Isolation of halotolerant bacteria from the rhizosphere of *Ceanothus velutinus* may lead to contributions in plant health in saline conditions.

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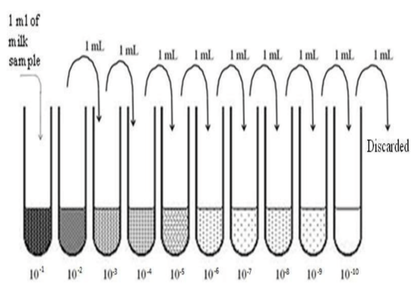
INTRODUCTION

The recent rise in the average global temperature has been a driving force over the past few years for rising soil salinity. This condition presents a hostile environment to many plant species that have not previously been exposed to these conditions. The rhizosphere, a layer of soil attached to the roots of a plant, contains microorganisms that may contribute to the plants' abiotic and biotic stress resistance. These microorganisms are known as Plant Growth Promoting Rhizobacteria (PGPR). These can play a significant role in contributing to plant stress resistance. Some native plants have shown a strong ability to resist harsh or acclimate abiotic and biotic stressors such as drought, cold temperatures, heavy metal contaminations, and more. In this study, we have selected a native resilient plant indigenous to the InterMountain West region of North America, known as *Ceanothus velutinus* (Snowbrush). We aim to try to isolate halotolerant bacteria from the rhizosphere of Snowbrush. Samples were collected from the rhizosphere of Snowbrush plants from the Tony Grove region of Logan Utah.

METHODOLOGY

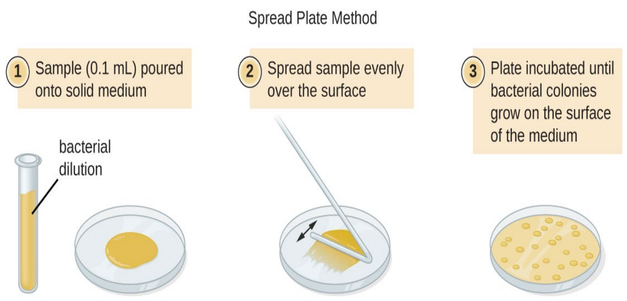
The root samples of the Snowbrush plant from three different locations in Tony Grove, Utah were collected. Rhizosphere soil separated from the roots. The soil samples were diluted to a 10:95 ratio in water. These dilutions further serially diluted five times in a 1:10 ratio. y diluted five times in a 1:10 ratio.

Serial Dilution



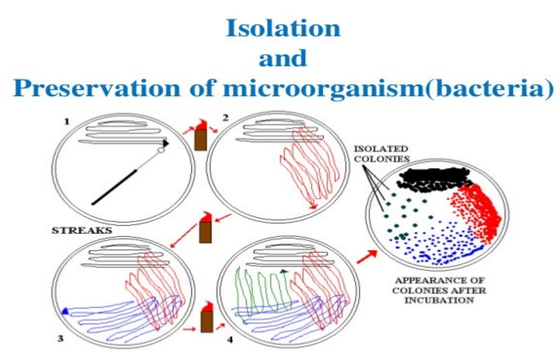
The dilutions of 10^-3, 10^-4, 10^-5 were then plated onto nutrient agar media with six different concentrations of sodium chloride (2%, 4%, 6%, 8%, 10% W/V) with 0% as a control.

Spread Plate



Once Isolated colonies were grown, each colony was put through a gram stain, a catalase test and described and documented, to be able to pick unique colonies for the next phase of the experiment .

Streak Plate



All unique colonies were put through colony PCR were the gene of interest was amplified and duplicated. PCR products were ran on a agarose gel in gel electrophoresis to test the success of the PCR. Products that failed to produce a band on the gel were put through PCR again to produce a positive product. After this each Product was purified with TE buffer and Magnesium chloride, and then tested for adequate concentration and then diluted down to a more appropriate dilution if concentrations were too high. PCR products were then subject to 16s rRNA sequencing. Once results of sequencing were received, they were then put through a blast search and will then be further identified

RESULTS

Sample	0% NaCl 10^-4	0% NaCl 10^-5	2% NaCl 10^-4	2% NaCl 10^-5	4% NaCl 10^-4	4% NaCl 10^-5	6% NaCl 10^-4	6% NaCl 10^-5	8% NaCl 10^-4	8% NaCl 10^-5	10% NaCl 10^-4	10% NaCl 10^-5
1920 (1)	162	185	64	160	0	1	0	0	0	0	0	0
1920 (2)	73	12	48	1	31	2	0	1	0	0	0	0

Table 1: Total number of colonies observed in each of sample and concentration plated.

A rhizosphere soil sample from two plants of one location 1920m was grown on nutrient agar media with 6 different concentrations of sodium chloride.
All 0% concentrations were used as a control and in each of those plates there were dozens to hundreds of colonies isolated depending on the sample. At 2% NaCl concentration there was still a very high amount of colonies being able to grow in the media. For example 1920 (2) 10^-4 in 2% had 48 colonies, and 1920 (1) 10^-5 in 2% had 160 colonies. As concentrations increased the number of colonies able to proliferate decreased. By 4% NaCl most samples were down to or near only a single colony being manifested. With one exception being 1920m (2) 10^-4 on 4% having 31 colonies. Only one sample managed to grow a single colony in 6%. That being 1920m (2) 10^-5. No colonies were observed at or past 8% NaCl concentration. Each unique colony type from each media and dilution were streaked on the same media and concentration in order to isolate a single species of bacteria to a plate.

JD 1	JD 3	Streptomyces sp.	Streptomyces rhizosphaerihabitans	Streptomyces adustus	
JD 2	JD 7				
JD 3	JD 8	Flavobacteriales bacterium	Pedobacter ginsenosidimutans	Flavobacterium sp.	
JD 4	JD 9	Priestia aryabhattai	Priestia megaterium	Bacillus sp.	
JD 5	JD 10				
JD 6	JD 11				
JD 7	JD 15	Bacillus pumilus	Bacillus zhangzhouensis	Bacillus australimaris	Bacillus circulans
JD 8	JD 17	Rhodococcus erythropolis	Rhodococcus sp.		
JD 9	JD 18	Bacillus thuringiensis	Peribacillus simplex	Brevibacterium frigoritolerans	Bacillus sp.
JD 10	JD 19	Rhodococcus erythropolis	Rhodococcus sp.		
JD 11	JD 20	Peribacillus simplex	Brevibacterium frigoritolerans	Bacillus thuringiensis	
JD 12	JD 23	Bacillus thuringiensis	Bacillus stercoris	Bacillus sp.	Bacillaceae bacterium
JD 13	JD 25				
JD 14	JD 28				
JD 15	JD 29	Rhodococcus sp.	Rhodococcus erythropolis		
JD 16	JD 30				
JD 17	JD 31	Bacillus sp.	Bacillus safensis	Bacillus pumilus	
JD 18	JD 32	Bacillus safensis	Bacillus pumilus	Bacillus sp.	Bacillus zhangzhouensis
JD 19	JD 33				
JD 20	JD 34				
JD 21	JD 35	Staphylococcus sp.	Staphylococcus equorum	Staphylococcus haemolyticus	

The 16s rRNA sequencing results were put through a blast search and the possible species above (this is an ongoing process and is incomplete as of now). Using these possibilities and the recorded descriptions and characteristics we will identify each species for each colony and further complete several metabolic tests as needed to identify any colony that is ambiguous or inconclusive with the data that is on hand.

DISCUSSION

The purpose of this study is to isolate halotolerant bacteria from native plants that survive in harsh conditions. After isolation, purification, and identification, these microbes will be tested on the model plant *Arabidopsis thaliana* with various salt treatments to see their role in the plant's growth and development under salt stress.

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