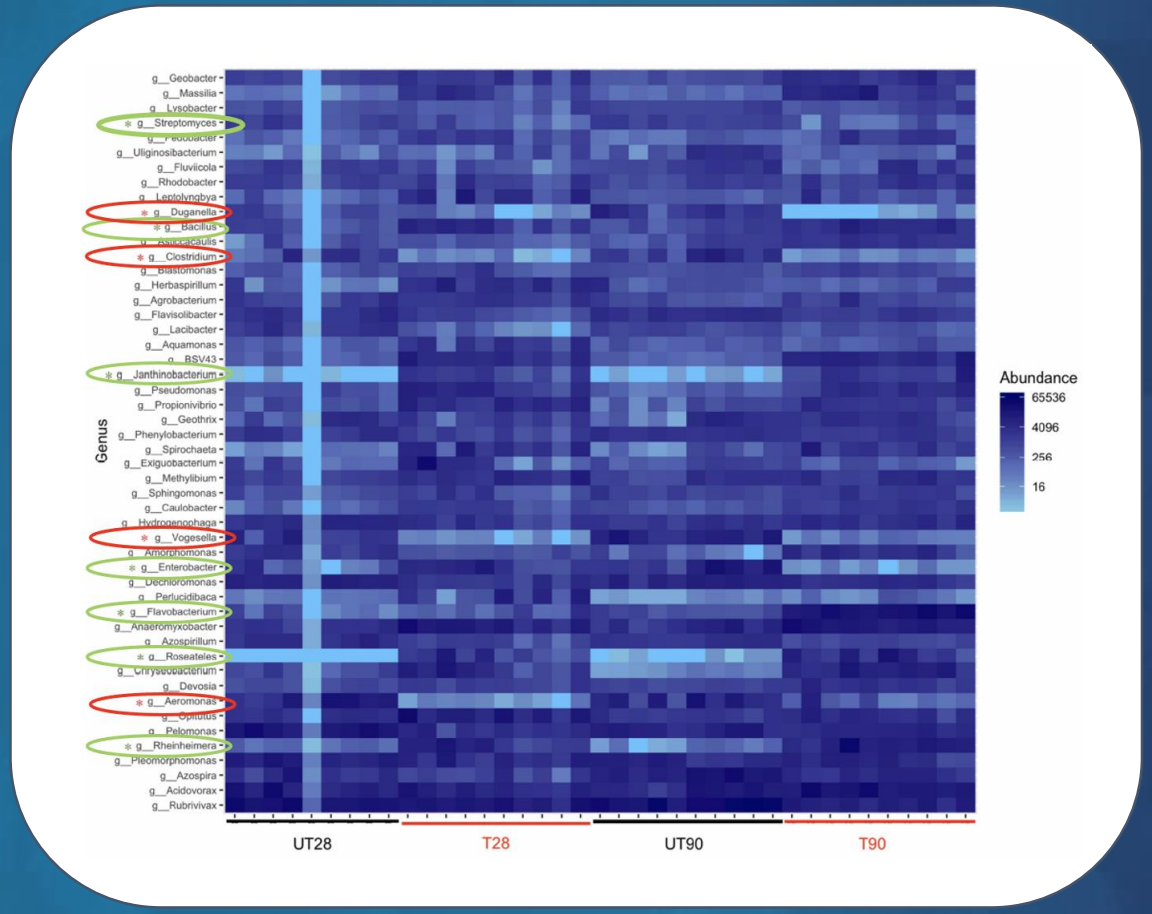
ST9 results in the microbiota



We highlight the significant distinctions

Higher Presence in **treated** (samples)

Flavobacterium
Bacillus
Paenibacillus
Bradyrhizobium

PGPRs

Higher Presence in **untreated** (samples)

Clostridium
Duganella
Vogesella
Aeromonas

Patógenos

Heatmap Poblaciones microbianas en el suelo (% secuencias leídas).
UT: sin tratar T:tratado a 28 y 90 días de la inoculación

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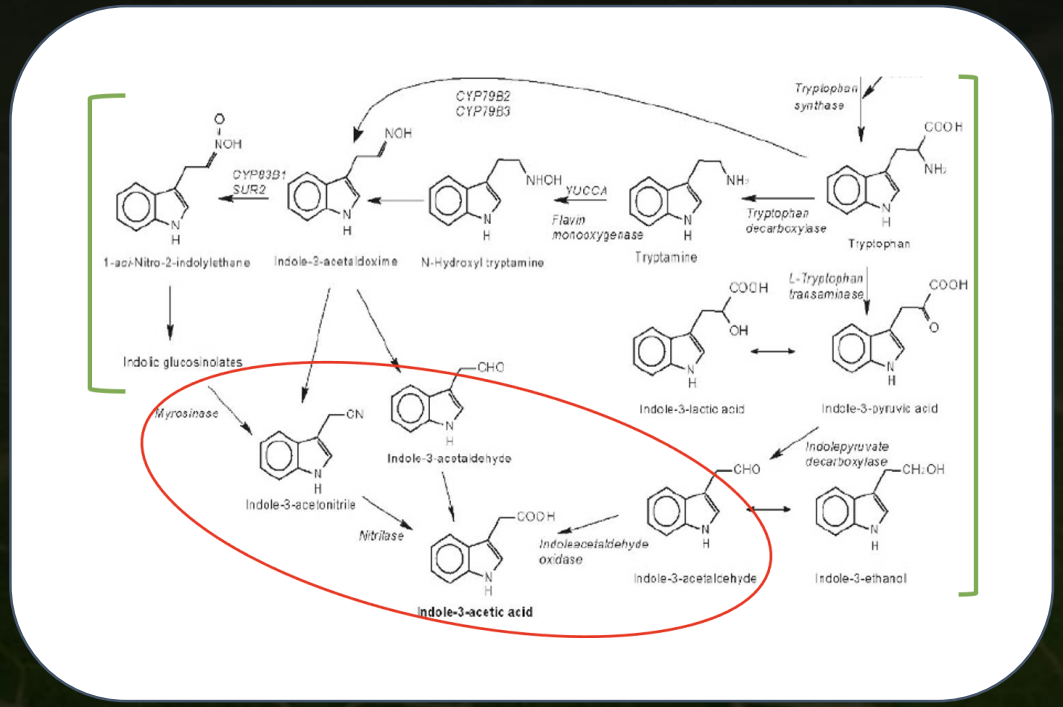
Auxins
Production and Induction

- There are multiple pathways
- ST9 utilizes tryptophan as a precursor

1. Trp. Indol (derivates)
2. Indol. Indol Acetico Acid (IAA)



Moreno Casco, J. (2010). Biorremediación de suelos contaminados con hidrocarburos. En Biotecnología ambiental (pp. 145-165). Pearson Educación

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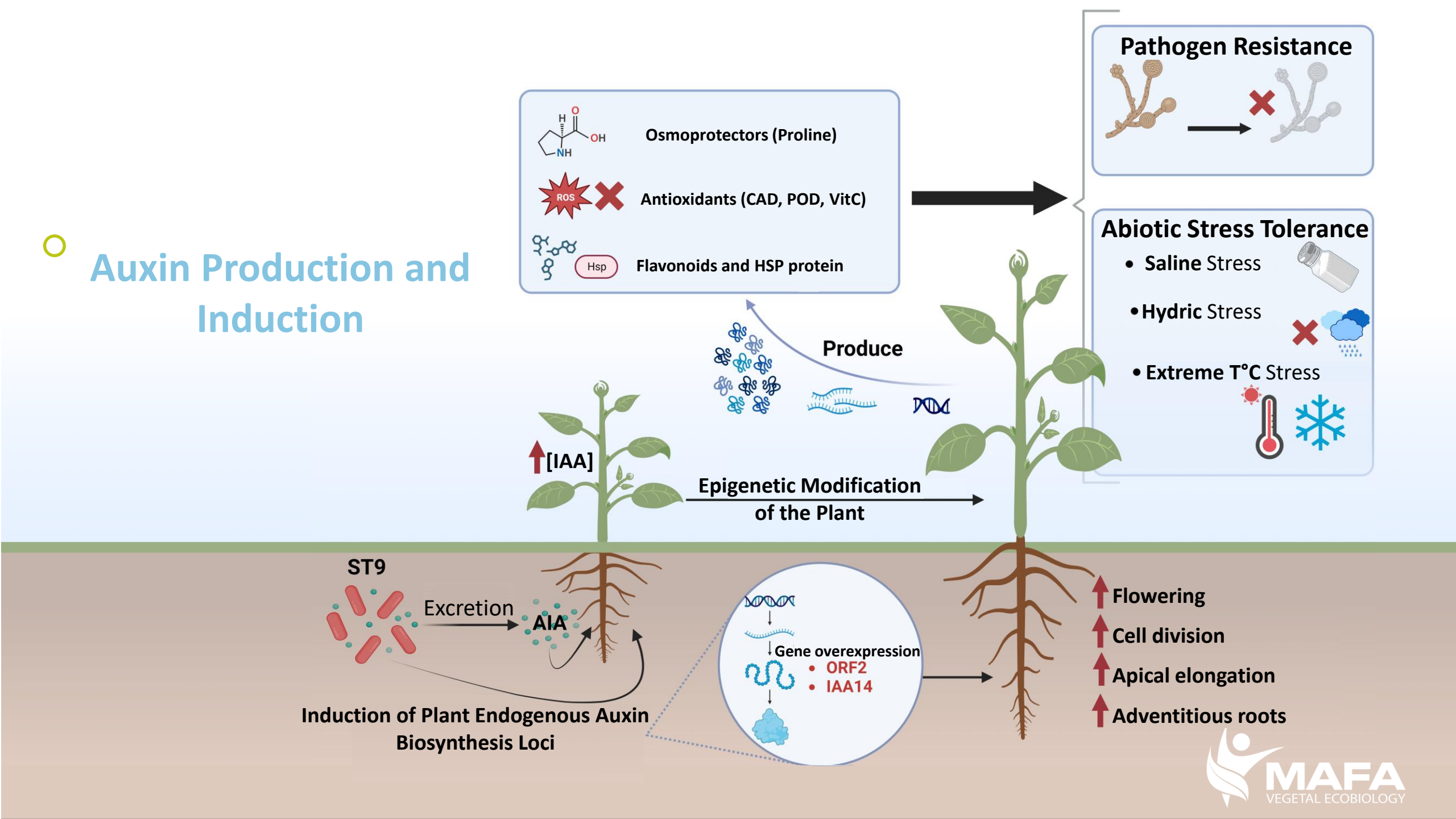
Auxin Production and Induction



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RESULTS Auxins-ST9

Control

Treated

- Stimulation of the radicular growth

Control

Treated

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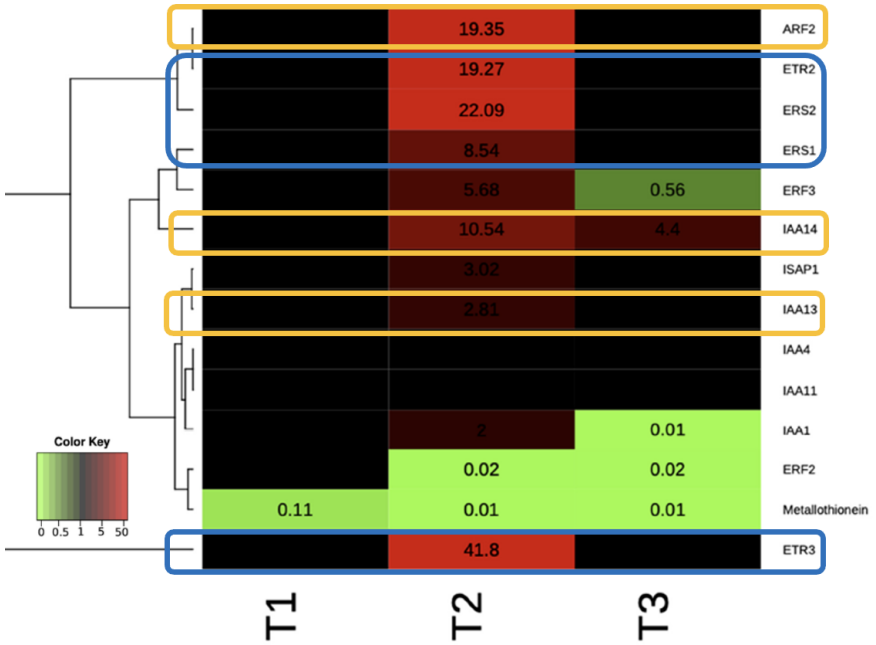
Differential epigenetic study in crops

- Analysis of the expression of 14 plant genes inoculated with ST9.



Elevated gene expression:

- ERS1
- ERS2
- ETR2
- ETR3

Regulation and signaling of **Ethylene**

Increase in tolerances

- **Saline stress**
- **Water stress**

Elevated gene expression:

- ARF2
- IAA14
- IAA13
- ETR3

Regulation and signaling of **Axunis**

Induction of Auxin biosynthesis in plants

Heatmap: ST9 induce Differential Plant Genetics expresión ($2^{-\Delta\Delta CT}$)

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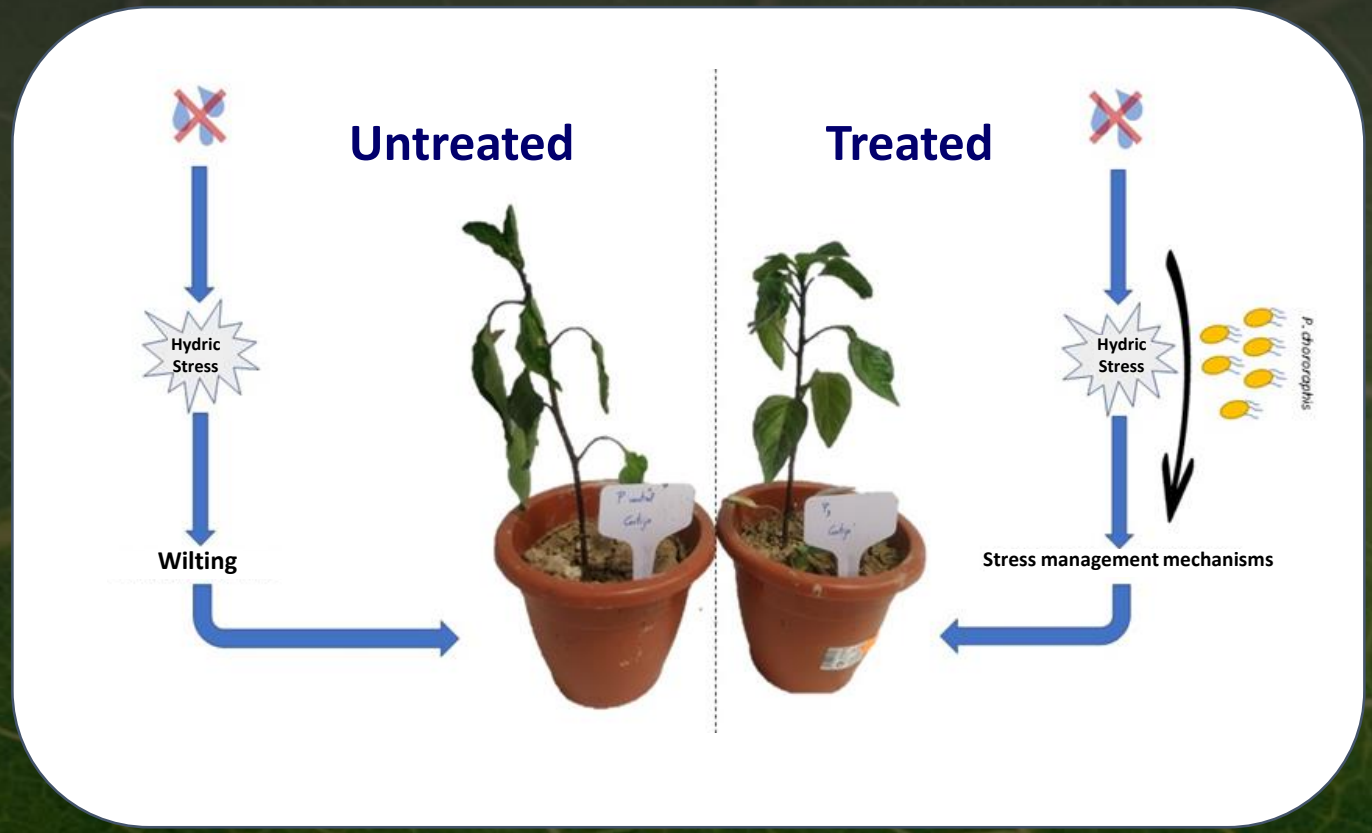
Reduction of water/saline stress

- Induction of water/saline stress.
- Results after treatment with ST9.



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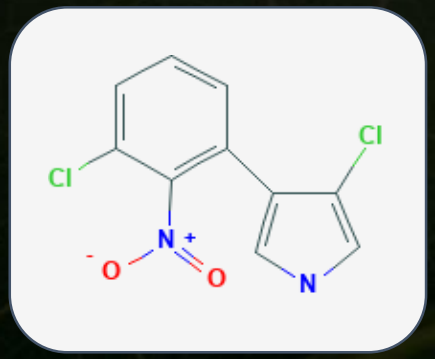
Biologic Control

- In addition to microbiota stimulation, biofilm, pioverdin production, proteases, and chitinases.
- Production of antifungal substances.



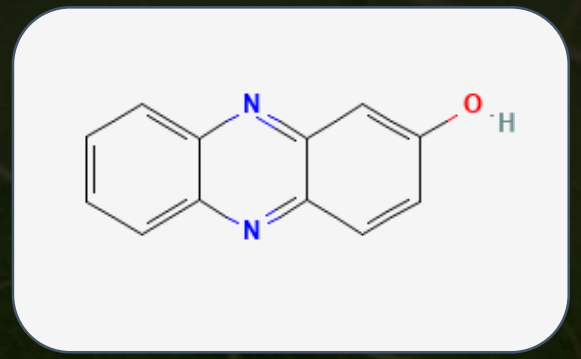
Pirrolnitrin

Nomenclature

- 2-hydroxy phenazine
- Pirrolnitrin
- 2-hexyl, 5-propyl resorcinol (HRP)
- Phenazine 1-carboxylic acid (PCA)

Antimicotics

Provide biological control agent function



2-hydroxy phenazine

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Biologic Control

- Proven effective biological control against

Aspergillus nodulans

Magnaporthe grisea

Fusarium graminearum

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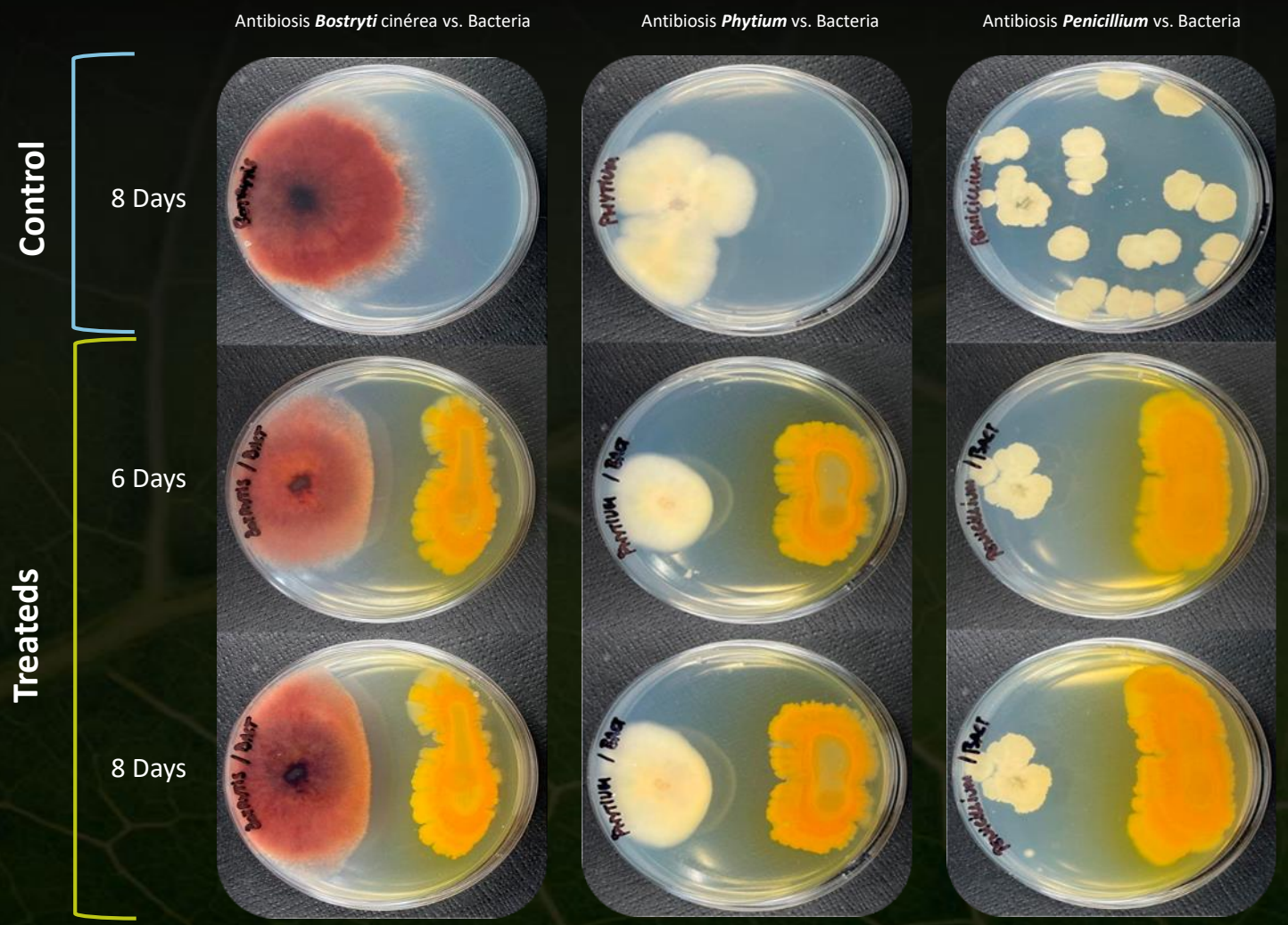
Biologic Control

- Proven effective biological control against:
  - *Botrytis cinerea.*
  - *Phytium.*
  - *Penicillium.*



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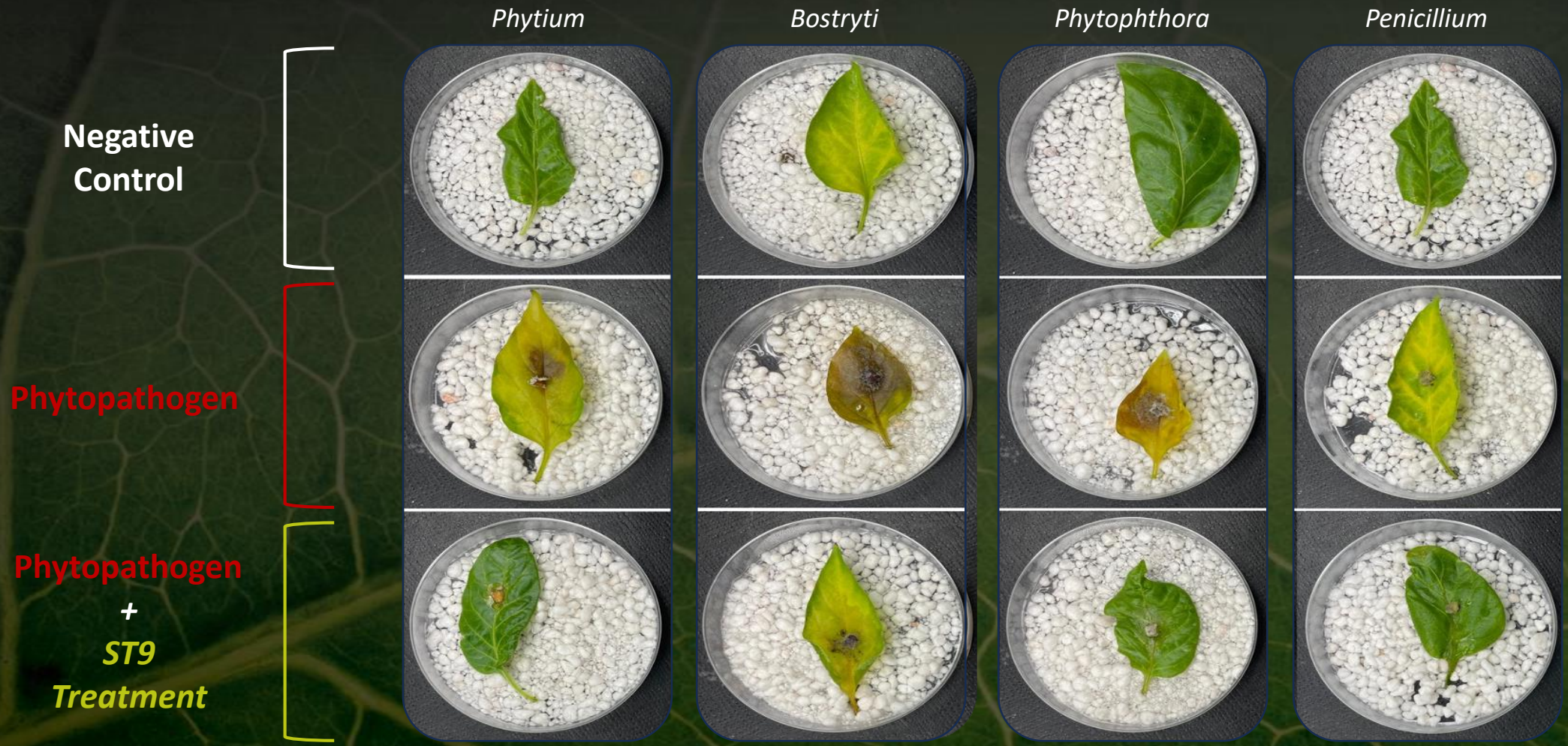
Biologic Control



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CONCLUSIONS

- The soil is a living entity.
- It is essential to mitigate the environmental harm caused by agriculture through the adoption of innovative technologies.
- PGPRs, a solution to current challenges
- ST9 demonstrates the characteristics to be the solution to the new challenges in agriculture.

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Thank you for your attention

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