Plant growth-promoting bacterial consortia isolated from halophytes to improve crop response to salinisation and climate change

*<u>**Racioppo A.**</u>, Guerrieri A., Speranza B., Botticella L., Conversa G., Corbo M.R.

email: angela.racioppo@unifg.it

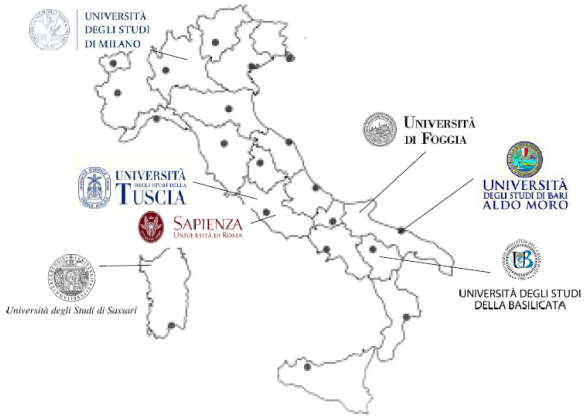
Department of the Science of Agriculture, Food, Natural Resources and Engineering, University of Foggia, Via Napoli 25, Foggia, Italy

18-20 September, 2024
Bari, Italy

Task 7.2.1 Identification of sustainable animal derived resources, crops, ornamental and medicinal plants (M1-M24)

Task Leader: UNISS, Sara Melito

Animal derived resources, crops, ornamental and medicinal plants adapted to Italian marginal areas will be tested for their ability to promote an integrated economic development and valorisation of the landscape



Research Activity

UNISS: Identification, characterization and valorization of crops suited to marginal areas.

UNIBA: Identification of sustainable animal derived resources, crops, ornamental and medicinal plants.

UNIFG: Identification of plant- and animal-derived products for the sustainable valorization of Foggia province marginal areas.

UNIBAS: Enhancement of sustainable animal resources.

UNIMI: Identification, characterization and valorization of agro-biodiversity plants in mountain areas

UNITUS: Identification, characterization and valorization of crops in marginal areas.

UNIRM: Minor cereals and legumes for the exploitation of marginal agricultural areas: selection of sorghum and yellow-pea genotypes with high adaptability to stresses and high productivity.

agritech

National Research Center for Technology in Agriculture

Production and growth of plants are affected by environmental conditions

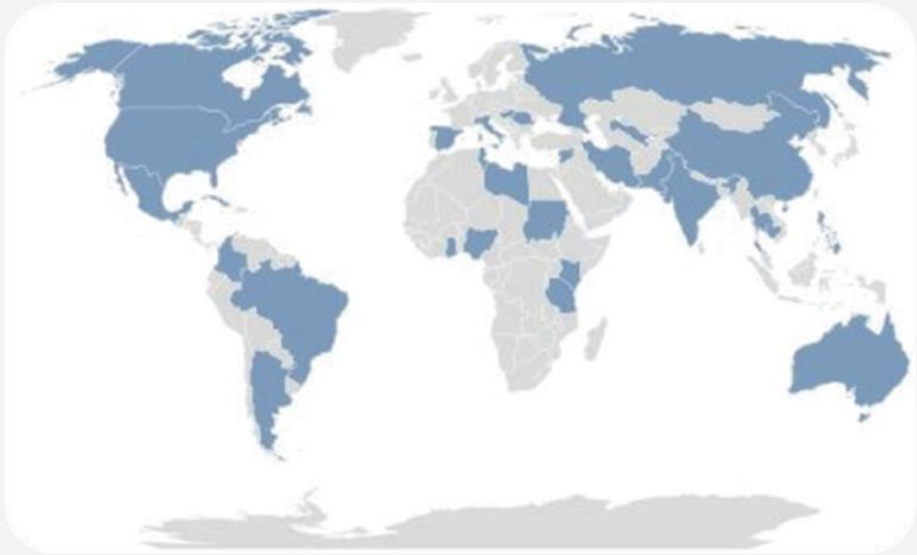
Salinity

Water deficit

Heat stress

Heavy metals

Among these, **SALINITY** can be considered one of the most serious threats to agricultural production and food security, as it affects more than 20% of agricultural land and is expected to increase to 50% by 2050.



Food and Agriculture Organization of the United Nations

Salt-affected soils

A global concern reducing agricultural productivity

HEALTHY SOILS

A healthy soil is able to sustain the productivity, diversity, and environmental services of terrestrial ecosystems.

Good and stable aggregates

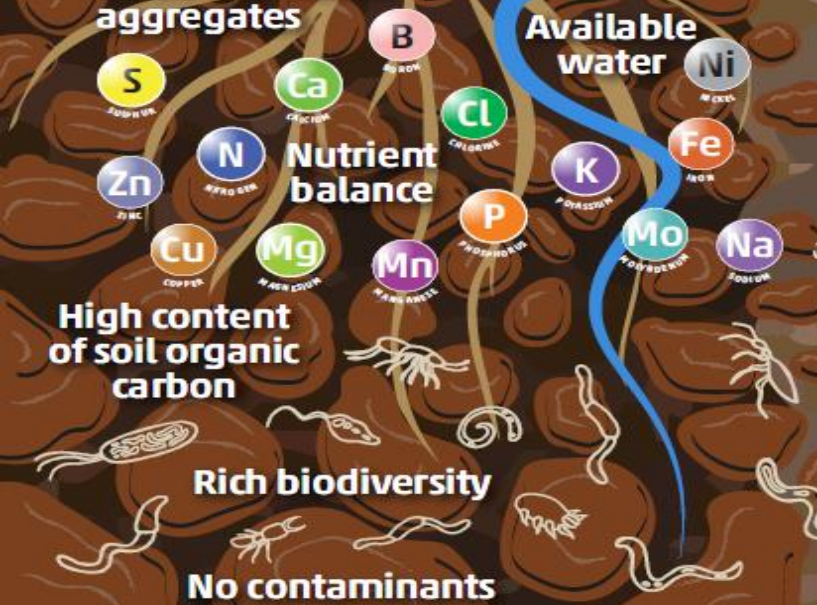
Available water

Nutrient balance

High content of soil organic carbon

Rich biodiversity

No contaminants



Accumulated salts

SALINE SOILS

Ca^{2+} Mg^{2+} Na^{+} SO_4^{2-} Cl^{-} HCO_3 CO_3^{-2}

Saline soils have excessive levels of soluble salts.
It can negatively impact or inhibit plant growth and can be toxic to life.

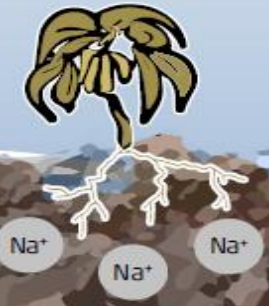
Na_2SO_4 NaCl

Nutrient imbalance

Less biodiversity

Less available water

Improper water management (insufficient water supply, poor water quality, reuse of brackish water and bad drainage systems)



SODIC SOILS

Na^{+} Na^{+} Na^{+}

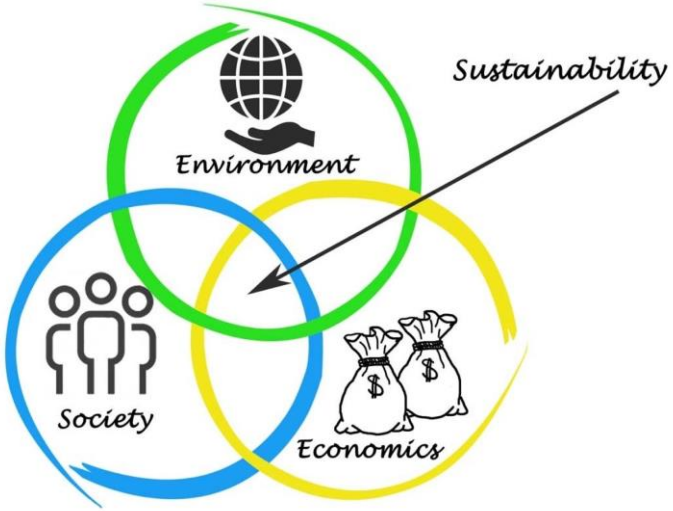
Sodic soils have a high amount of adsorbed sodium.
It leads to degradation of soil structure and inhibits plant growth.

Massive structure in the subsoil

Less biodiversity

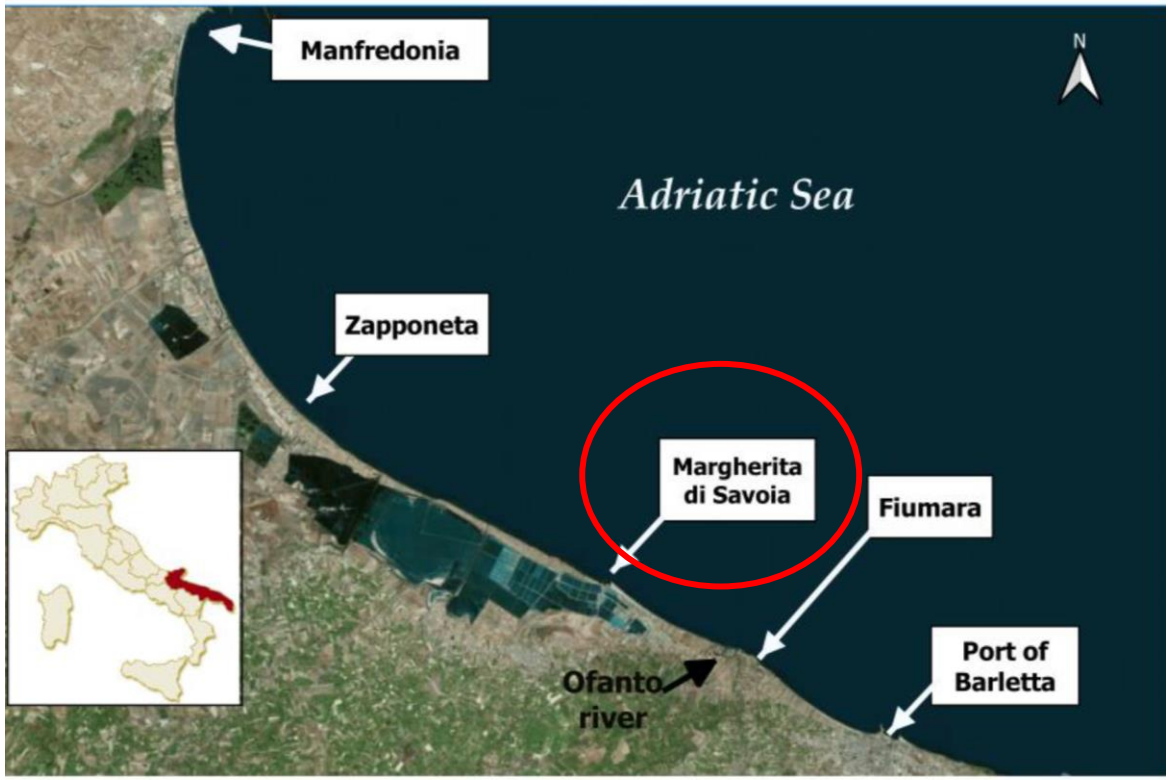
Nutrient imbalance

GLOBAL SOIL PARTNERSHIP



Plant Growth Promoting Bacteria (PGPB)

Halotolerant plant growth promoting bacteria are able to enhance plant growth by increasing soil carbon, nitrogen and mineral availability and uptake.

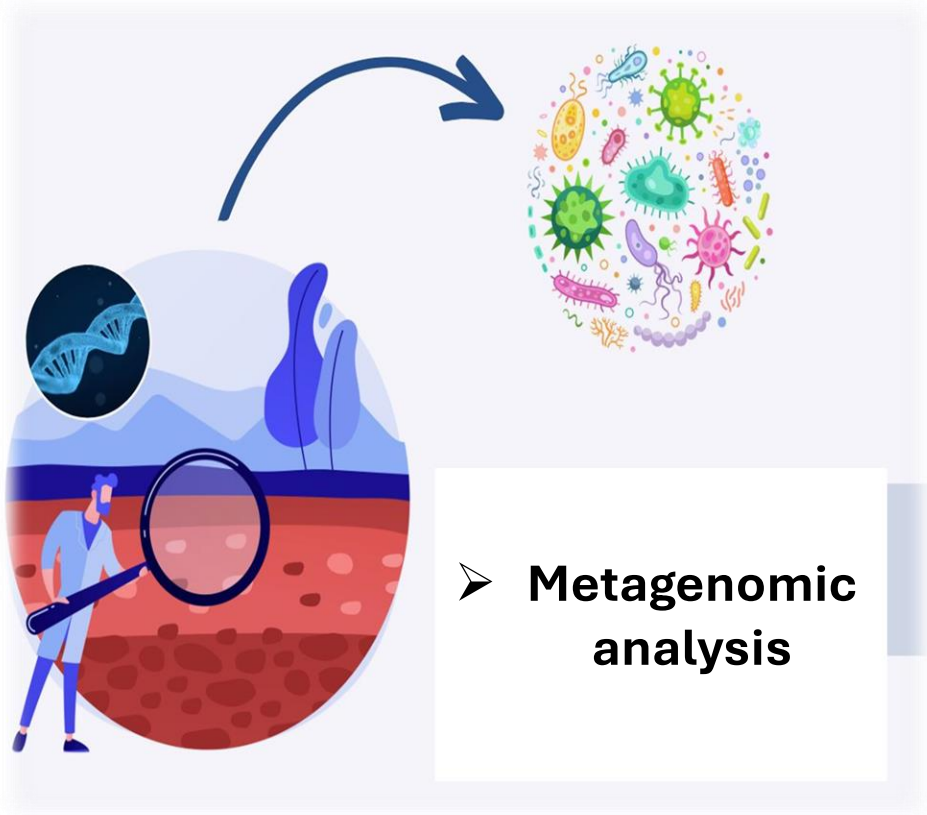


Coastal and saline regions, including the area of **Margherita di Savoia saltworks**, north-eastern Apulia (Italy), have proven to be a natural source of beneficial microbes adapted to high salinity.

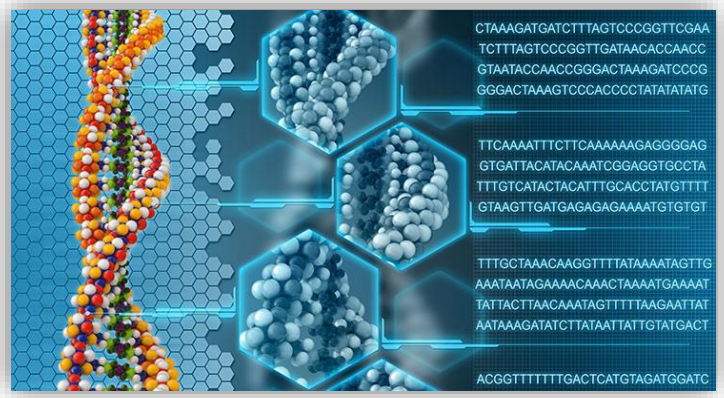
Aim
Research

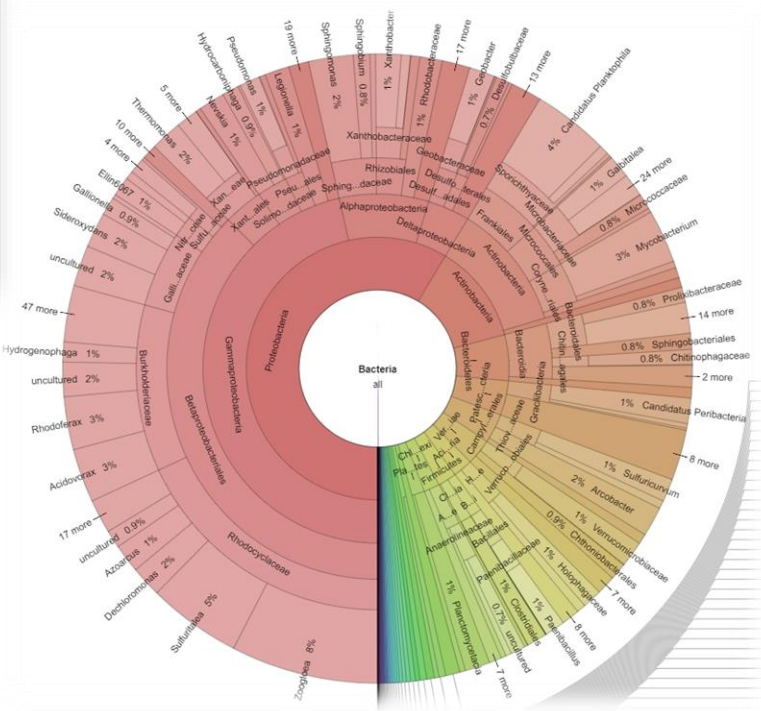
The aim of the present study was the isolation, characterization and selection of potential **PGPB** from ***Cakile maritima*** plants collected in **two sites** located in **Margherita di Savoia** (N 41, 431624; E 16, 008846 - N 41, 427654, E 007945) to be used as potential biofertilizer.

Activity 1:



- **Metagenomic analysis**



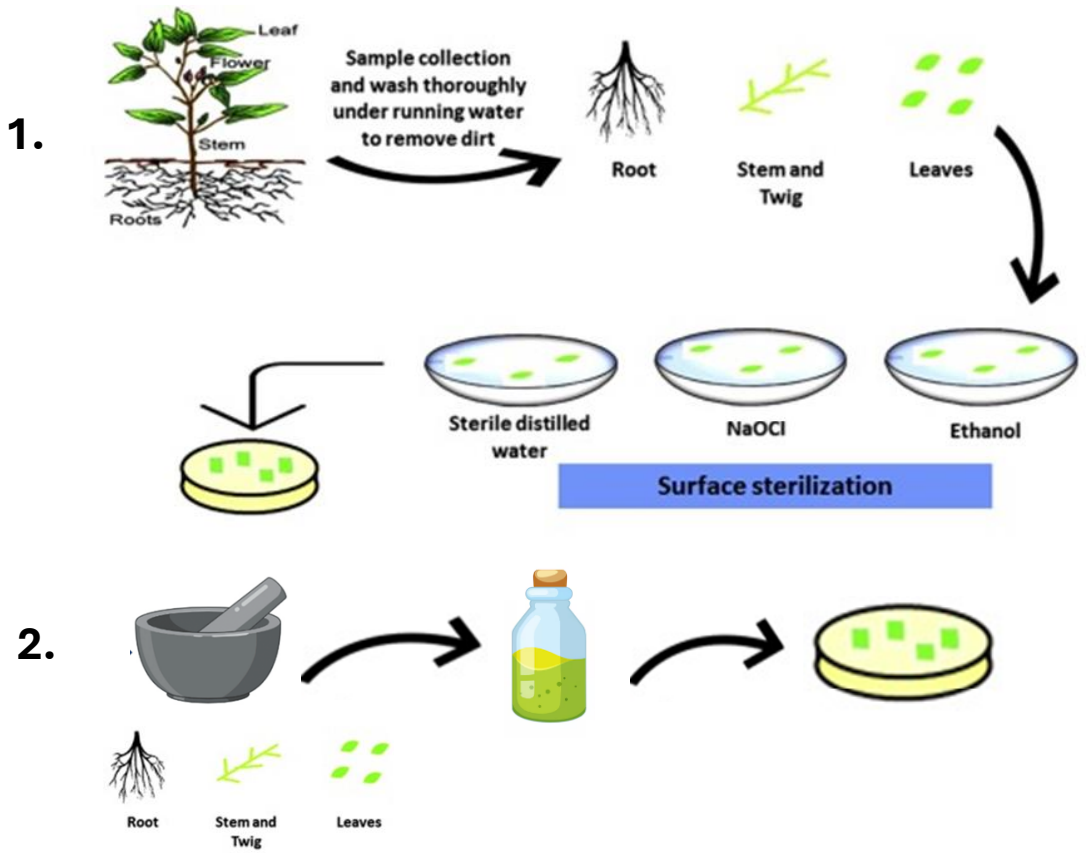


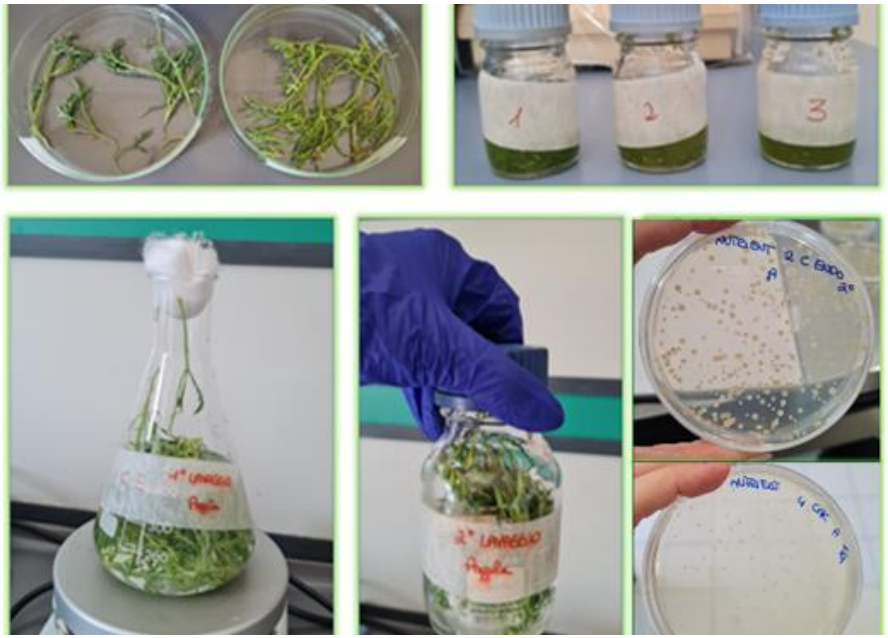
➢ Isolation and Characterization

Microbiological sampling was carried out in triplicate at three phenological stages of plant life cycle during the year 2023:

- ✓ Seedling
- ✓ Vegetative growth
- ✓ Flowering

Endophytic cell isolation (Christakis et al., 2021)





Rhizosphere cell isolation

3 distances from rhizosphere (0 mm, 1-3 mm and 5-10 cm)

- **Mesophilic bacteria**
- **Pseudomonads**
- **Spore-formers**
- **Actinobacteria**
- **Nitrogen-fixing bacteria**

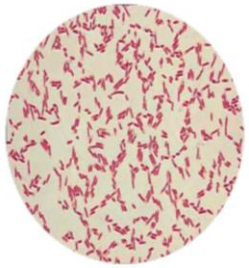
Christakis CA, Daskalogiannis G, Chatzaki A, Markakis EA, Mermigka G, Sagia A, Rizzo GF, Catara V, Lagkouvardos I, Studholme DJ, Sarris PF. Endophytic Bacterial Isolates From Halophytes Demonstrate Phytopathogen Biocontrol and Plant Growth Promotion Under High Salinity. Front Microbiol. 2021, 4;12:681567.

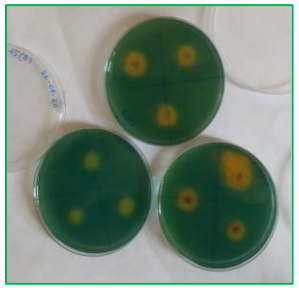
Isolated **180** bacteria:

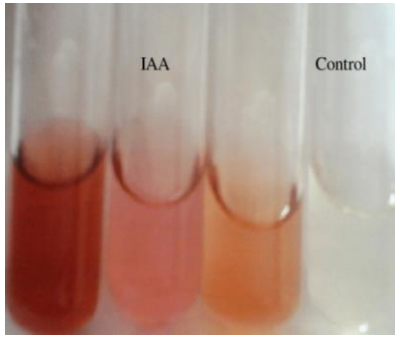
150 Rhizobacteria

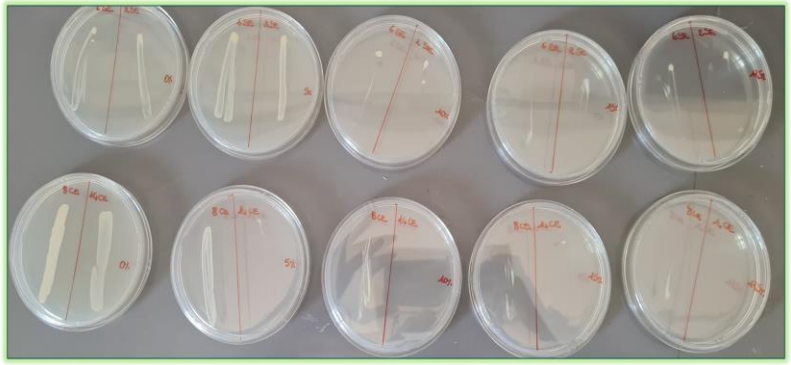
30 Endophytes

Characterization





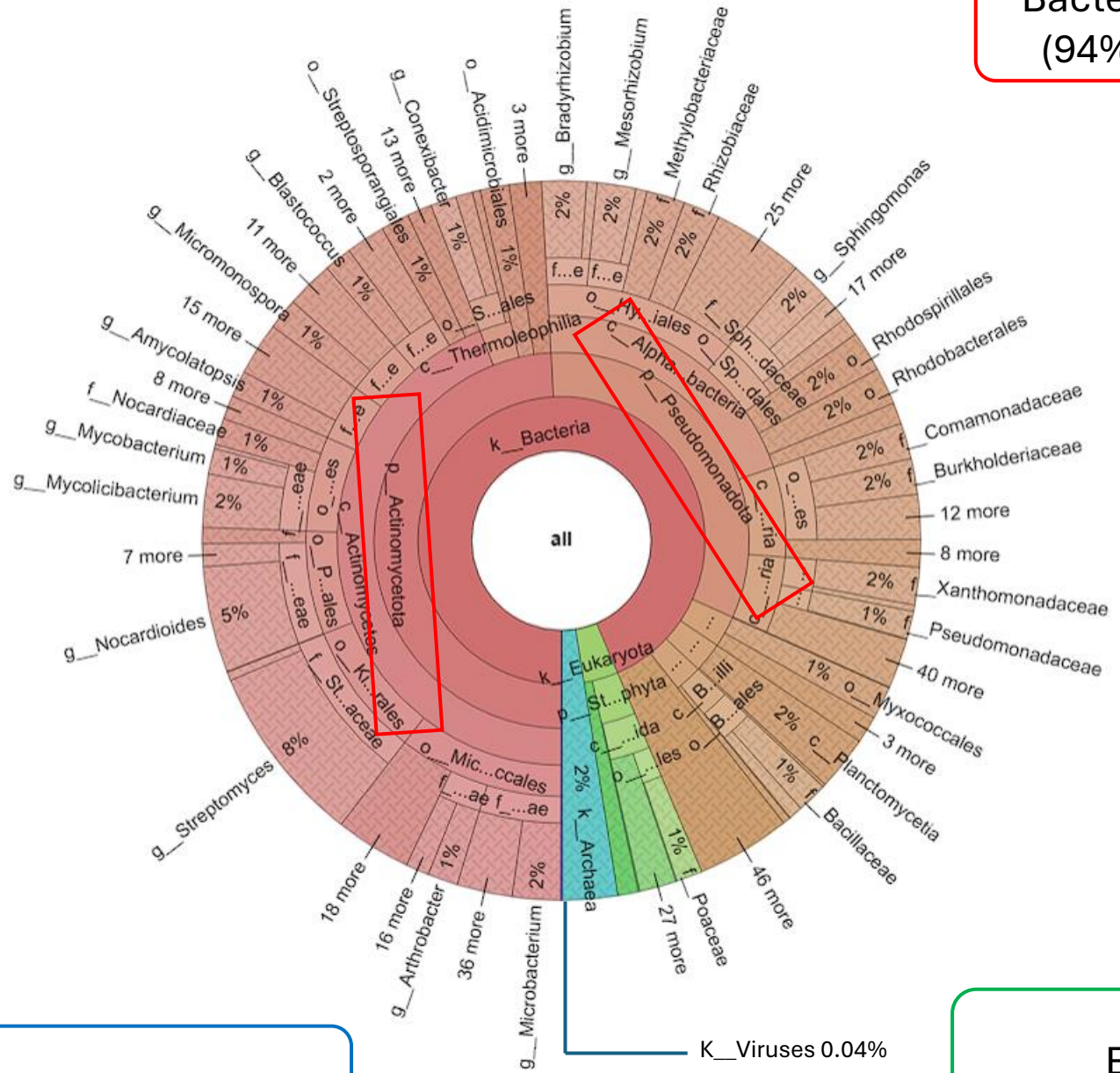




phenotypic tests (microscopic observation, Gram staining, spore production, catalase, oxidase, urease)

qualitative tests (ammonium production, nitrification, P-solubilization, silicon solubilization, IAA production, siderophores production, salt tolerance (NaCl ranging from 5, 10, 15, and 17.5%, w/v)).

Results



Bacteria (94%)

ACTINOMYCETOTA (52%)
PSEUDOMONADOTA (35%)

ACTINOBACTERIA (44%)
ALPHAPROTEOBACTERIA (21%)
PROPIONIBACTERIALES (7%)
BETAPROTEOBACTERIALES (6%)
GAMMAPROTEOBACTERIA (5%)

Streptomyces (8%)
Bradyrhizobium (2%)
Mycobacterium (1%)
Pseudomonas (1%)
Bacillus (1%)

Archea (2%)

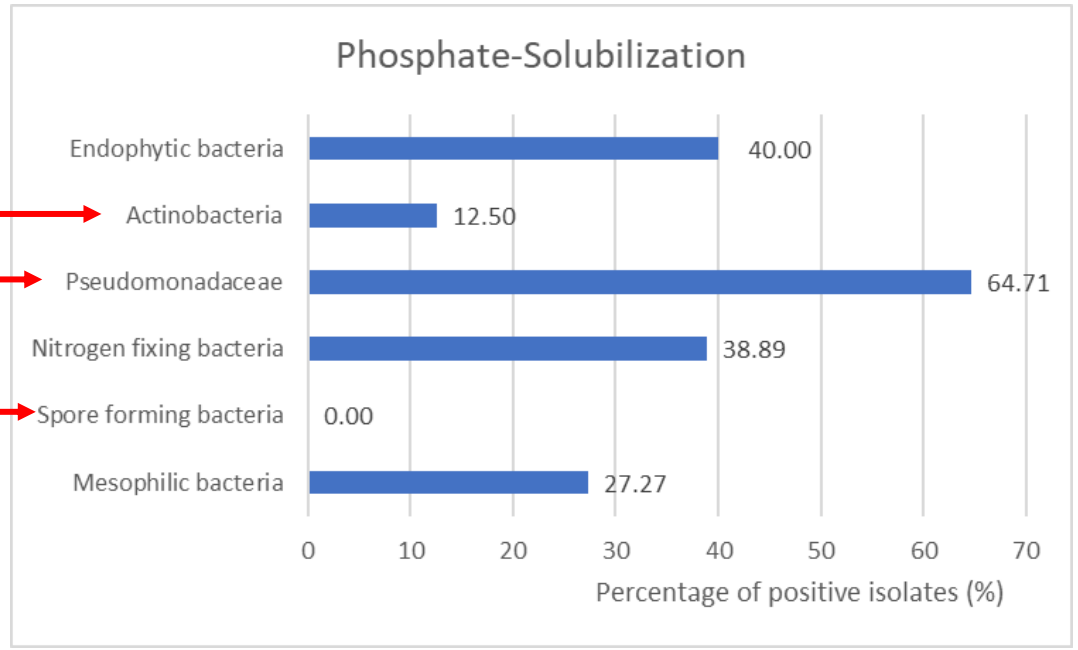
Eukaryota (4%)

Table 1. Results for the phenotypic characterization of the 180 isolates from sea rocket (*C. maritima*).

Bacteria	Number of isolates from sea rocket	Gram positive	Catalase	Oxidase
Mesophilic bacteria	41	15	33	7
Spore forming bacteria	26	18	18	6
Nitrogen fixing bacteria	22	10	12	4
Pseudomonadaceae	23	3	21	12
Actinobacteria	38	15	31	12
Endophytic bacteria	30	4	21	4

- ✓ Gram-positive bacteria accounted for about 40% of the population of presumptive mesophilic bacteria, nitrogen-fixing bacteria and actinobacteria; pseudomonads were mainly Gram-negative;
- ✓ A positive response to oxidase was mainly found among the pseudomonads and actinobacteria;
- ✓ Response to H_2O_2, the isolates were mainly catalase-positive, and this characteristic suggested their aerobic or aero-tolerant metabolism;
- ✓ Endophytic bacteria were mainly Gram-negative, oxidase-negative.

Figure 1. Strains positive to phosphate-solubilization.



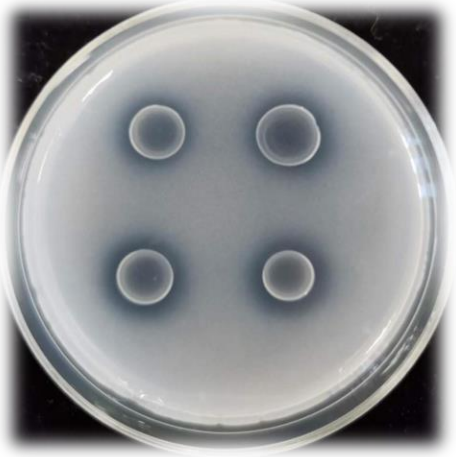


Figure 2. Strains positive to ammonium production.

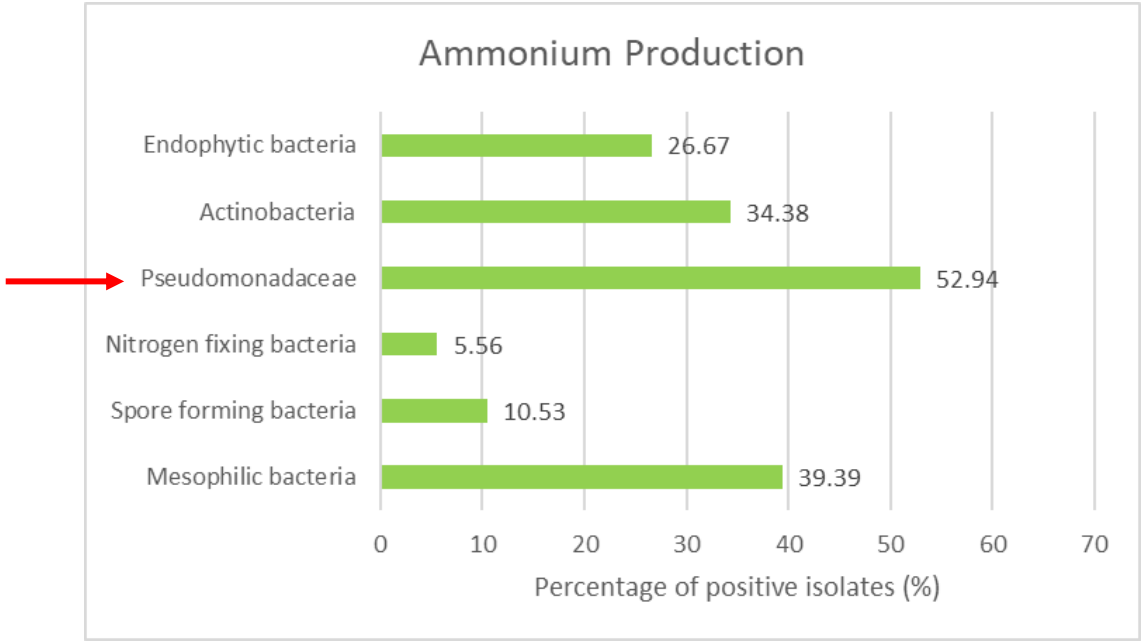


Table 2. Strains positive to silicon solubilisation, indole acetic acid production (IAA), siderophores production, salt tolerance.

Bacteria		Siderophores production	IAA	Silicon solubilization	Salt tolerance
Rhizobacteria	150	14	14	47	70
Endophytes	30	1	5	17	12
Tot.	**180**	**15**	**19**	**64**	**82**
		↑ 9%	↑	↑ 36%	↑ 46%

Figure 3. Salt tolerance.

The bacteria were also tested for their ability to grow at increasing levels of salinity (5%, 10%, 15%, 17.5%).

all isolates were able to grow at **5%** salinity

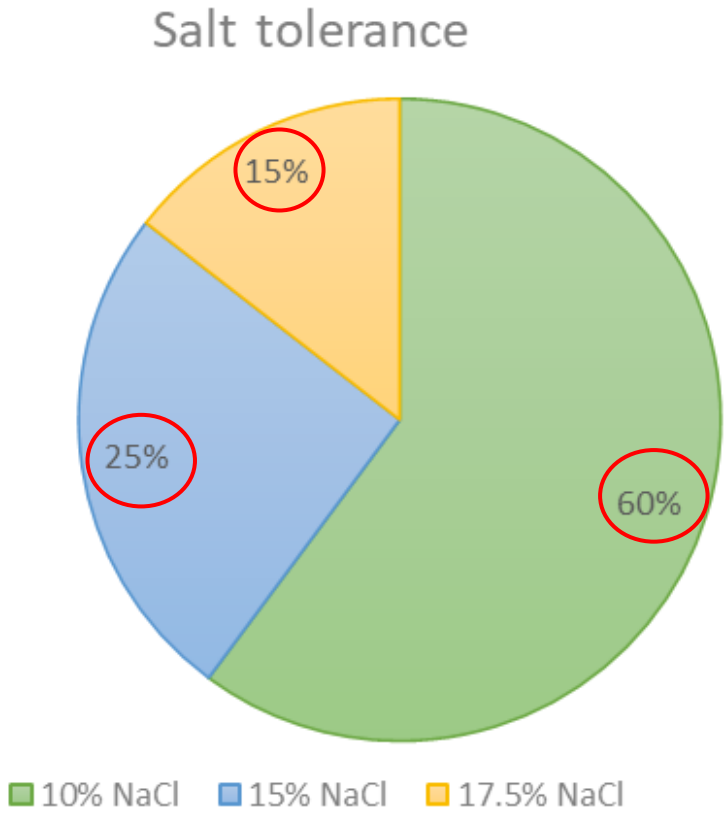
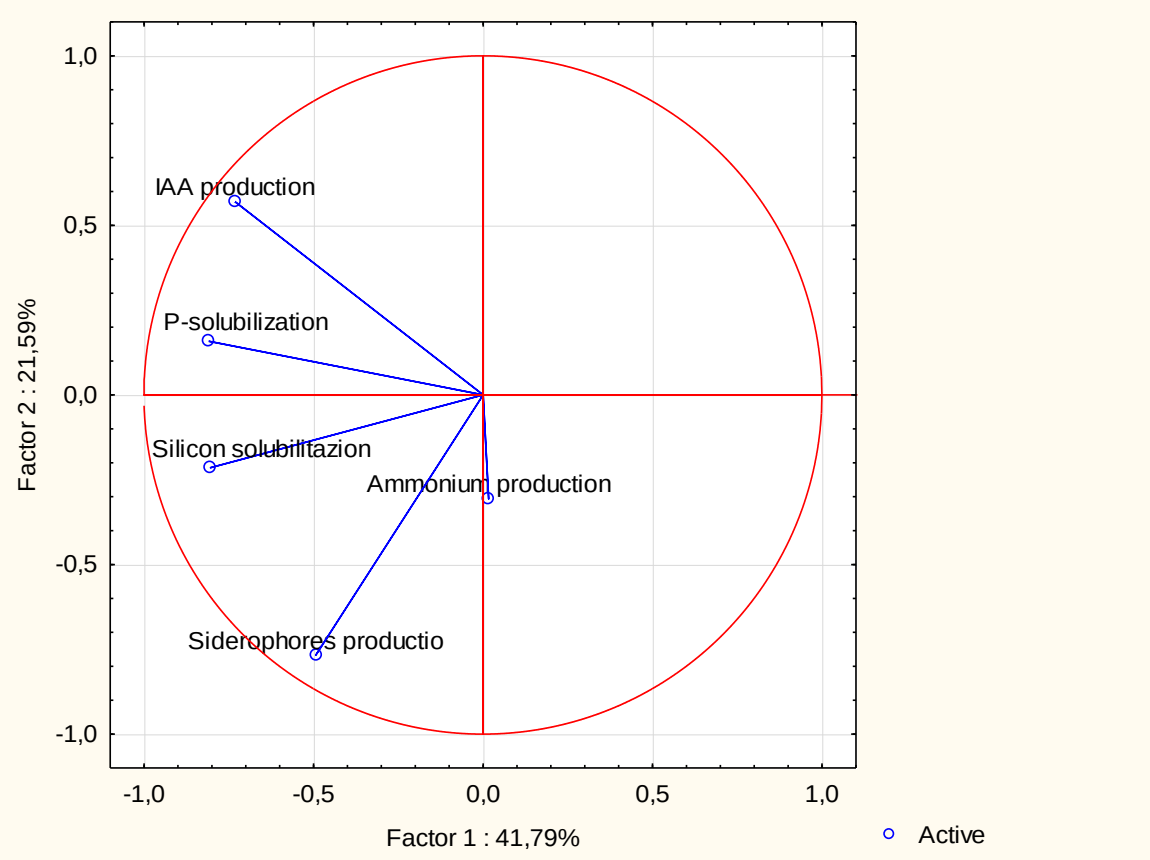
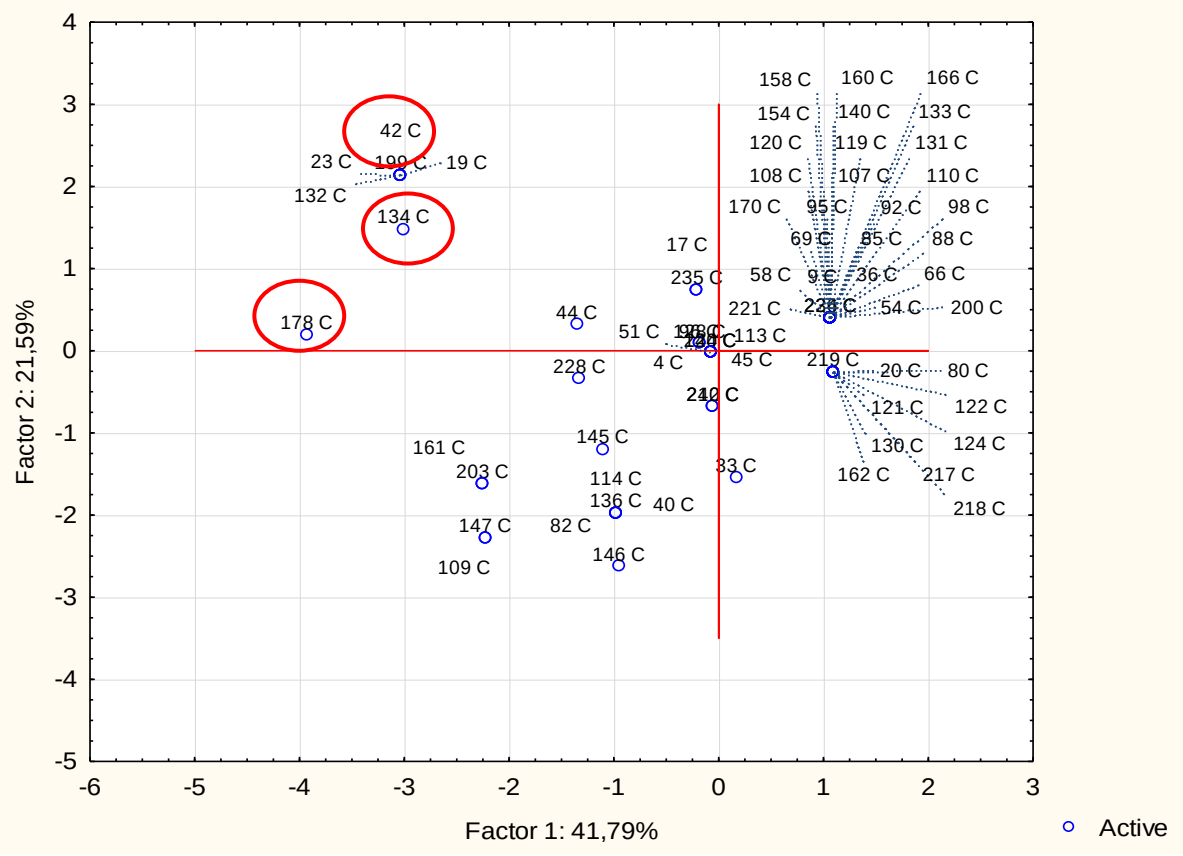
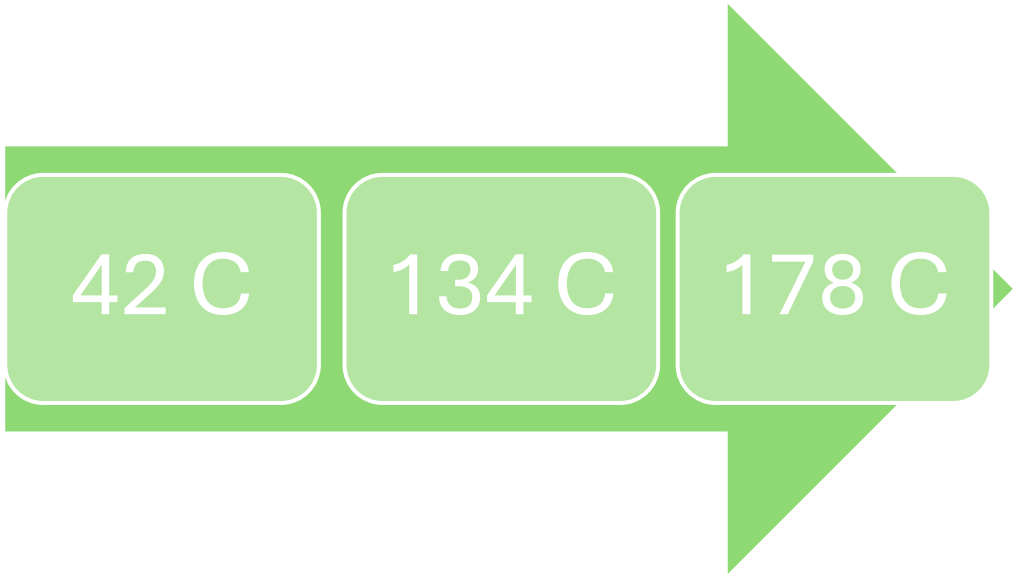


Figure 4. Principal component analysis run on the output to IAA production, P-solubilization, silicon solubilization, siderophores production, ammonium production.





3 MOST PROMISING STRAINS:



The isolates were identified by sequencing the 16S rDNA

Table 3. Characteristics of the most promising strains.

	Identification	Ammonium	Phosphate	Salt	Silicon	IAA	Siderophores
42C	*Pantoea*		+	+	+	+	
134C	*Bacillus*	+	+	+	+	+	
178C	*Pantoea*		+	+	+	+	+

Activity 2:

Preliminary validation in greenhouse

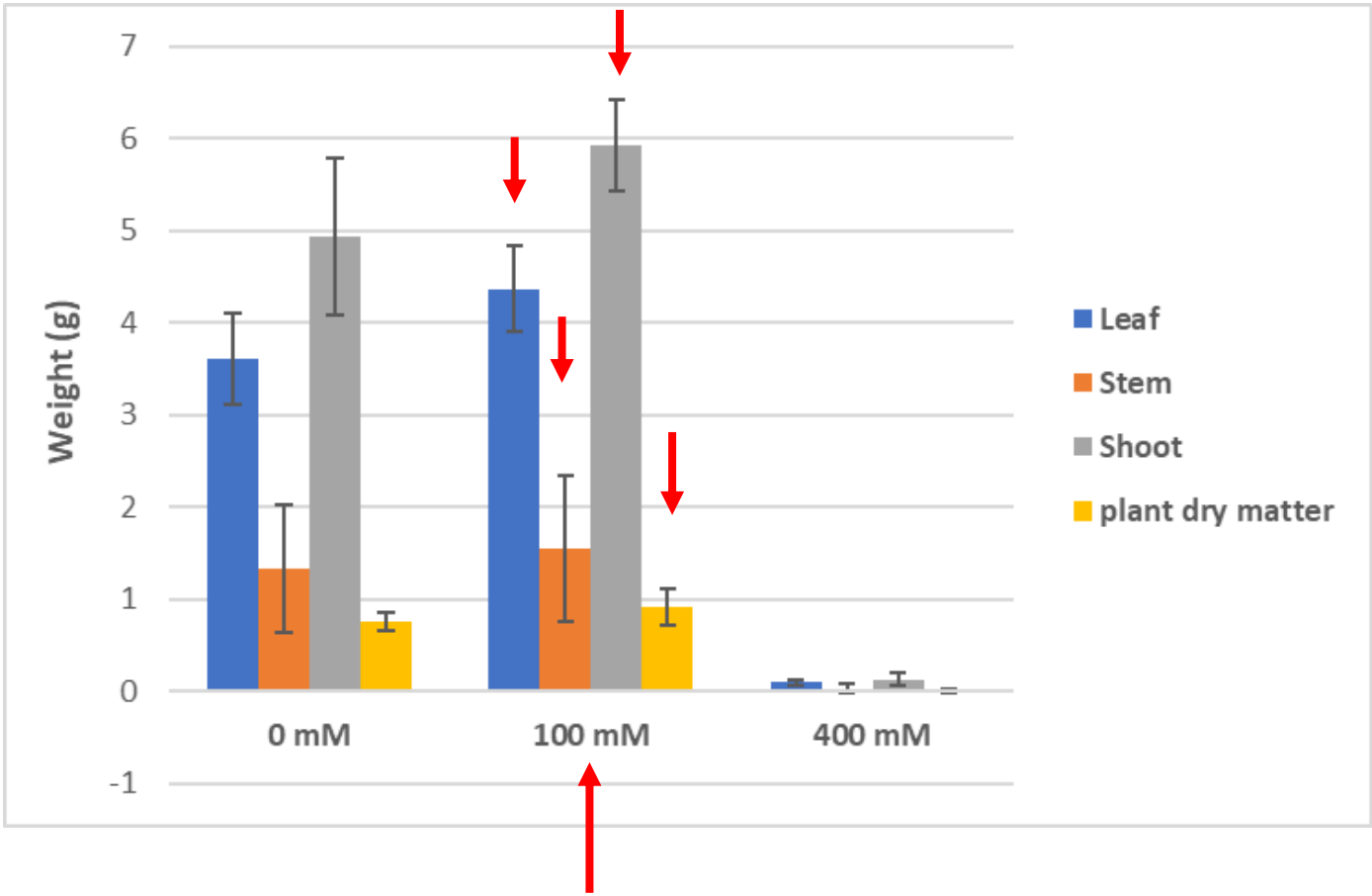
- Bacterial inoculation (BI) was performed on four-week-old plants of *Cakile maritima* grown in pots filled with inert sand (1L), irrigated with Hoagland's nutrient solution and then supplemented with saline solution added with 0, 100, or 400 mM of NaCl.

Results

MICROBIOLOGICAL ASSAY

Initially, the soil viable count was approximately 6 log CFU/g; soil inoculation resulted in a 1-log increase in the viable counts of the most important groups at the end of the assay.

Figure 5. Fresh and dry weight of inoculated plants.



Conclusions

1. • Selected 3 promising strains from indigenous halophytes plants
2. • Possessed several plant growth promoting traits
3. • In vitro and in vivo evidence suggest the potential of these strains for biofertilizer application

01 The estimation of a PGPB-potential to a biofertilizer application, requires a long way of greenhouse and field experiments

02 Future studies concerning greenhouse and field applications as biofertilizer are in progress

Department of the Science of Agriculture, Food, Natural Resources and Engineering,

University of Foggia, Via Napoli 25, Foggia, Italy