

## Introduction

- Ceanothus velutinus* is an evergreen shrub native to the Intermountain West region of the US
- The center for water-efficient landscaping (CWEL) recommended this plant for sustainable landscaping as its moderately drought tolerant
- Roots of these plants form a symbiotic relationship with Plant growth-promoting endophytic bacteria (PGPE) that aids in nutrient uptake, lowers ethylene levels under stress conditions, production of metabolites and thus plays a vital role in plant growth and development.
- They are more beneficial because of their close association with the plants and are used as biofertilizers to substitute chemical fertilizers



## Objectives

- Isolation and characterization of endophytic bacteria from the nodules
- Isolation of Frankia and testing for nodulation in Snowbrush
- Studying the effect of identified PGPE on plant growth and development in alfalfa & maize

## Materials and methods

### 1. Nodule extract

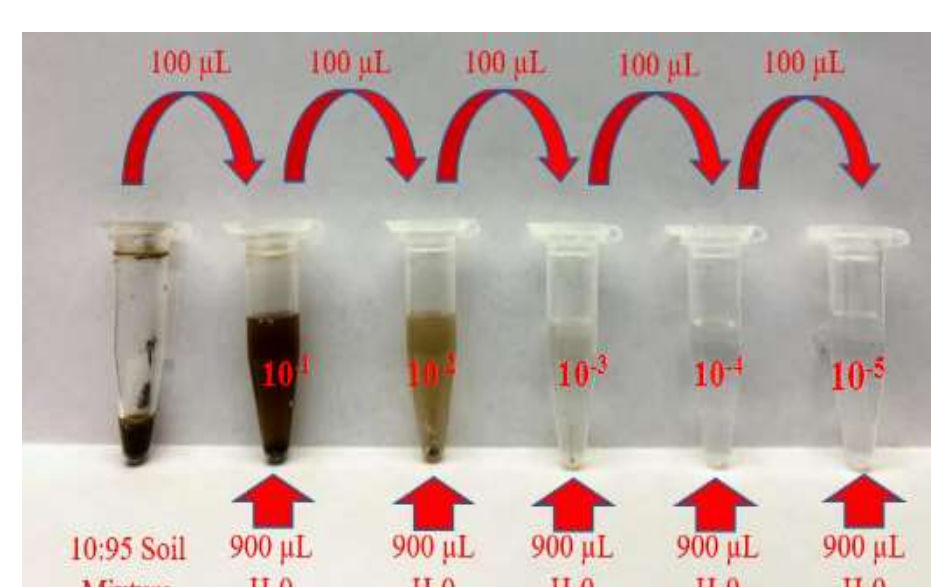
Root nodules from green-house grown *Ceanothus velutinus* were surface sterilized, ground and particles were suspended in autoclaved water.



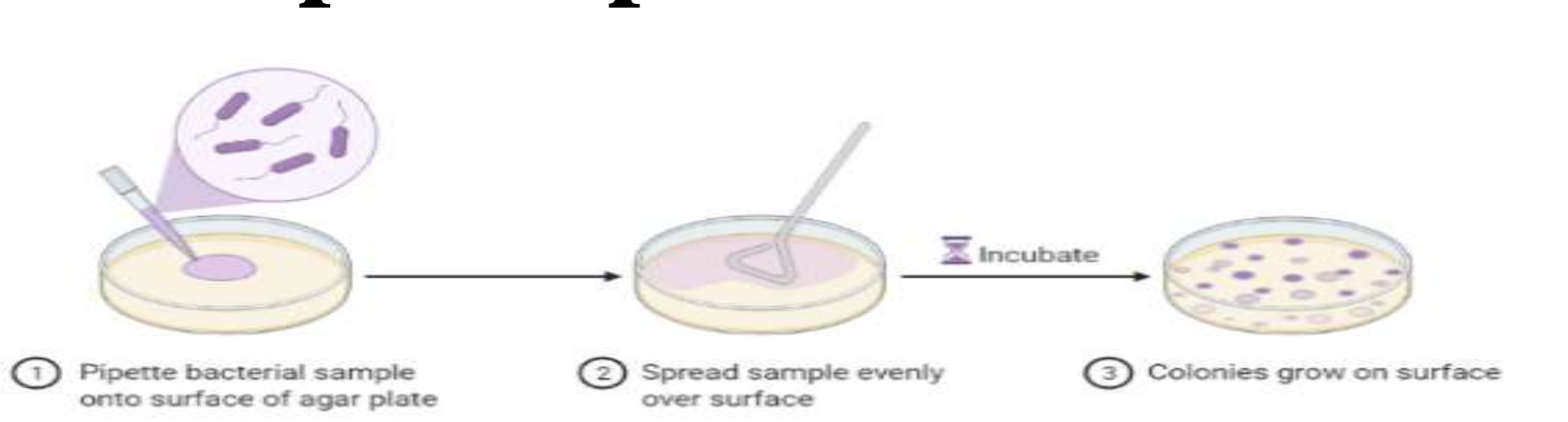
### 2. Isolation

#### 2.1 Serial dilution

The extract was serially diluted to a scale of concentrations



#### 2.2 Spread plate method



### 3. Purification – Streak Plating



### 4. Identification

Colonies were identified by 16S rRNA sequencing and BLAST search

### 5. Morphology



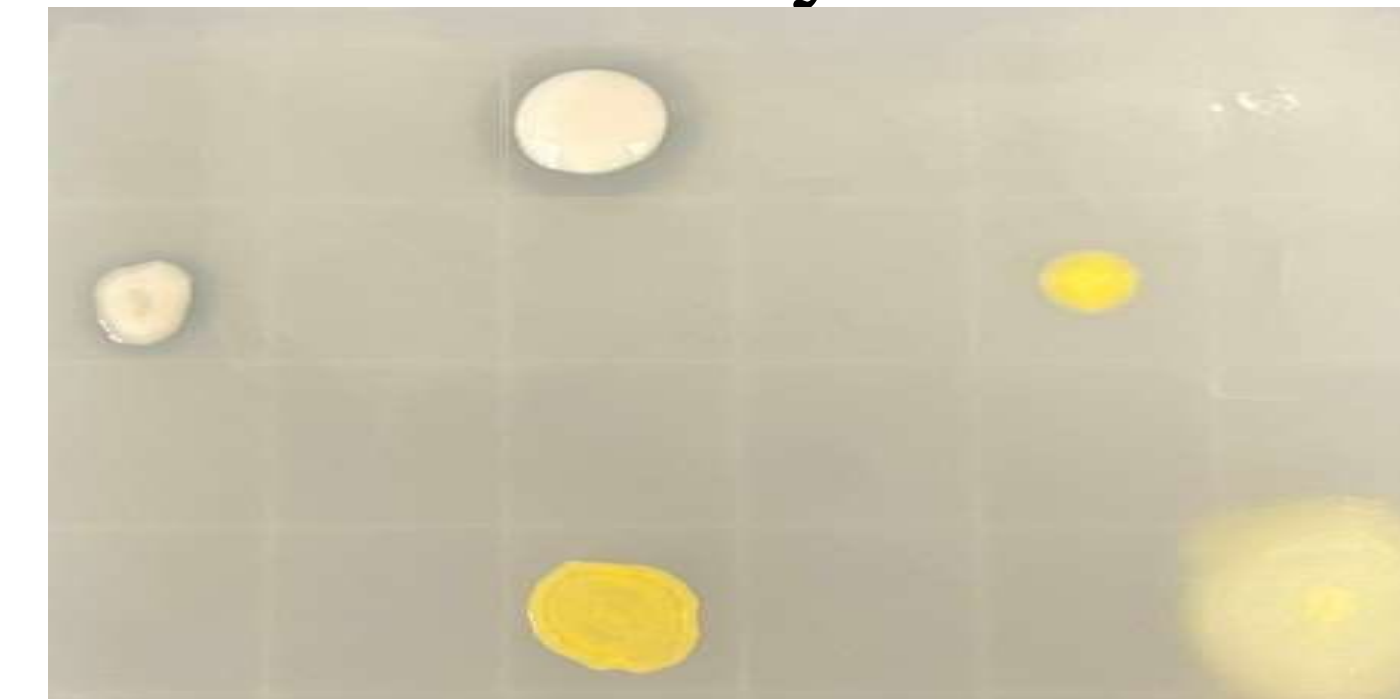
### 6. Biochemical characterization

Each identified colony was tested for following characteristics NF, PS, SP. Positive result – A clear halo around the bacterial colony Negative result – No halo

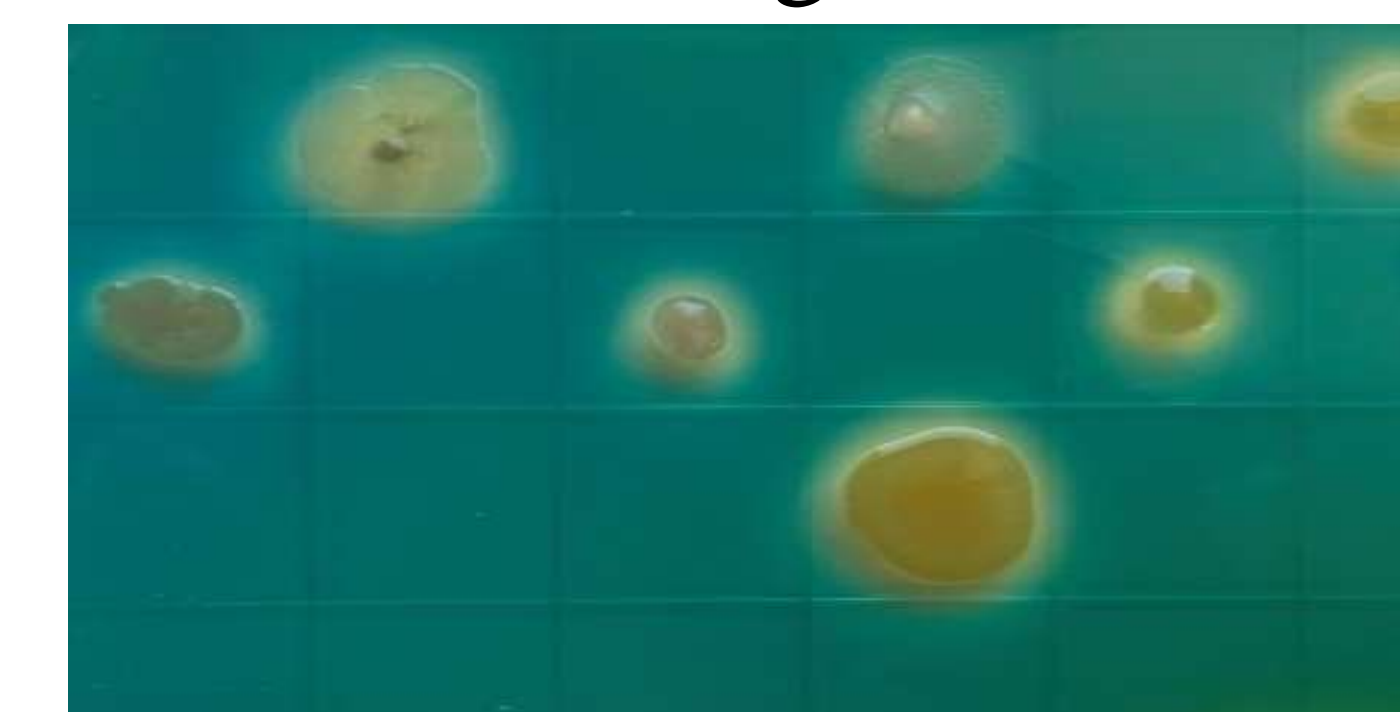
i) Nitrogen fixation on Norris Glucose Nitrogen Free media



ii) Phosphate solubilization on Pikovskaya's media



iii) Siderophore production on CAS Agar



## Results

A total of one hundred and six colonies were isolated from the nodules by using four different media (¼ Nutrient agar, ¼ Tryptic soy agar, Yeast mannitol agar and Frankia basal media)

Table 1: Isolates showing NF, PS, and SP from different media

Media	Total isolates	No. of isolates sequenced	NF	PS	SP
¼ NA	32	18	78%	11%	83%
¼ TSA	36	21	86%	14%	57%
YMA	18	7	71%	14%	100%
FBM	20	7	29%	29%	57%

Table 2: Isolates showing all the three characteristics

Bacterial Isolates	16S rRNA sequencing	NF	PS	SP	Plant growth promoting activities (literature study)
TKE - 1	<i>Streptomyces misionensis</i>	++	+	+	Phytopathogen biocontrol
TKE - 27	<i>Paenibacillus alba</i>	+	+	++	Not determined
TKE - 30	<i>Pseudomonas chlororaphis</i>	++++	+	+++	Biocontrol agent
TKE - 31	<i>Leclercia adecarboxylata</i>	++++	+	+	Drought tolerant
TK_NE_1 5	<i>Paraburkholderia dioscoreae</i>	+++	+	++	Biofertilizer

Clear halo zone diameters produced by bacteria grown on different media: (-) no clear zone, (+) 5-10 mm, (++) 10-15 mm, (+++) 15-22 mm, (++++>22 mm

## Conclusion

- Most of the identified PGPE belonged to the genus *Bacillus*, *Streptomyces*, *Variovorax* and *Paenibacillus*
- From the total isolates, 66% of identified PGPE showed NF, 17% PS, and 74% SP
- Unable to isolate Frankia by using Frankia basal media
- But, identified Frankia by metagenomic study confirms that Frankia is present in the nodules of Snowbrush

## Future work

- Cocktail of identified PGPE will be tested on alfalfa and maize
- Ultracentrifugation will separate nodule vesicles, and Frankia will be isolated
- Identified Frankia will be tested for inducing nodulation in snowbrush under green-house conditions