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## Identification and Isolation of Halotolerant Endophytes in *Ceanothus velutinus* May Lead to Plant Health in Saline Conditions

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# Halotolerant Endophytes

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



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### Introduction

- Plant-microbe relations are integral to plant survival and crop productivity.
- Part of a plant’s microbiome is endophytes, the microbes that inhabit the plant and form a symbiotic relationship. These microbes promote a plant's growth and development and help them cope with abiotic stressors such as salinity.
- In Utah, soils are high in salinity. Climate change has increased soil salinity worldwide, and water availability is becoming scarcer leading farmers to use more saline sources to irrigate.
- Identification of halotolerant or salt-tolerant endophytes that promote plant growth in saline conditions leads to the discovery of a tool for farmers to use in saline soils and degraded water for irrigation.



Nitrogen-fixing root nodule of *Ceanothus velutinus*, which contains endophytes that assist in plant growth and survival.

### Methods

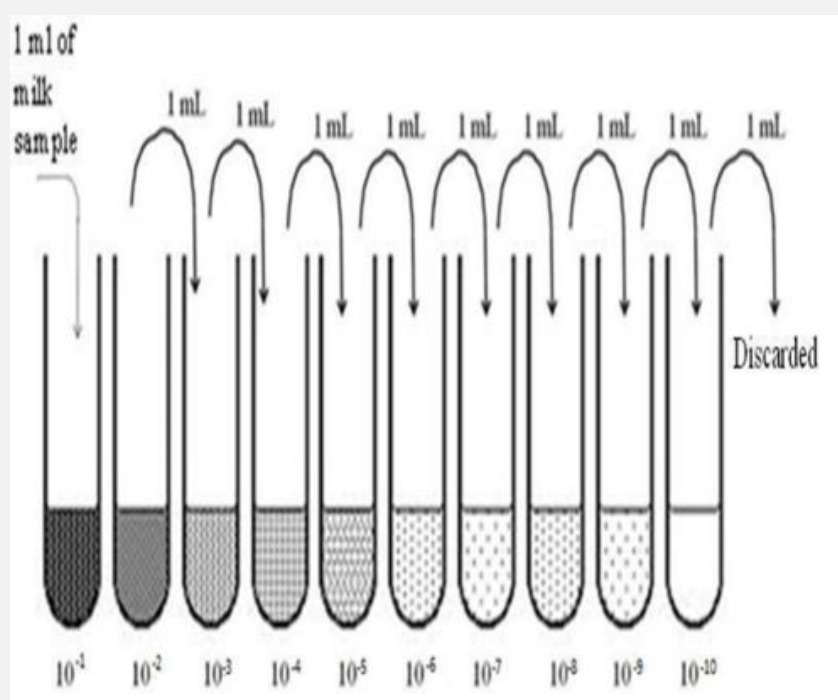
#### 1. Nodule extract

Root nodules from native plant *Ceanothus velutinus* were ground and particles were suspended in autoclaved water.

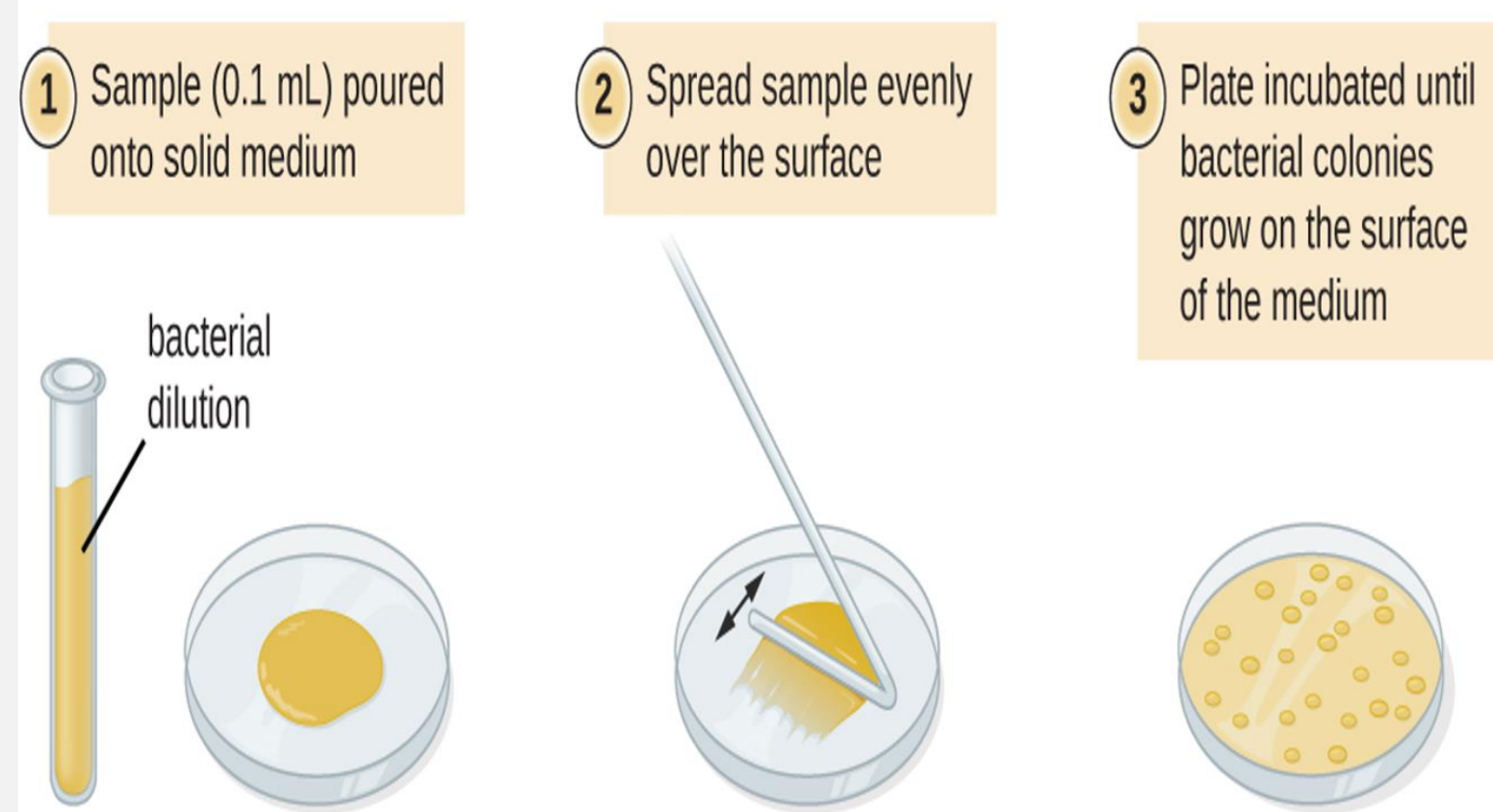
#### 2. Isolation

##### 2.1 Serial dilution

The extract was diluted to a scale of concentrations.

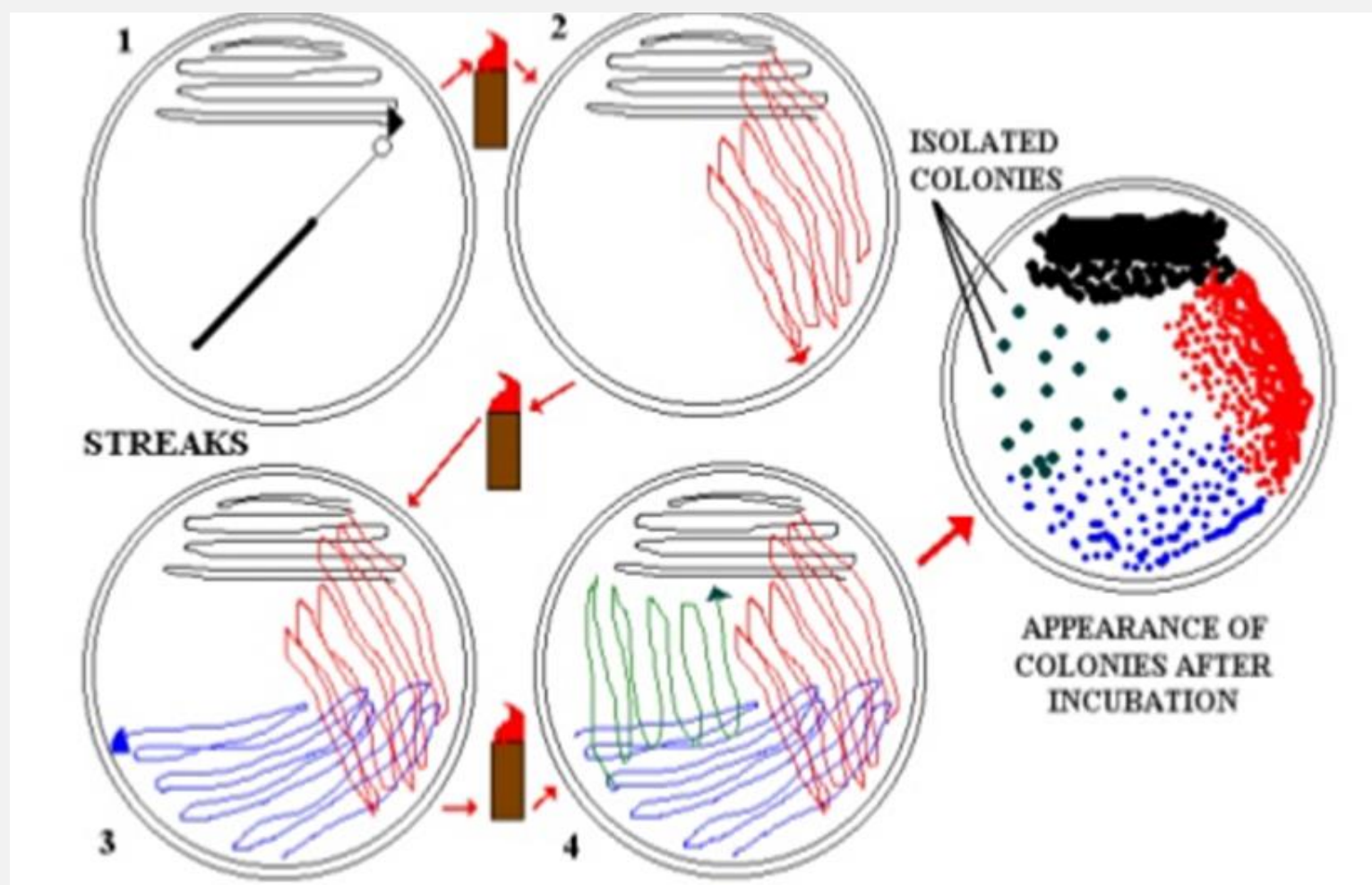


##### 2.2 Spread Plating of Dilutions



#### 3. Purification

Streak Plating method of purification

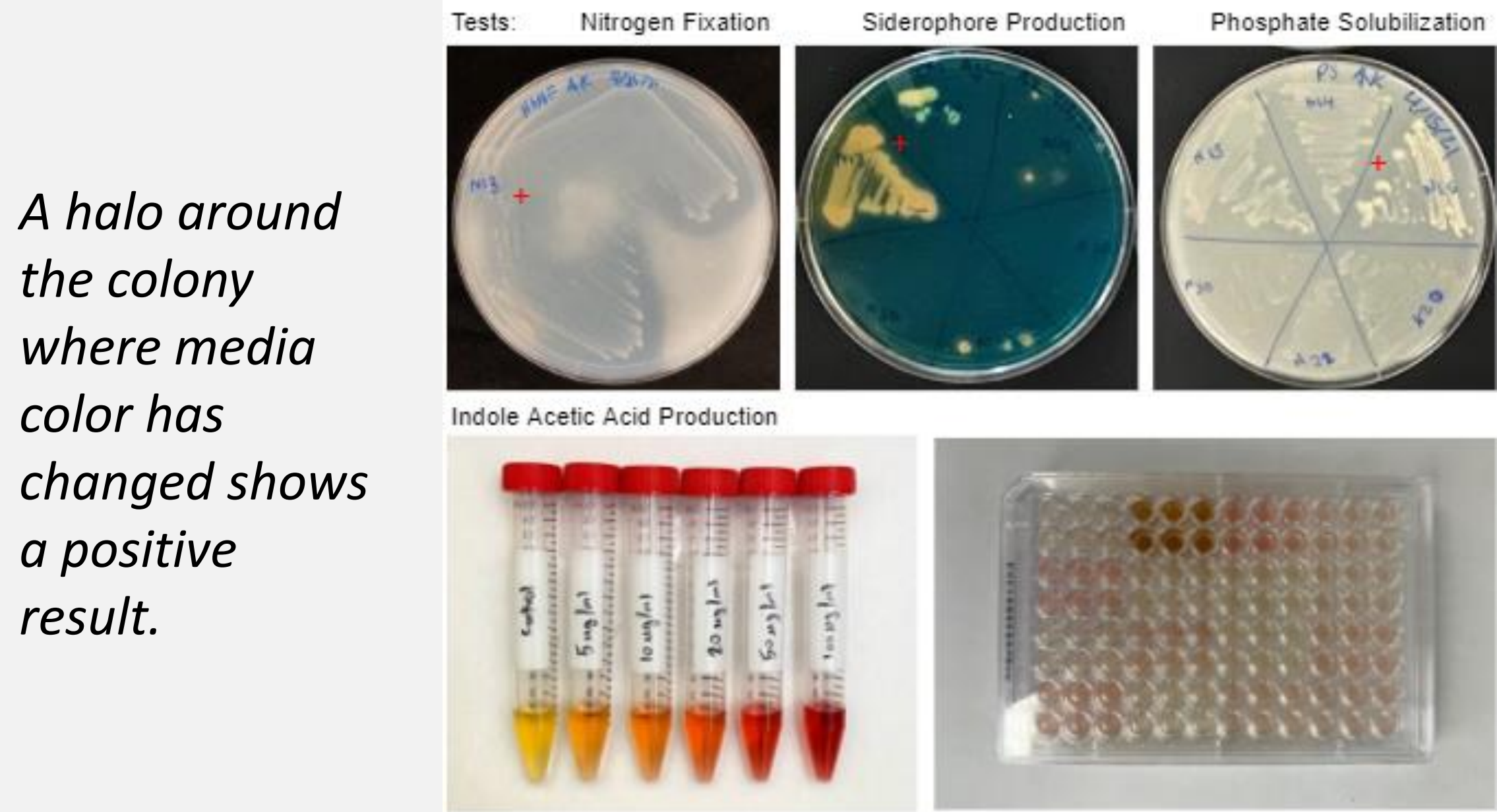


#### 4. Identification

Colonies were identified through 16SrRNA DNA sequencing and BLAST search.

#### 5. Biochemical Characterization

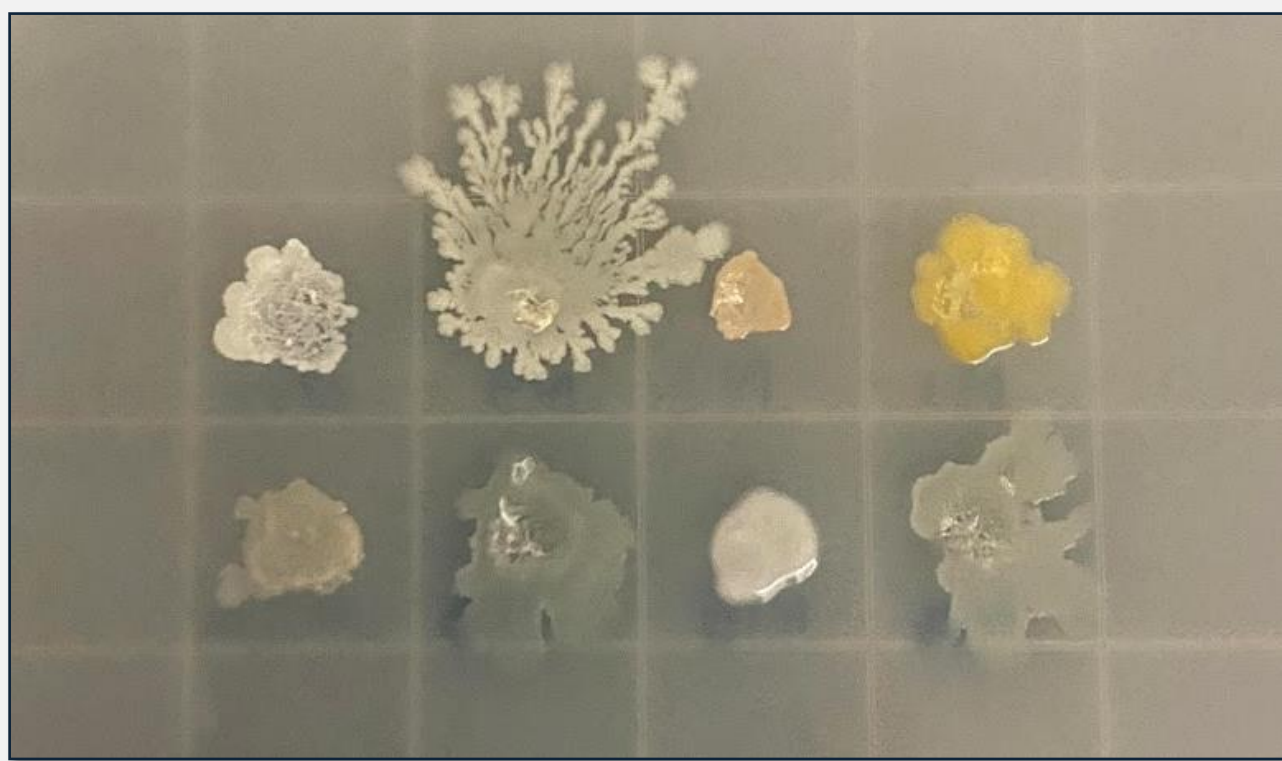
Each colony was tested for the following properties using media screening or chemical assays.



A halo around the colony where media color has changed shows a positive result.

### Results

- Fourteen colonies were isolated from the nodule of *Ceanothus velutinus* and found to be tolerant to at least 2% of NaCl.
- Endophytes isolated from Snowbrush roots in a previous study were also screened on salt, making a total of 37 colonies that have been screened for halotolerance and biochemical characteristics.
- All 37 colonies have been sequence by 16SrRNA and Identified by BLAST search.



Morphological Diversity of phosphate solubilizing endophytes from the nodules of *Ceanothus velutinus*..

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

Table 1: Percent of Isolated Microbes which grew on Nutrient media with added concentrations of NaCl.

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

Table 2: Through screening and chemical procedure, colonies were identified with the outlined plant-growth-promoting characteristics.

### Conclusion

- Identified Plant-Growth Promoting Bacteria PGPB belonged to the genus *Streptomyces*, *Pseudomonas*, *Anthrobacter*, and *Bacillus*.
- The identified endophytes are currently being tested on the model plant *Arabidopsis thaliana*, *Zea mays* (maize), and *Medicago sativa* (alfalfa) under saline conditions.
- Growth characteristics such as biomass, stomatal conductance, net photosynthetic rate, relative water content, and electrolyte leakage will be measured and compared.

Identification of a plant growth benefiting halotolerant endophyte can lead to the development of biofertilizers /biostimulants for saline soils and be used as a tool in sustainable and productive crop production for these harsh environments.