Halotolerant Endophytes

Identification and characterization of plant-growth promoting microbes in saline conditions

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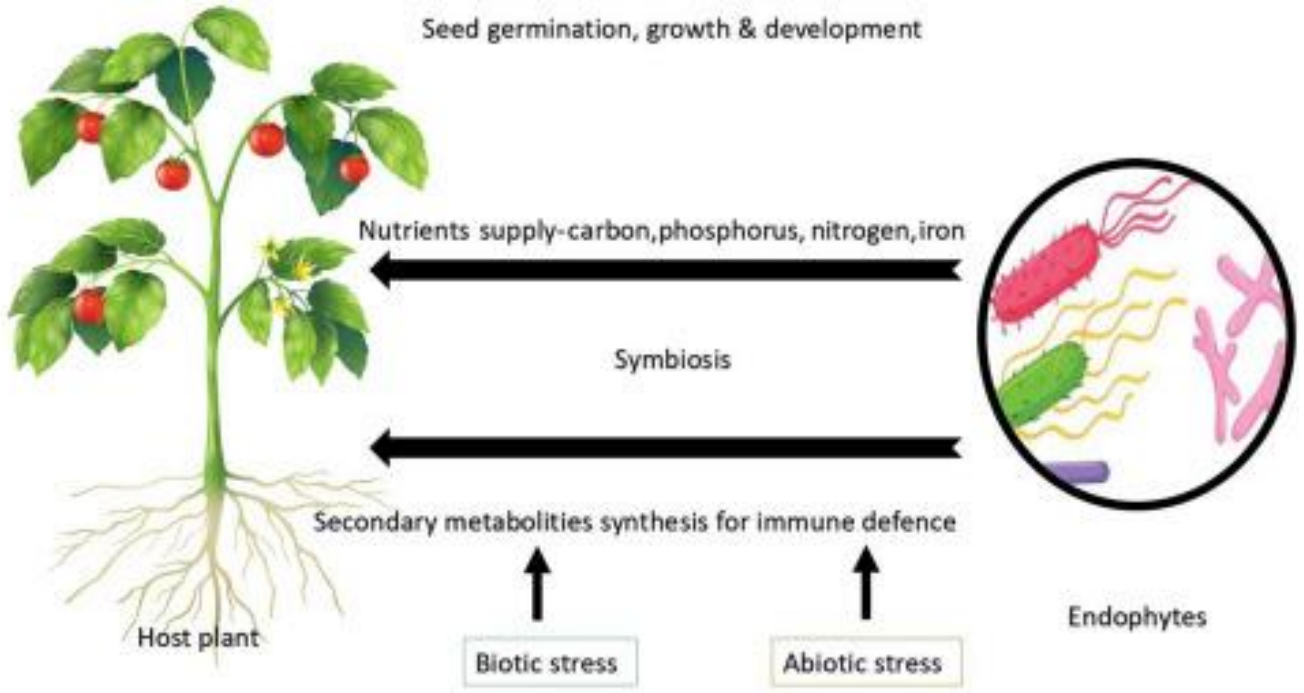
Endophytes: Microorganisms living between the cells of a Plant

Symbiosis

- Microbes provide available nutrients and hormones to the plant
- Plant provides energy to the microbe in biomaterials

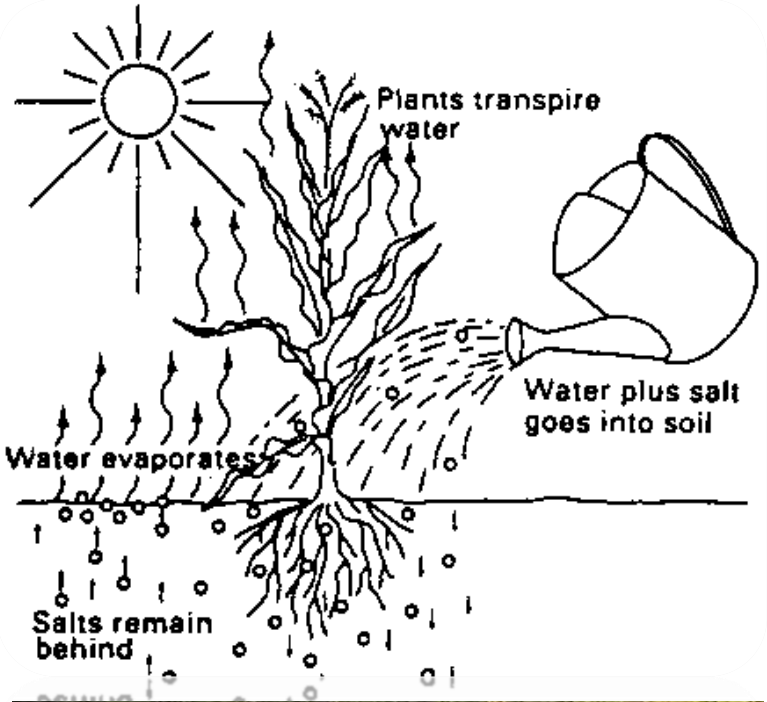


https://www.sciencedirect.com/topics/immunology-and-microbiology/endophyte

Salinity in Utah and Across the World

Salt is a concern for farmers and for food security.

- Increased incidences of drought are forcing the use of more saline irrigation.



Endophytes Extracted from *Ceanothus Velutinus* (Snow Brush)

- Native to the Intermountain West
- Resilient to dry, harsh conditions
- Found in previous studies to have a beneficial microbe biome

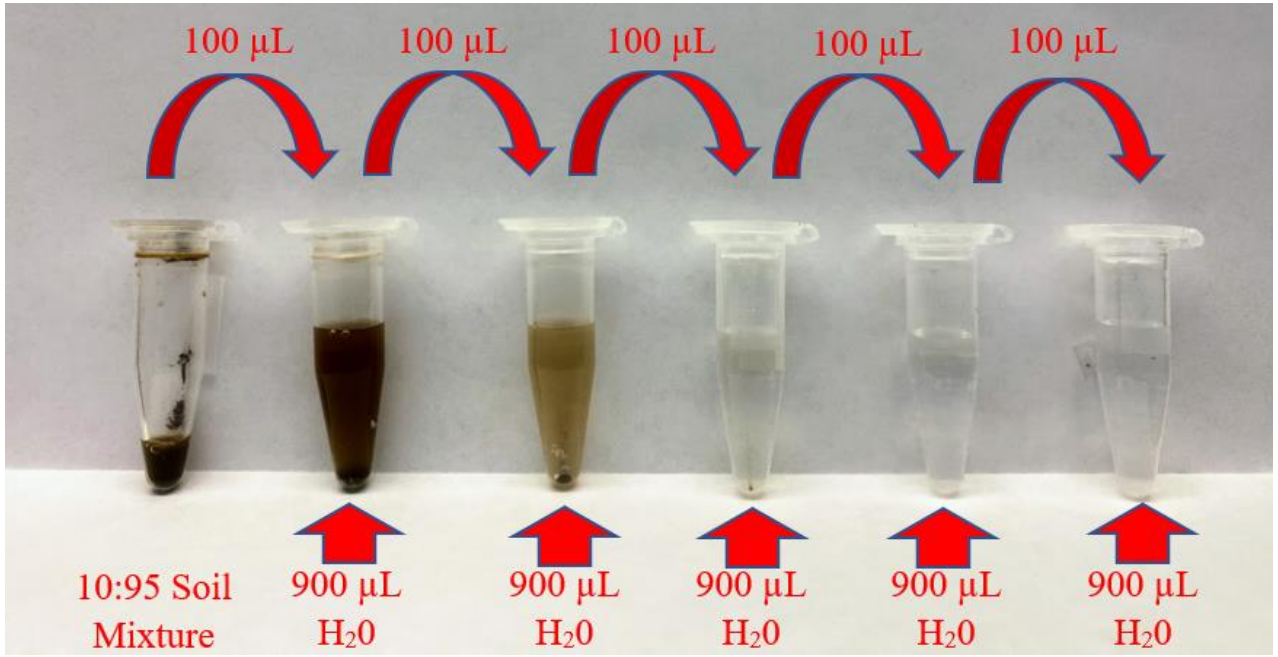
Extraction of Endophytes

1. Grinding

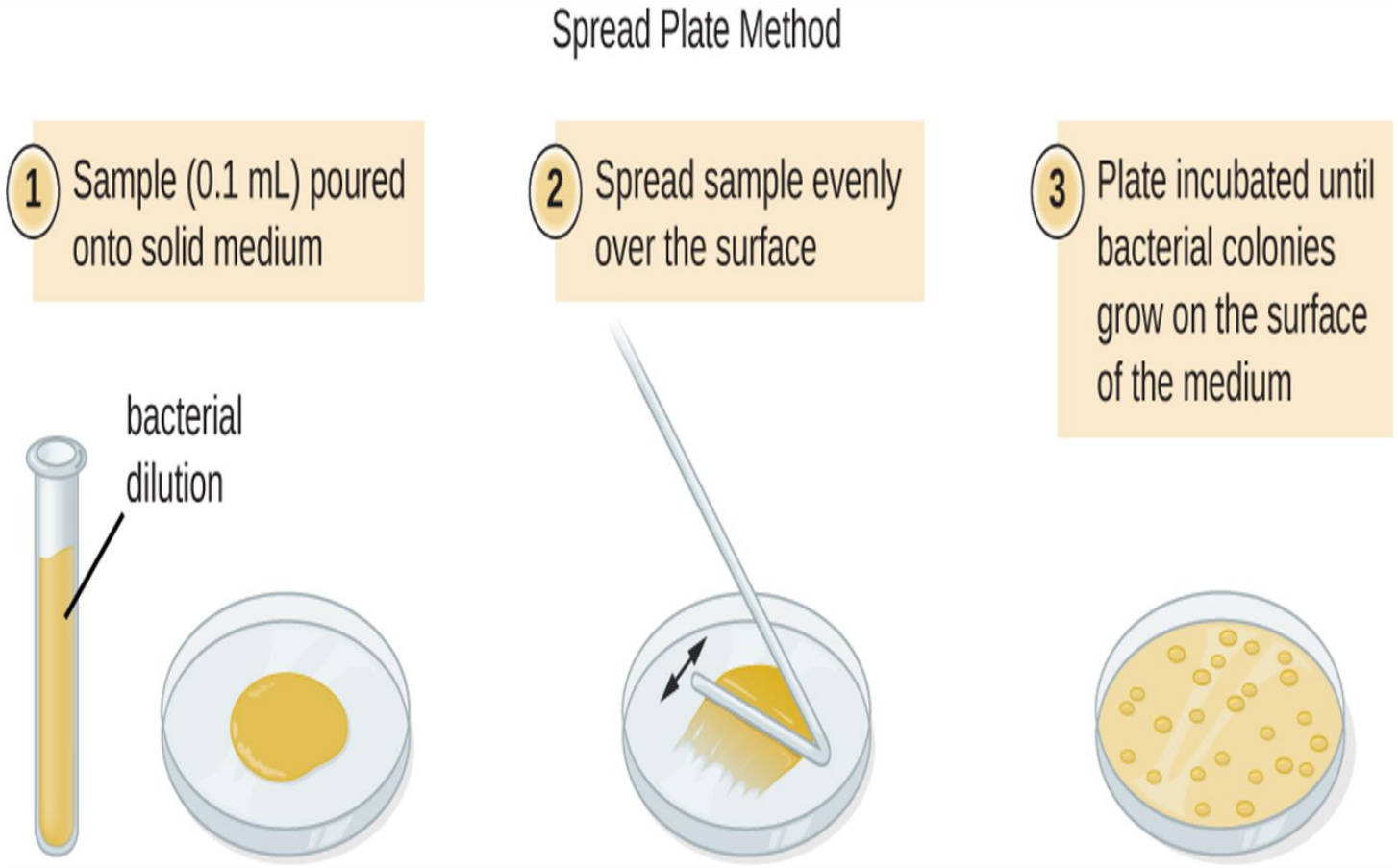
Root

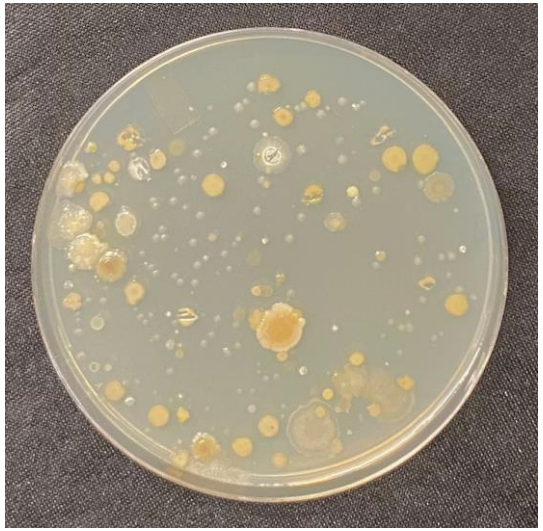
Nitrogen-Fixing Nodule
induced in greenhouse

2. Dilution



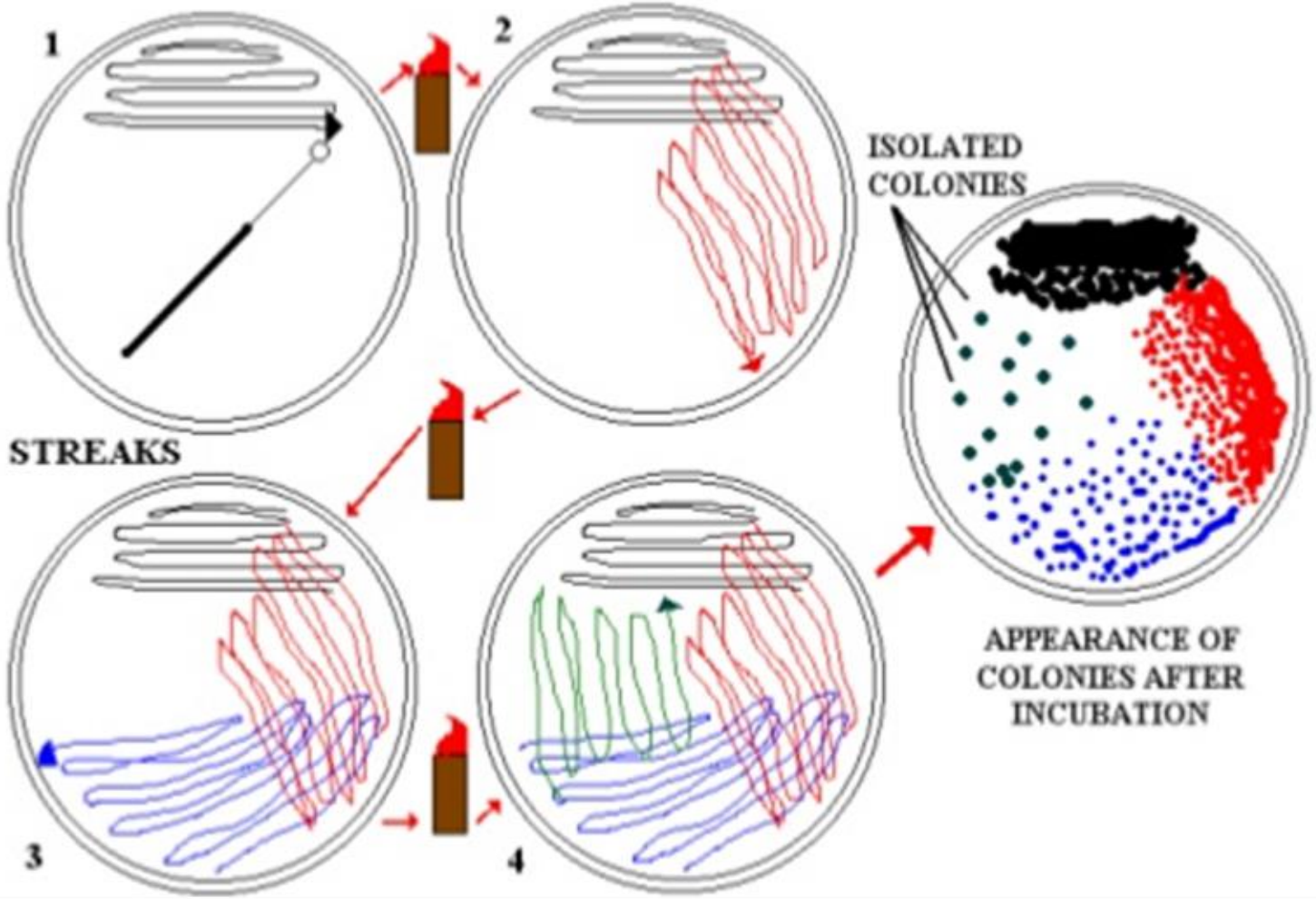
Isolation of Endophytes

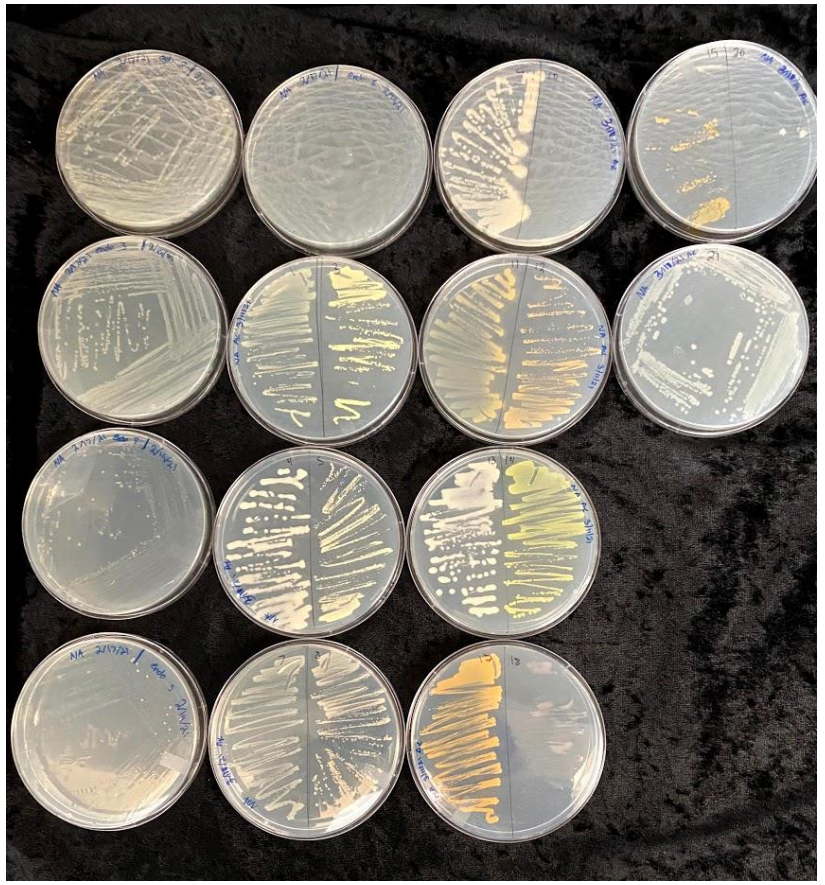




Purification of Bacterial Cultures

Streak Plate Method

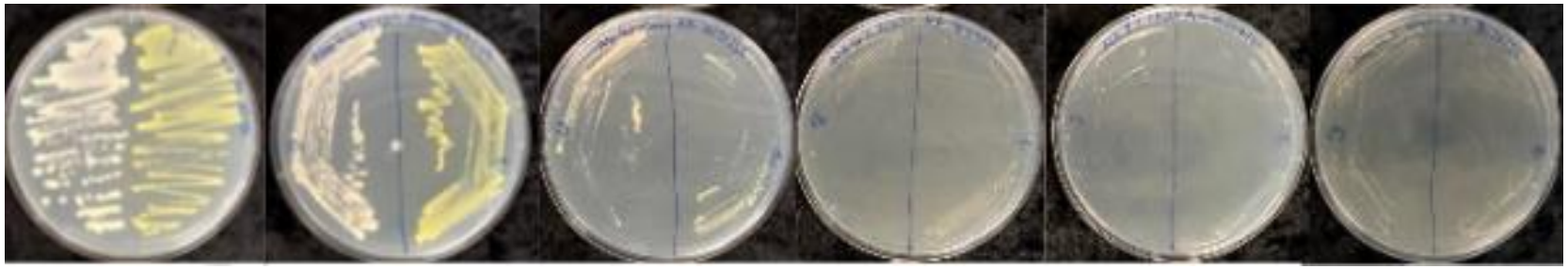




Media: ¼ NA + NaCl

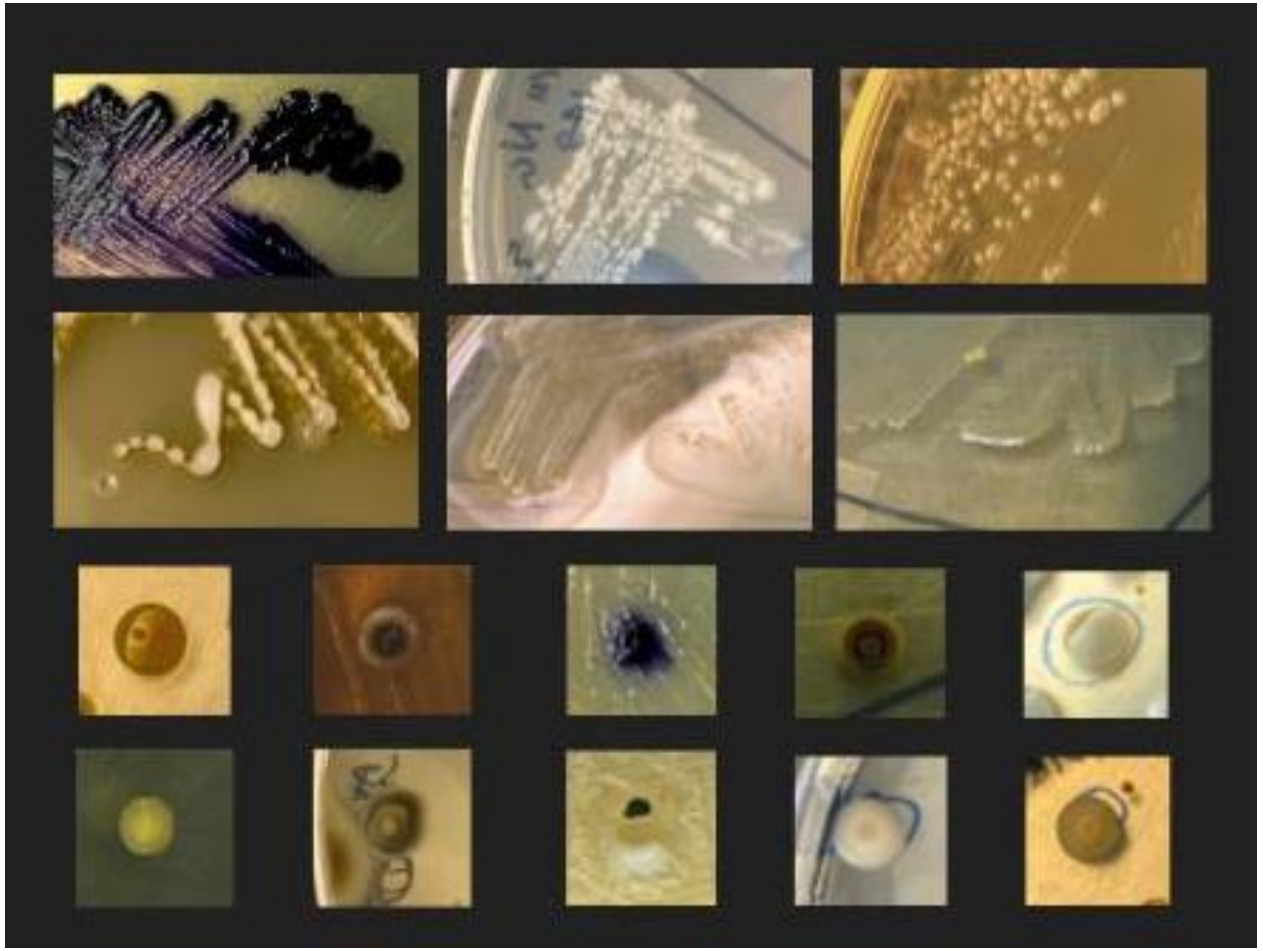
Screening for Halotolerance

Isolating on Concentrations of NaCl



0% 2% 4% 6% 8% 10%

Morphological Characteristics

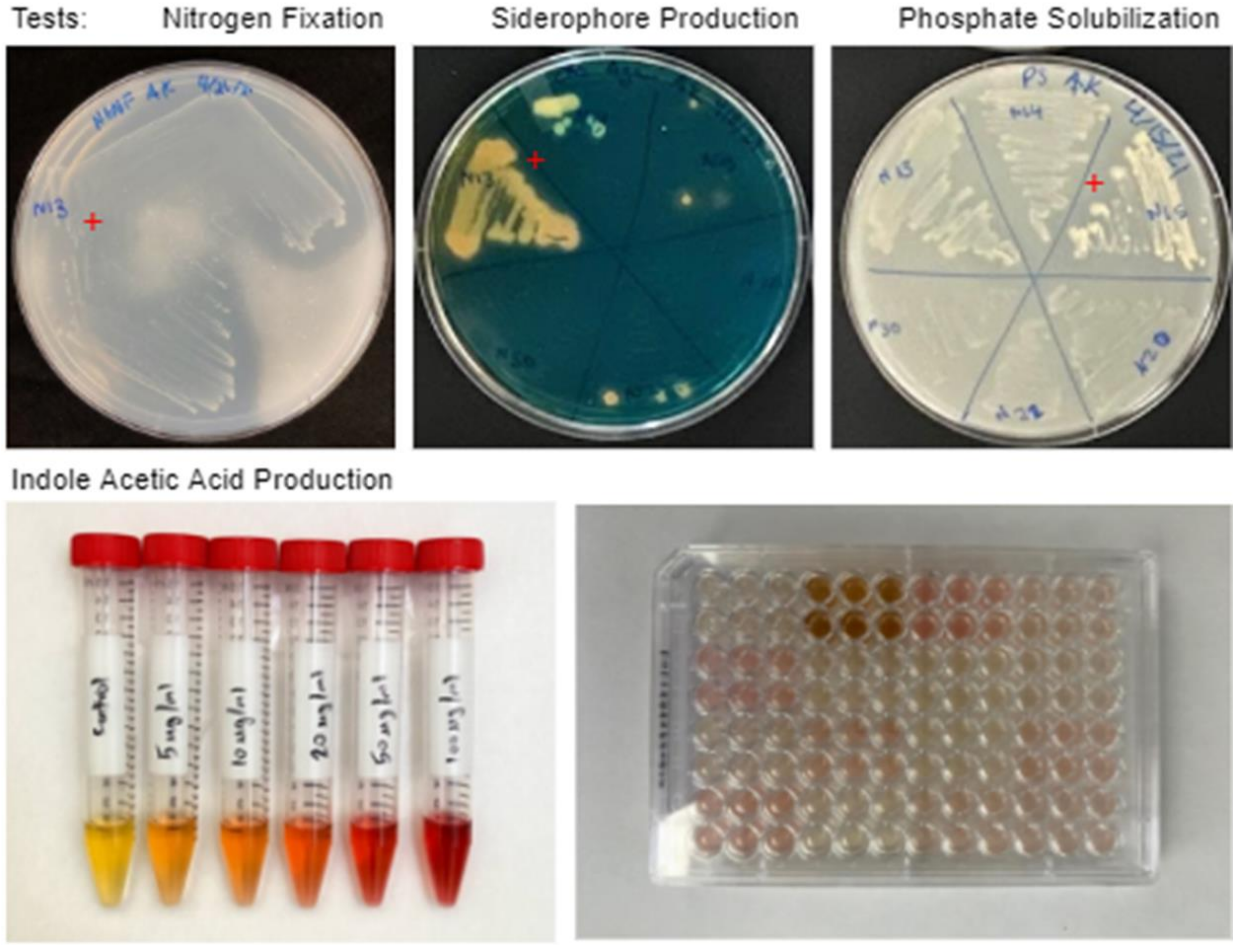


The following characteristics of each colony were recorded:

- Color
- Size
- Texture
- Opacity
- Morphology

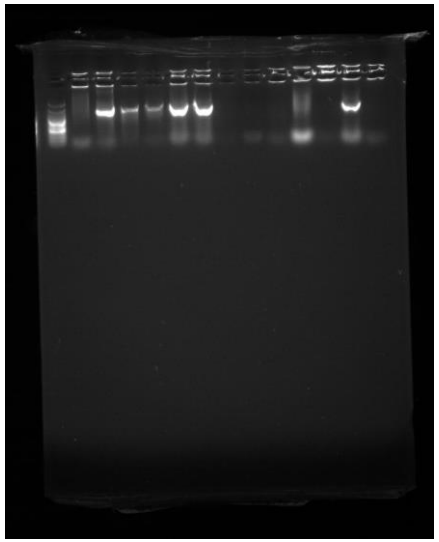
Biochemical Characterization

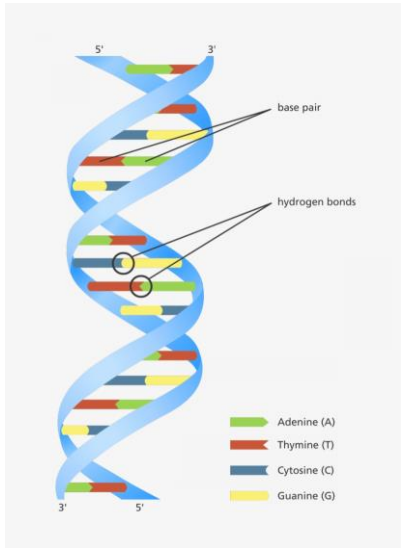
- Nitrogen Fixation using Norris Glucose media
- Siderophore Production using CAS media
- Phosphate Solubilization using Pikovskaya Agar
- Calorimetry Assay for IAA production



Sequencing and Identification

1. DNA extraction and Amplification
2. Sequencing
3. Blast Search against 16SrRNA database on NCBI





Results

36 Total Colonies:

- 14 from Nodules
- 22 from Roots

NaCl Concentration (%)	Number of colonies	% Microbes
2%	37	100
4%	30	81
6%	19	51
8%	6	16
10%	1	3

Plant Growth Promoting Activity of Isolates

Biochemical Characteristic	% Microbes
Produce Siderophore	51
Solubilize Phosphate	24
Fix Nitrogen	14
Produce Indole Acetic Acid	24

Nodule Endophytes

Genus	Salinity Tolerance	Siderophore Production	Phosphate Solubilization	Nitrogen Fixation	IAA Production
Massilia	2%	++	+	-	4.51±0.10
Anthrobacter?	6%	++	-	-	17.41±1.00*
Delfitia	4%	++	-	-	2.02±0.19
N/A	2%	++	-	-	0.70±0.08
Brevundimonas	4%	+	-	-	3.47±0.18
Pseudomonas	4%	++	++	-	1.67±0.11
Paenibacillus	2%	-	-	-	7.51±0.06
N/A	4%	-	+	-	0.79±0.17
Bacillus	8%	-	+++	-	14.30±2.37*
Pseudomonas	4%	+	+++	+++	13.64±0.11*
Bacillus	4%	+	+	-	0.46±0.07
Shinella	4%	++	+	-	4.78±0.07
Bacillus	8%	-	-	-	5.90±0.12
Peribacillus?	4%	++	-	-	6.73±0.08

Root Endophytes

Genus	Salinity Tolerance	Siderophore Production	Phosphate Solubilization	Nitrogen Fixation	IAA Production
Serratia	6%	+	-	+++	2.97±0.06
Serratia	6%	+	-	+++	2.56±0.06
Pseudomonas	2%	+++	+	-	3.75±0.05
Pseudomonas	2%	++++	-	++	5.94±0.01
Pseudomonas	2%	-	-	+	3.59±0.04
Actinobacteria	4%	-	-	-	4.82±0.46
Micrococcus	10%	-	-	-	10.83±0.15
Rhodococcus	6%	-	-	-	0
Micrococcus	8%	+	-	-	10.86±0.07
Anthrobacter?	6%	-	-	-	3.71±0.23
Rhodococcus	6%	-	-	-	0.25±0.06
Stenotrophomonas	6%	++	-	-	1.35±0.05
Bacillus	8%	-	-	-	1.19±0.37
Stenotrophomonas	4%	++	-	-	2.42±0.08

Root Endophytes

Genus	Salinity Tolerance	Siderophore Production	Phosphate Solubilization	Nitrogen Fixation	IAA Production
Rhodococcus	6%	+	-	-	1.25±0.06
Rhodococcus	6%	-	-	-	0.33±0.08
Microbacterium	6%	-	-	-	3.54±0.03
Rhodococcus	6%	+	-	-	3.05±0.06
Pedobacter	6%	-	-	-	0
Streptomyces	2%	-	-	-	?
Streptomyces	8%	-	-	-	0
Paenibacillus?	2%	-	-	-	0

Testing on Alfalfa: Treatment Groups

1. Cocktail
   - Four halotolerant bacteria, one high in each growth characteristic

Genus	Salinity Tolerance	Siderophore Production	Phosphate Solubilization	Nitrogen Fixation	IAA Production
Serratia	6%	+	-	+++	2.56±0.06
Stenotrophomonas	6%	++	-	-	1.35±0.05
Anthrobacter?	6%	++	-	-	17.41±1.00
Pseudomonas	4%	+	+++	+++	13.64±0.11

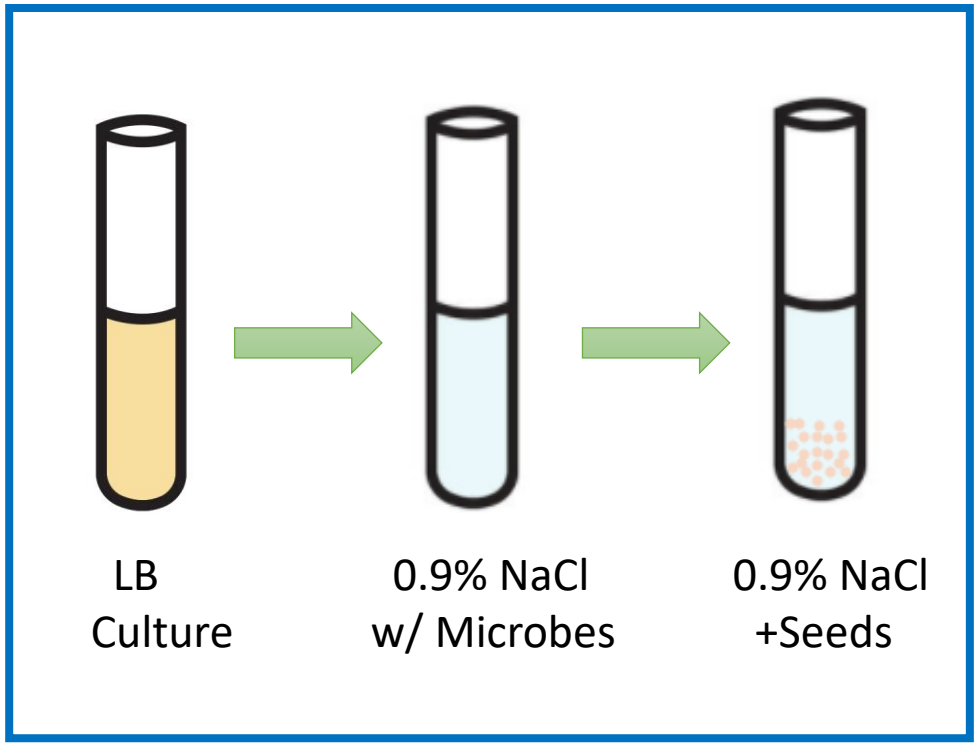
2. All 14 Endophytes from the Nodule

Inoculating Alfalfa Seeds

Medicago sativa

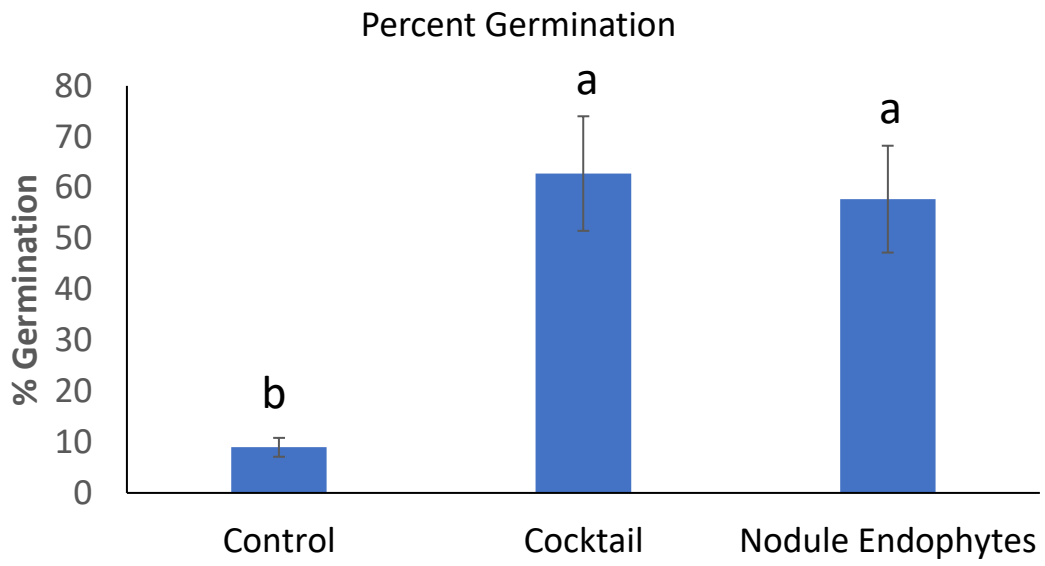
- Endophytes were cultured in LB to a density of 1.5 at 600nm
- Cells were pelleted and resuspended in 0.9% NaCl
- Surface Sterilized seeds put in the solution overnight to incubate at 28 Degrees C

Control = Sterile 0.9% NaCl



Planting & Salt Treatment

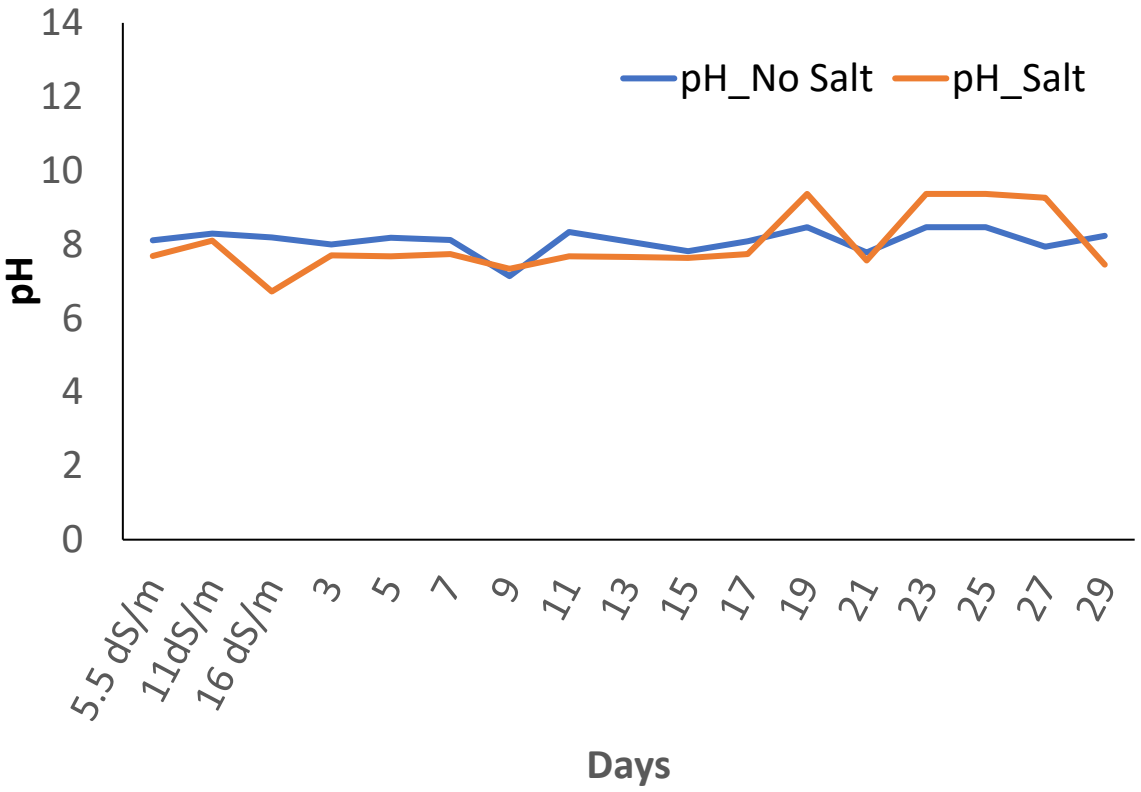
- Seeds planted in autoclaved vermiculite, watered with ½ Hoagland's solution
- At germination, plants were re-inoculated with Bacterial solution by pouring
- Salt treatment began two weeks after germination (2 NaCl: 1 CaCl salt in ½ Hoagland solution, adding salt gradually to increase over 3 days until it reached 16.5 ds/m)

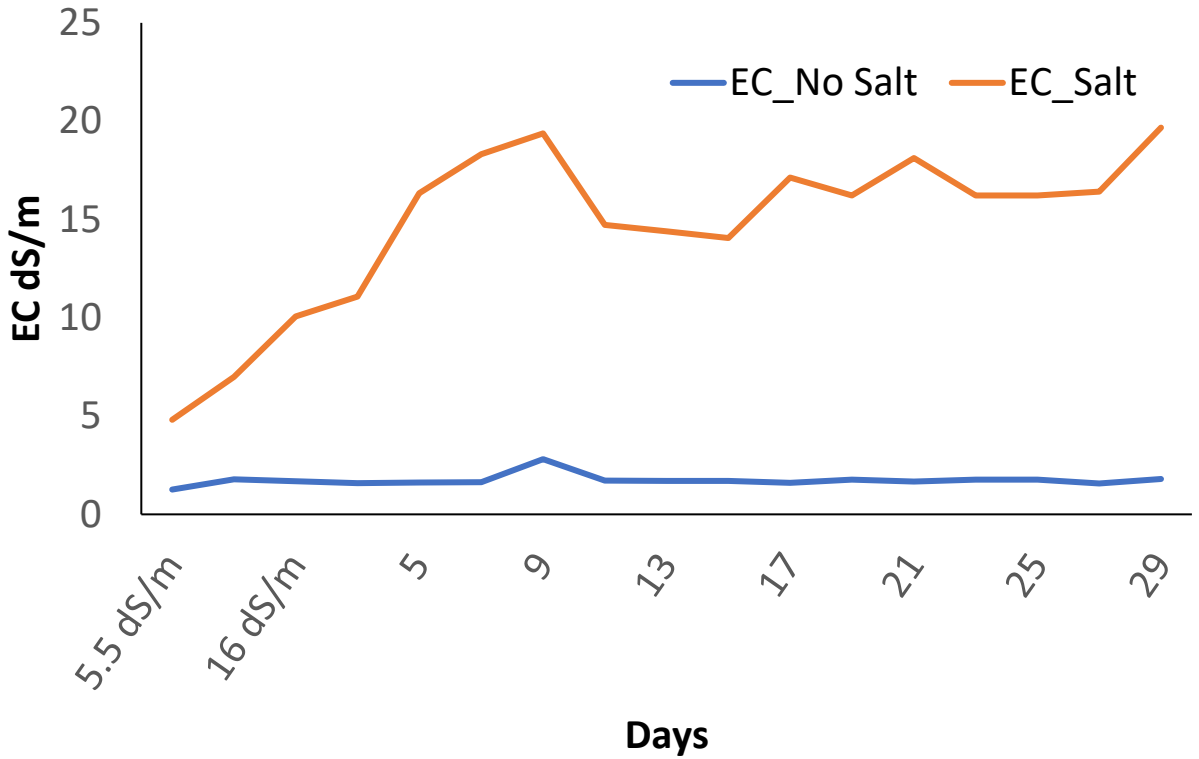


Plant growth assays

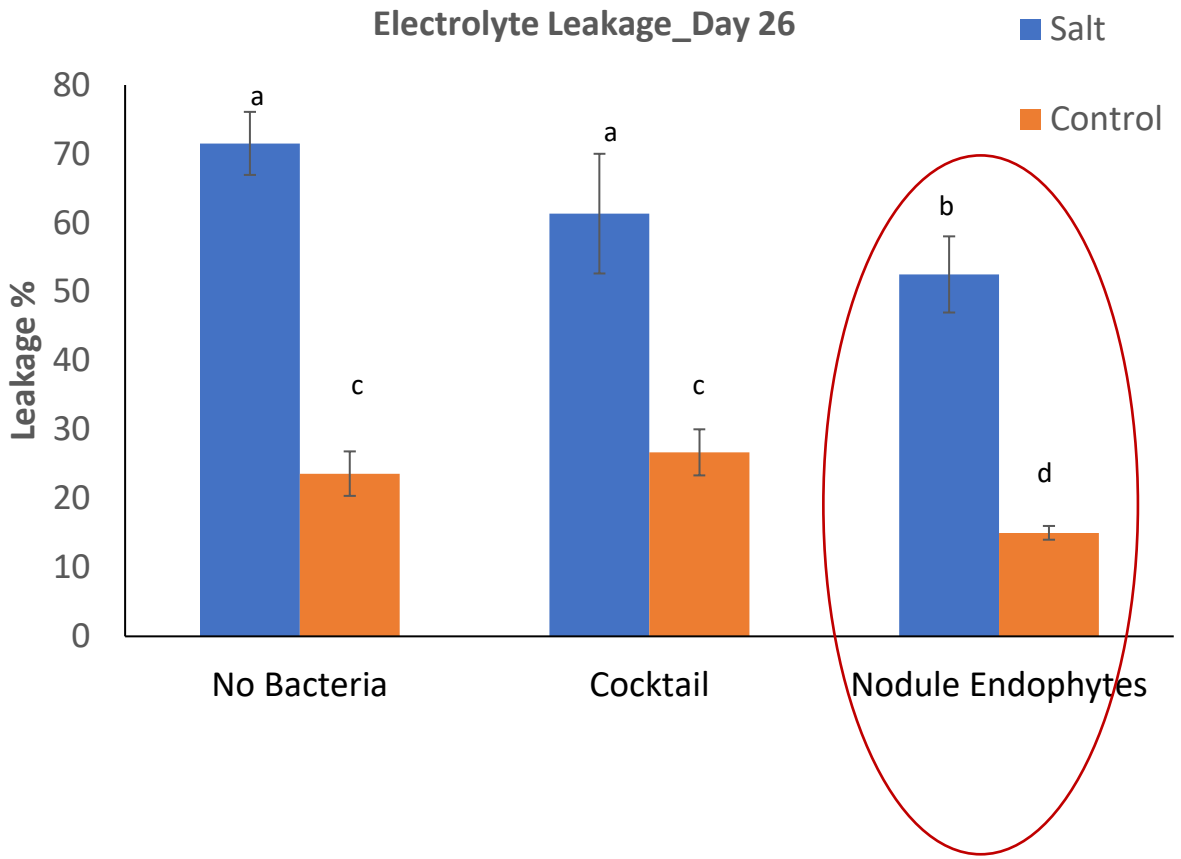
- EC and pH of leachate
- Electrolyte Leakage
- Stomatal Conductance
- Photosynthetic rate
- Biomass
- Gene expression analysis of salt responsive genes,
  - SOS family (*SOS1, SOS2,SOS3*), *NHX1* and *NHX2*, *HKT1*
  - Proline (*P5C*)
  - Catalase
  - ABA biosynthesis (*NCED3*)

EC and pH
EC of all plants maintained between 16-19 dS/m





Electrolyte Leakage Assay



ANOVA Single Factor

- No Bacteria vs. Nodule Endophytes *in salt*

  P-value ~ 0.0247

- No Bacteria vs. Nodule Endophytes *in no salt*

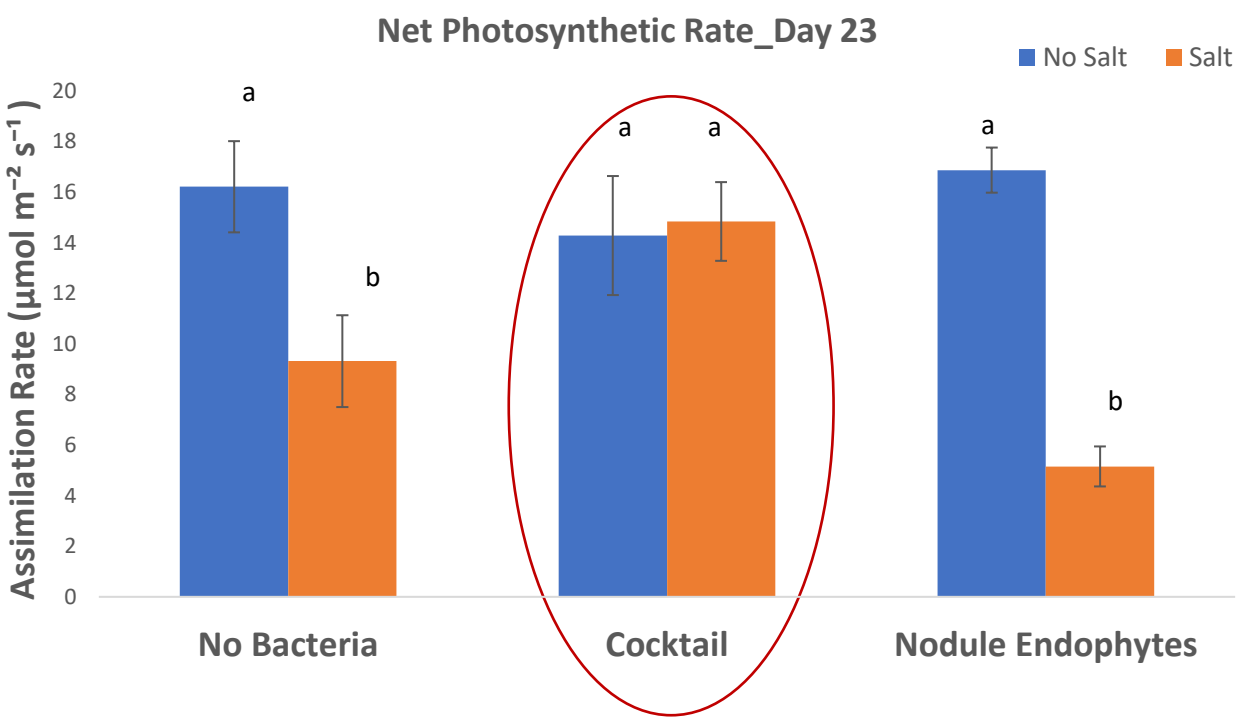
  P-value ~0.0235

- No Bacteria vs. cocktail Endophytes *in no salt*

  P-value ~0.0034

Nodule endophyte inoculation showed significant reduction in EL in plants compared to control irrespective of salt treatment.

Net Photosynthetic Rate

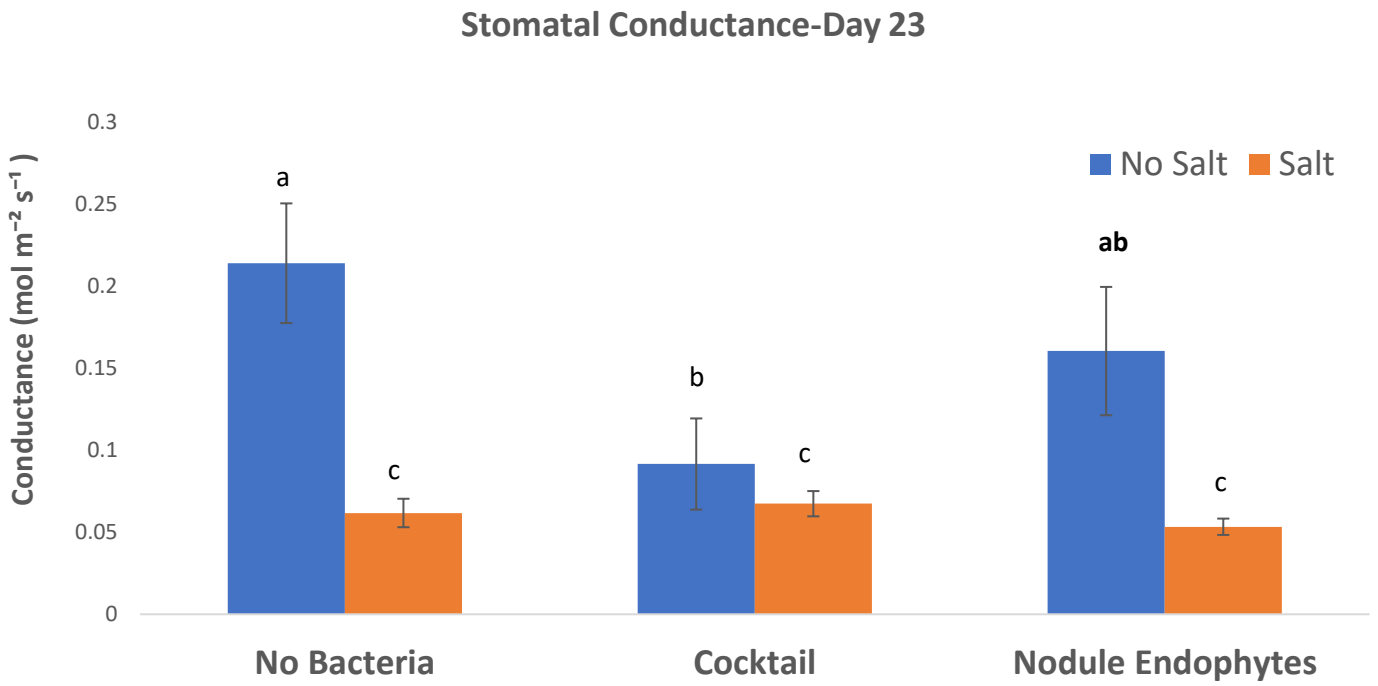


ANOVA Single Factor

- Cocktail *in no salt* vs. Cocktail *in salt*
  P-value ~0.9999

There is not a significant difference in photosynthetic rate between Cocktail plants treated salt and Cocktail plants treated without salt.

Stomatal Conductance

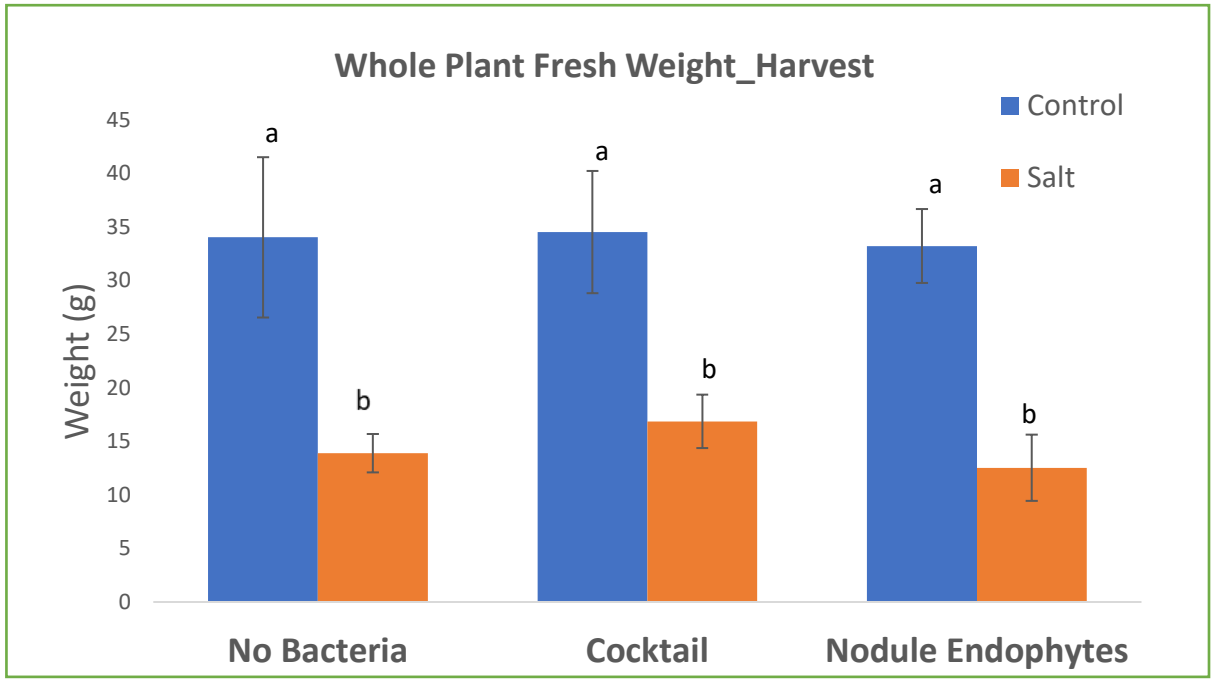


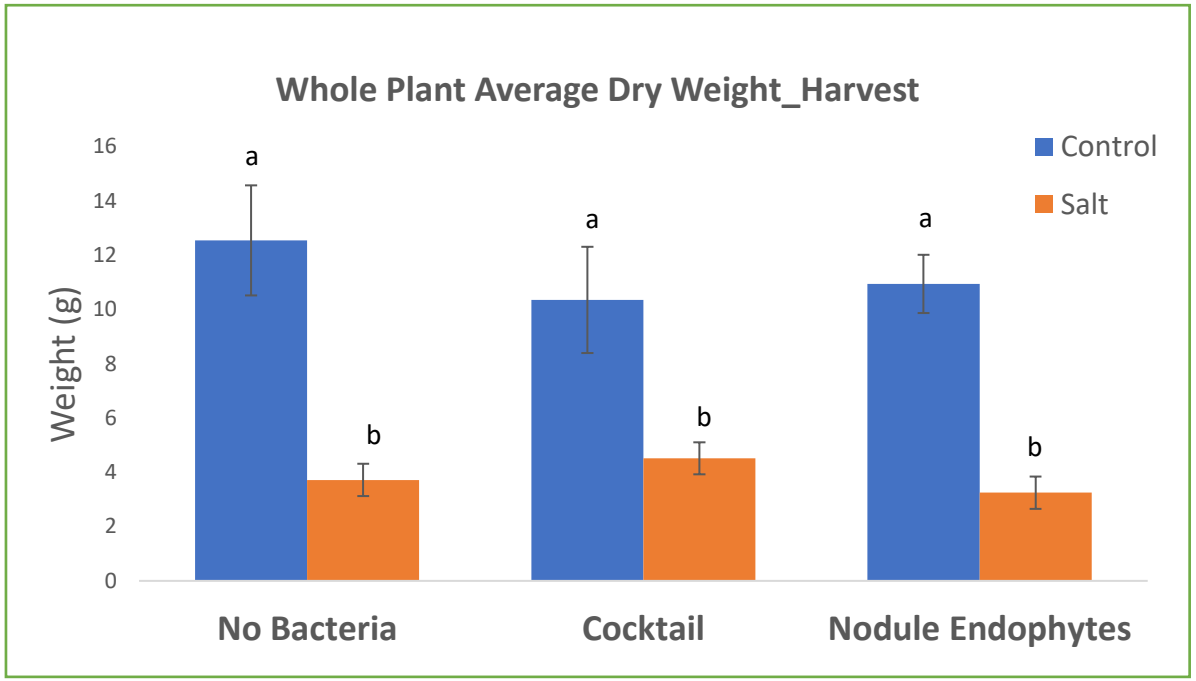
ANOVA Single Factor

- No Bacteria vs. Cocktail *in no salt*
  P-value~0.0124

- No significant difference between the bacterial treatments *in salt*
- Cocktail showed significantly less conductance than No Bacteria *in no salt*

Biomass

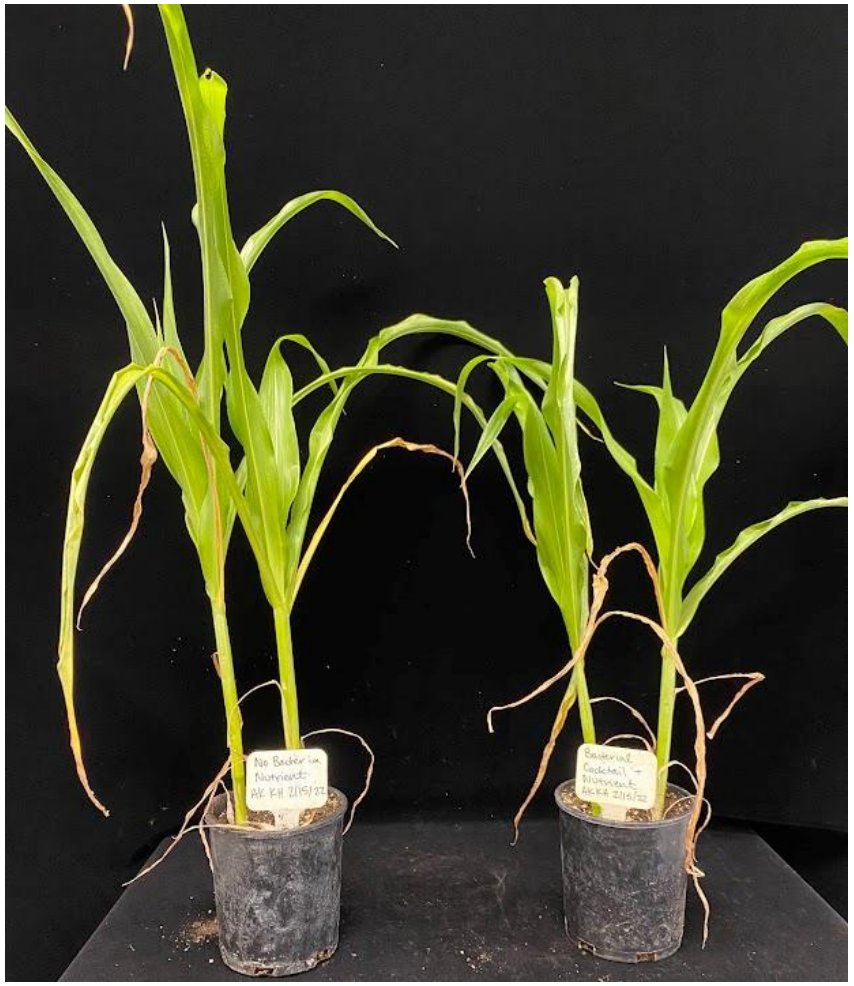




- No significant difference between the bacterial treatments within the salt and no salt groups
- Inconclusive result because of genetic diversity between plants

Testing Cocktail Inoculation on Corn

Zea mays



No Bacteria Cocktail

No Salt

No Bacteria Cocktail

Salt

Conclusion

1. Isolation of endophytes from nodules and roots under salt concentrations
    - 100% colonies grew in 2% NaCl
    - 50% grew in 6%
    - 3% grew at 10%
2. Plant growth promoting activities shown by biochemical assays
    - Most isolates showed plant growth promoting activity for at least one characteristic measured
3. Selection and testing of 4 bacteria cocktail and of nodule endophytes for on alfalfa and cocktail was tested on corn

Conclusion

4. Testing on Crops
    - Bacterial inoculation has been observed to enhance seed germination.
    - Nodule endophytes may help plants maintain electrolyte leakage with and without salt treatment.
    - Cocktail endophytes may help plants maintain photosynthetic rate under salt stress.
    - Cocktail endophytes may negatively affect the plant growth in corn.

Next Steps

- Screening microbes on model plants *Arabidopsis thaliana* and *Medicago truncatula* for plant growth and development
    - **Nearly 100 isolates to screen** which have plant growth promoting activities species
    - The microbes will be tested in cocktail of different bacteria

How we plan to improve our experiment on crops in the future:

- The screened bacteria (positive for plant growth and development) will be tested on crop plants for plant growth and development under stress

- Alfalfa plants will be propagated by cutting propagation to get uniform genotype to test the microbes.

Statement of Purpose

We hope to discover beneficial endophytes that can be used as **bio-fertilizers** and *provide a tool for growing food in saline conditions.*

Acknowledgements

Thank you to…

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- My Lab mates
- The Masters Students of the Plants, Soils, and Climate Department at USU

Questions?