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Halotolerant Rhizosphere Bacteria: Isolation of Rhizosphere Bacteria From Native Utah Plant *Ceanothus velutinus*

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Halotolerant Rhizosphere Bacteria

Isolation of Rhizosphere Bacteria from Native Utah Plant *Ceanothus velutinus*

Introduction

- Increasing incidences of drought forced farmers to use the secondary, degraded water for irrigation. These water sources are rich in salt concentrations.
- This project has started with the hopes isolate bacteria from the rhizosphere of a native to Utah plant, *Ceanothus velutinus* (Snowbrush), which helps the plant survive the saltier conditions of Utah.
- The roots and rhizosphere samples were collected from Tony Grove in Logan, Utah. The samples were collected from three different elevations (1920m, 1950m, and 2289m).
- The rhizosphere bacteria were isolated at 37°C and 28°C on 2,4,6,8 and 10% NaCl concentration in Nutrient Agar media.
- The isolated colonies from the first screen were identified by 16SrRNA sequencing and characterized for nitrogen fixation, phosphate solubilization, and siderophore production.

Methods

Isolation of Rhizosphere bacteria

Serial Dilution:

The rhizosphere soil was separated from the roots and resuspended in sterilized water.

The resuspended rhizosphere soil serially diluted. 100ul of soil suspension added to 900ul of sterilized water and so on to get dilutions from 10⁻¹ to 10⁻⁶

Spread plate

100ul each from the last three dilutions were spread plated on the 0,2,4,6,8 and 10% NaCl on Nutrient Agar media.

Two rounds of screening were done, one at 37°C and another at 28°C.

Purification of bacteria by streak plate

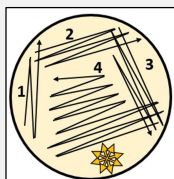
After four days of incubation, the unique colonies were purified by the streak plate method.

Identification

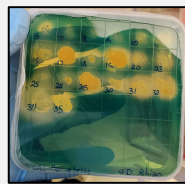
Each colony was stored in 25% glycerol stock at -80°C and sequenced for 16srRNA to identify.

Characterization

- Nitrogen Fixing bacteria were screened on Norris Glucose agar media.
- Siderophore positive colonies screened on CAS media agar.
- Phosphate solubilization colonies screened on Pikovskaya's Agar.



An diagram of streak plating



Siderophore production

Results



Spread plate examples of 6 and 8 percent nutrient agar



Isolated colonies that were streaked on 2% Nutrient Agar

Bacteria	Nitrogen Fixation	Siderophore Production	Phosphate Solubilization
JD3	-	+	+
JD7	-	-	-
JD8	-	-	-
JD9	-	-	-
JD10	-	-	-
JD11	-	-	-
JD15	-	+	-
JD17	-	-	-
JD18	-	+	+
JD19	-	-	-
JD20	-	+	-
JD23	-	-	-
JD25	-	+	-
JD28	-	+	-
JD29	-	-	+
JD30	-	+	-
JD31	-	+	-
JD32	-	+	-
JD34	-	+	-
JD35	-	-	-

Biochemical characterization of twenty isolates

Conclusion

- At 2% NaCl concentration there was still a very high amount of colonies being able to grow in the media.
- As concentrations increased, the number of colonies able to proliferate decreased.
- 4% NaCl most samples were down to or near only a single colony being manifested
- 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 on the first screen
- Twenty unique colonies were isolated, purified, and identified.
- None of the isolates showed nitrogen fixation ability.
- 50% of isolates showed siderophore production.
- 15% isolates showed phosphate solubilization.
- Two isolates JD3 and JD18 showed siderophore production and phosphate solubilization ability.
- Bacterial isolate JD34 was able to grow in 6% salt concentration nutrient agar and showed siderophore production.
- In the second screen, a total of 135 colonies were isolated from three locations at various salt concentrations. Out of those three colonies were isolated at 6% and 8% nutrient agar from 1950m.
- Unique isolates will be isolated from different salt concentrations will be sequenced for 16SrRNA and tested for Nitrogen fixation, phosphate solubilization, siderophore production
- Isolated and identified bacteria will be applied to the rhizosphere of new plants (*Arabidopsis* and *Medicago*) and be tested to see their aid in contribution to plant health and varying levels of soil salinity.



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