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Isolation and Purification of Bacterial Species from Rhizosphere of Native Plant Ceanothus velutinus (Snowbrush)

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Isolation & purification of bacteria from rhizosphere of native plant *Ceanothus velutinus* (Snow Brush)

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Introduction

Due to the ever-changing climate, plants face stressors that limit their growth. Therefore, it is vital to find new ways to protect plants from biotic and abiotic stressors such as pathogen infection, drought, heavy metal poisoning, and salinity. One approach to this is focusing on beneficial plant-microbe interaction, such as plant growth-promoting rhizobacteria (PGPR). These bacteria are found in the rhizosphere- the soil that is attached to the roots of a plant. Various PGPRs share a symbiotic relationship with plants and help plants cope with stresses. The purpose of this study is to isolate aerobic rhizobacteria off *Ceanothus velutinus* (snowbrush), a plant native to the Intermountain West region of North America that thrives in dry and harsh conditions.

Methods

The rhizosphere soil samples from the snowbrush plants were collected from Tony Grove, Utah, from three elevations: 1920m, 1950m, and 2289m. The rhizosphere soil samples were also collected from the cuttings plants of Snow Brush treated with native soil from the 1920m and 2289m in the greenhouse.

1.Isolation- Samples were diluted with an initial ratio of 10:95, then further serially diluted to 1:10 of dirt to water five times. The last three dilutions were plated on five different growth mediums by the spread plate method to the amount of 100ul, and incubated in 28℃. Different media were chosen based on their ability to grow different types of bacteria. Media include ¼ Tryptic Soy Agar, ¼ Nutrient Agar, Yeast Mannitol Agar, Actinomycetes Isolation Agar, and Minimal media.

2.Purification- The colonies were picked and purified by the streak plate technique.

3.Characterization- The purified colonies have been characterized by Gram staining, catalase activity, and morphological characteristics such as color, consistency, opacity, and texture.

4.Identification- So far, ten randomly selected bacterial species were sequenced for the 16s rRNA variable region and identified by BLAST against the 16s rRNA database at NCBI.

Figure 1. *Ceanothus velutinus* (snowbrush) cuttings at 3 months (left) and 6 months (right) after treatment with native soil

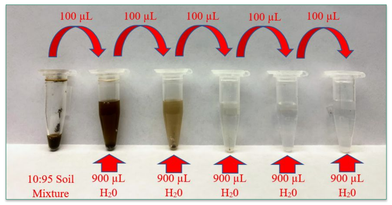

Figure 2. Serial Dilution

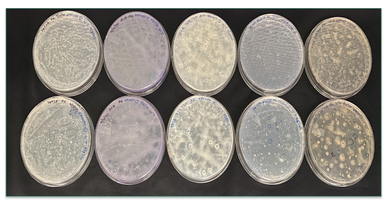

Figure 3. Growth of cultures from 1950m elevation samples

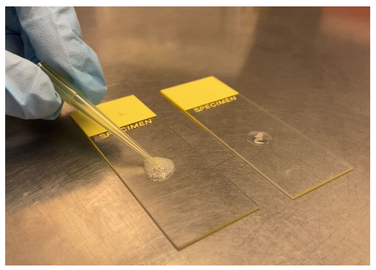

Figure 4. Catalase testing- catalase positive bacteria (left) and catalase negative bacteria (right)

Results and Conclusions

- Over 10,000 colonies were cultured.
- A variety of colors, shapes, and textures were grown. Colonies were purple, yellow, orange, tan, or secreted a brown pigment. Textures ranged from mucus-like to dry and cracking.
- 197 colonies were purified. So far, 10 bacterial species have been identified by 16SrRNA sequencing.
- The bacterial species identified by BLAST and their roles are listed in Table 1. Many of the isolated colonies belong to the genus Streptomyces.
- *S. ciscaucasicus* is known to accumulate zinc, a heavy metal that can be toxic when in excess.
- S. *Viridochromogenes* removes reactive oxygen species which can have toxic effects.
- *S. atratus* has antifungal properties that protect certain trees from rot.
- *S. pseudovenezuela* and *S. ederensis* produce a variety of antibiotic substances which can decrease the risk of disease.
- Other identified bacterial species included *Variovax ginsengisoli* which holds myriad capabilities such as removal of excess nitrates, synthesis of indoleacetic acid, a common phytohormone, as well as degrades many pollutants, which increases disease resistance.
- We are working on isolating more bacteria species from the rhizosphere of snowbrush, identifying and validating their functions.

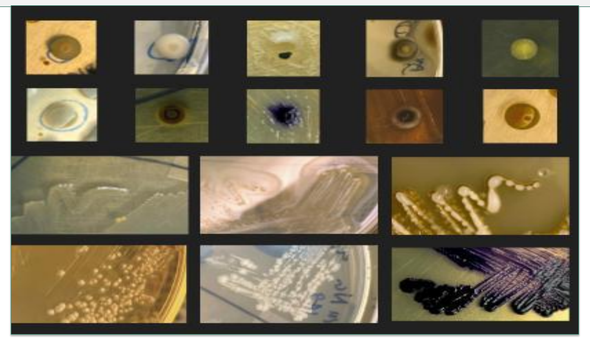

Figure 5. Examples of various unique bacterial species cultured across different dilutions and media of the *Ceanothus velutinus* rhizosphere

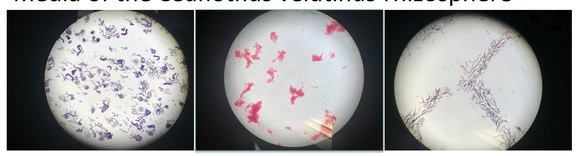

Figure 6. Gram stain-result from gram-positive and gram-negative.

BLAST results	Role
Variovax, Variovorax ginsengisoli	PGPR, denitrification, Degradation of pollutants, nitrile-converting enzymes, regulation of auxin
Streptomyces canus, Streptomyces ciscaucasicus	Zinc Biosorption
Streptomyces prunicolor, Streptomyces canadensis	Produces anti-cancer product Pironetin
Kitasatospora gansuensis	IAA Production
Streptomyces ederensis, Streptomces umbrinus	Antibiotic production of Tetrangomycin
Terrabacter spp.	Nutrient mineralization
Streptomyces atratus	Biocontrol agent
Streptomyces viriodochromogenes, Streptomyces bobli	PGPS, Antioxidant activity (catalase and superoxide dismutase)
Streptomyces bobli, Streptomyces pseudovenezuelae, Streptomyces galilaeus	Antibiotic (chloramphenicol and jadomycin) and secondary metabolite production
Janthinobacterium lividum	Hserlactone production (secondary metabolite), antifungal metabolite production- violacein

Table 1. Identification of 10 bacterial species and their respective roles as plant growth promoters