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The Plant Growth Promoting Ability of the Microbiome of *Ceanothus velutinus* from the Intermountain West Region

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THE PLANT GROWTH PROMOTING ABILITY OF THE MICROBIOME OF *CEANOTHUS VELUTINUS* FROM THE INTERMOUNTAIN WEST REGION

By

Jyothsna Ganesh

A thesis submitted in partial fulfillment
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ABSTRACT

The plant growth promoting ability of the microbiome of *Ceanothus velutinus* from the Intermountain West region

by

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Due to the ever-changing climate and deterioration of the earth's ecosystems, abiotic (drought, salinity) and biotic stresses (pathogen infection) gravely affect plant growth. Native plants offer an option for decreasing the negative effects of these stresses on urban landscapes. These native plants may be useful as ornamental plants in landscapes as they are well accustomed to the environments found in their native habitats. The Center for Water-Efficient Landscaping at Utah State University has released a list of plants that can be used for low water use landscaping. One such native plant is *Ceanothus velutinus* (snowbrush ceanothus). They are evergreen plants that can grow in dry and harsh conditions and are native to the Intermountain West region of North America. This study focused on the effects of rhizosphere and endosphere microorganisms on the growth and

development of snowbrush ceanothus plants. The first objective is to characterize the microbial community diversity using a comparative metagenomic study for the bulk soil, rhizosphere, and endosphere of the snowbrush ceanothus plant. Metagenomic analysis of the rhizosphere and endosphere communities of snowbrush ceanothus plants treated with native soil and propagated from cuttings and seedlings were also carried out. The second objective is to determine the effect of native soil on the callus formation, rooting, and survival of snowbrush ceanothus cuttings under greenhouse conditions. The effect of native soil on the growth, development, and nodule formation was also observed for snowbrush ceanothus plants under greenhouse conditions. The amount of macro and micronutrients including nitrate, phosphorus, carbon, potassium, zinc, manganese, copper, and iron for the native and greenhouse soil was measured and growth parameters of the plants (treatment and control) were determined including plant height, width, numbers of primary and secondary shoots. The final objective is to isolate bacterial species from the rhizosphere soil of snowbrush ceanothus plants from the native habitat and from greenhouse grown plants treated with native soil and from the endosphere of snowbrush ceanothus roots collected from their natural habitat. These bacterial isolates were characterized for siderophores production, indole acetic acid production, catalase activity, fixation of atmospheric nitrogen, and solubilization of phosphate.

(196 pages)

PUBLIC ABSTRACT

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Due to the ever-changing climate and deterioration of the earth's ecosystem, environmental stresses like abiotic (drought, salinity) and biotic stresses (pathogen infection) gravely affected plant growth. Native plants are a great way of improving these effects on the urban landscape. They can be used as ornamental plants in landscaping as they are accustomed to their natural environment. The Center for Water-Efficient Landscaping at Utah State University has released a list of plants to be used for low water use landscaping. One such native plant is *Ceanothus velutinus* (snowbrush ceanothus). They are evergreen plants that can grow in dry and harsh conditions and are native to the Intermountain West region of North America. This study focused on the effect of rhizosphere and endosphere microbiome on the growth and development of snowbrush ceanothus plants. A comparative metagenomic study in the bulk soil, rhizosphere, and endosphere of snowbrush ceanothus revealed the microbial diversity and presence of several plant growth promoting rhizobacteria (PGPR). So next, the effect of this native soil was observed on the growth and development of snowbrush ceanothus under the greenhouse conditions. Inoculation of native soil to the propagation media enhanced the rooting and survival rate of snowbrush ceanothus cuttings. The inoculation of native soil in the snowbrush ceanothus plants developed from cutting propagation and seedlings in the greenhouse revealed an improved growth compared to control plants. The metagenomic study of the rhizosphere

and endosphere of snowbrush ceanothus plants treated with native soil revealed the presence of several PGPR that were absent in the control plants. Nodulation was observed for the first time in snowbrush ceanothus plants grown in the greenhouse and inoculated with native soil. So finally, an attempt was made to isolate as many PGPR species as possible from the rhizosphere and endosphere of snowbrush ceanothus plants. Many of these isolates tested positive for one or more specific traits such as siderophore production, indole acetic acid production, catalase production, nitrogen fixation, and phosphate solubilization. The isolates were further tested for their plant growth-promoting properties in plants. We found many of these bacterial isolates could potentially be used as bio-fertilizers or bio-stimulants.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Environmental deterioration and increase in human population have increased the food demand globally (Glick, 2014). Environmental stresses, such as salinity and drought pose a great threat to sustainable agriculture as it affects plant health and crop productivity (Tuomisto et al., 2017). While soil salinity diminishes growth and development by affecting morphological, physiological, and biochemical aspects of plant growth (Gupta and Huang, 2014), drought is another major environmental stress that harms plants and declines crop productivity s (Bodner et al., 2015). It is an anticipation that by 2050 drought will be the lead problem in plant growth due to the increase in climate change (Vinocur and Altman, 2005).

A combination of stresses during the crop growth period has led to a severe loss of productivity (Mahalingam, 2015; Narsai et al., 2013; Prasad et al., 2011; Suzuki et al., 2014). Biotic stresses are something that plants have to fight (Pimentel et al., 1991), and abiotic stresses influence biotic stresses (Coakley et al., 1999; Peters et al., 2014; Scherm and Coakley, 2003), threatening the economic viability of crop production.

Throughout evolution, plants have adapted to stresses by fine-tuning their molecular, cellular, and developmental activities. The complete knowledge of how plants cope with these stresses is still unknown.

Every organism in the ecosystem interacts with its physical and biological environment (Gupta et al., 2017). Despite being immobile, plants interact with their biotic

environment, and the plant-microbe interaction is one of the prominent forms (Cheng et al., 2019). Microbial populations such as plant growth promoting rhizobacteria (PGPR) interact with plants in many different ways and affect their growth and development directly or indirectly (Ortíz-Castro et al., 2009).

Plant growth-promoting microorganisms are beneficial microbes present in and around plants that help plant growth and development. Endophytes are microorganisms that reside inside the plant. Microorganisms are also present on the surface of the above-ground part of the plant (phyllosphere) or around the roots of a plant that are influenced by soil (rhizosphere) (Mendes et al., 2013; Montañez et al., 2012; Wu et al., 2005). The potential of microbes to enhance plant growth can be harnessed to increase the growth and development of food crops, eventually increasing crop production. Therefore, it is essential to study the interactions of microorganisms with plant roots (Lugtenberg et al., 2002). The microorganisms associated with plant roots can dramatically increase plant resistance to abiotic and biotic stresses (Etesami and Beattie, 2017; V. S. Meena et al., 2017). Microorganisms can ameliorate stress conditions for crop plants and may pave the way to potentially promising options for sustainable agriculture (Etesami and Beattie, 2017; Shrivastava and Kumar, 2015).

Plant growth promoting rhizobacteria also protect plants from phytopathogens, act as bio-control agents (Mendes et al., 2011), and improve plant growth and development (Huang et al., 2014). Plant growth promoting rhizobacteria promote plant growth in two ways – direct and indirect mechanisms (Kumari et al., 2018). These direct mechanisms to promote plant growth are either the secretions of plant hormones such as auxins (indole acetic acid- IAA), cytokinins, gibberellins (GA_3), and ethylene (Bent et al., 2001; Chabot

et al., 1996) or enhancing nutrient availability by nitrogen fixation, phosphate solubilization, other mineral solubilization such as potassium and zinc, production of ACC, (1-aminocyclopropane-1-carboxylate) deaminase enzyme, and siderophore production (Ahmad et al., 2008; Habibi et al., 2014a; Reyes et al., 2002). The indirect mechanism involves the suppression of infection by pathogenic bacteria, fungi, nematodes, and viruses (Barea et al., 2005) by acting as bio-control agents (Romero et al., 2007).

Plant growth promoting rhizobacteria are found effective against salt stress in plants (Paul and Lade, 2014; Qin et al., 2016; Shrivastava and Kumar, 2015) by enhancing the uptake of potassium and accumulation of compatible solutes such as amino acids, polyols, ectoines, and betaines (C. Dimkpa et al., 2009; Lamosa et al., 1998). Several PGPRs have also been shown to increase tolerance to drought in plants (Kaushal and Wani, 2016; Kavamura et al., 2013). Drought tolerance induced by rhizospheric bacteria involves several biochemical and physiological changes (Kaushal and Wani, 2016).

Plant growth-promoting bacteria also possess the ability to act as bio-stimulants. Bio-stimulants are microorganisms that promote plant growth, improve crop productivity, help plants deal with biotic and abiotic stresses, and increase nutrient uptake (Silva et al., 2017). Bio-stimulants help in nitrogen fixation, humic substance production, and nutrient availability in soil, so plants have heightened access to nutrients (Macias-Benitez et al., 2020; Silva et al., 2017). Biostimulants are used in agriculture for promoting plant growth for years and have been exponentially increasing in the past decade (Silva et al., 2017).

Plant growth promoting rhizobacteria can help crops improve their health and productivity even under stress or nutrient-deficit environments. Bacteria-induced

production of IAA can lead to root architecture alterations. They can improve water and nutrient uptake, increase root surface area, and increase overall plant health (Etesami et al., 2015; Glick, 2012; Pii et al., 2015). According to previous literature, the majority of the PGPR species belonged to the genera- *Arthrobacter, Azospirillum, Bacillus, Burkholderia, Erwinia, Flavobacterium, Pseudomonas, Rhizobium,* and *Serratia*, etc. (Orhan et al., 2006).

Plants that thrive in a particular region and are well adapted to their edaphic and climate conditions are known as native plants. They are widely used for scientific research, for their ability to maintain viability and integrity in their native environment. The current study focuses on *Ceanothus velutinus*, commonly known as snowbrush ceanothus - a native plant of Utah in the United States. More than half of Utah's land consists of wildland areas filled with endemic species. Utah ranks among the top ten in the native species diversity in the United States (Stein, 2002). Many of these native plant species are known for their aesthetic value and ability to adapt to various environments and are very useful in conservational purposes such as rehabilitation and restoration of disturbed and degraded lands (Hooper et al., 2008). The farmers and ranchers of Utah have used these native plants in conservation by constructing snow shelters and windbreaks to increase livestock survival and crop production in rural areas (Hooper et al., 2008). These native plants have been used to rehabilitate or refurbish lands degraded by mining, soil erosion, fires, and intense cattle grazing. They have also used native plant species to restore wildlife habitats and essential wetlands (Hooper et al., 2008). Native plants such as silver buffaloberry and mountain mahogany are recommended for low water use landscaping (Centre for Water Efficient Landscaping (CWEL) (https://cwel.usu.edu/). *Ceanothus velutinus* (snowbrush ceanothus)

is an evergreen plant of the *Rhamnaceae* family. It is a branched, stout plant found in dry areas with sticky and shiny evergreen leaves and can fix nitrogen (Rupp and Wheaton, 2014). They are usually located at 2133- 2743 m elevation and are hard to grow from seeds. However, if the seeds of this plant are collected and appropriately treated, almost 80-90% of them will germinate (Rupp and Wheaton, 2014). The roots of snowbrush ceanothus propagated from cuttings are susceptible to rotting and hard to grow from cuttings in landscape media (Rupp and Wheaton, 2014).

The present investigation explores the rhizosphere and endophytic microbiome of *Ceanothus velutinus*. The effect of the soil from the rootzone of snowbrush ceanothus plants from Logan Canyon on the growth and development of snowbrush ceanothus plants grown in the greenhouse was studied by inoculating plants with native soil. We also investigated the effect of soil from the rhizosphere and endosphere of snowbrush ceanothus plants from Logan Canyon on the propagation of *Ceanothus velutinus* cuttings in the greenhouse. The metagenomic study of the rhizosphere and endophytic microbiome of the snowbrush ceanothus provided insight into the presence of PGPR and their potential role in the growth and development of the plant. The effect of soil from snowbrush ceanothus plants from Logan Canyon on the propagation of *Ceanothus velutinus* cuttings in the greenhouse was also studied. Lastly, isolation, identification, and characterization of bacteria from the rhizosphere and endosphere of snowbrush ceanothus plants from Logan Canyon and greenhouse conditions was carried out. The isolates were characterized based on nitrogen fixation, phosphate solubilization, siderophore, catalase, and indole acetic acid (IAA) production.

These can further be used as bio-fertilizers/biostimulants as chemical fertilizers have adverse effects on the environment. Bio-fertilizers/biostimulants are becoming very popular, especially in organic farming, to maintain soil health, plant growth and development, and sustainability. Using PGPR in bio-fertilizer or biostimulants can be eco-friendly, economical, and productive. Bacterial cultures of PGPR can be used as inoculants and lead to a sustainable agricultural technique (Kumari et al., 2018).

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CHAPTER II

METAGENOMIC STUDY OF THE MICROBIOME OF THE NATIVE PLANT *CEANOTHUS VELUTINUS* (SNOWBRUSH CEANOTHUS) AND THE EFFECT OF NATIVE SOIL ON THE GROWTH OF SNOWBRUSH CEANOTHUS UNDER GREENHOUSE CONDITIONS

Abstract

Biotic and abiotic environmental stresses affect plant health and reduce crop production. The rhizosphere microbiome of a plant plays a significant role in plant growth and development. In this study, we investigated the microbial diversity of bulk soil, rhizosphere, and endosphere of *Ceanothus velutinus* snowbrush ceanothus. *Ceanothus* is an evergreen native plant usually found in dry areas that thrives well in harsh conditions. The snowbrush ceanothus samples were collected from different locations of the Tony Grove region near Logan, Utah. The metagenomic analysis of 16S rRNA sequences revealed the presence of several potential plant growth-promoting rhizobacteria (PGPR) belonging to several genera including *Frankia, Mesorhizobium, Nocardia, Pseudomonas, Rhizobium,* and *Streptomyces*. Native soil inoculation may improve rooting and survival percentage of the cutting propagation in snowbrush ceanothus. Snowbrush ceanothus plants treated with native soil showed may improve overall growth. Nodulation was observed for the first time in snowbrush ceanothus plants under greenhouse conditions upon treatment with native soil. The microorganisms in the native soil were isolated and

tested for their role in plant-growth promotion. Potential biofertilizers and bio-stimulants strains were isolated that may eventually help with sustainable agriculture.

Highlights

- Presence of several plant growth-promoting bacteria in the rhizosphere and endosphere of snowbrush ceanothus plants
- Native soil inoculation potentially improves rooting and survival percentage in snowbrush ceanothus cutting propagation under greenhouse conditions
- Nodule formation observed in greenhouse grown snowbrush ceanothus plants possibly due to native soil inoculation
- **Keywords:** Plant growth-promoting rhizobacteria (PGPR), 16S rRNA sequencing, native soil, nodulation

1. Introduction

Environmental deterioration and increase in human population have increased food demand globally (Glick, 2014). Environmental stresses, such as salinity and drought pose a great threat to sustainable agriculture as it affects plant health and crop productivity (Tuomisto et al., 2017). While soil salinity diminishes growth and development by affecting morphological, physiological, and biochemical aspects of plant growth (Gupta and Huang, 2014), drought is another major environmental stress that harms plants and deceases the productivity of crops (Bodner et al., 2015). It has been anticipated that by 2050 drought will be the lead problem in plant growth due to climate change (Vinocur and Altman, 2005).

A combination of stresses during the crop growth period has led to a severe loss of productivity (Mahalingam, 2015; Narsai et al., 2013; Prasad et al., 2011; Suzuki et al., 2014). Biotic stresses are something that plants have to fight (Pimentel et al., 1991), and abiotic stresses influence biotic stresses (Coakley et al., 1999; Peters et al., 2014; Scherm and Coakley, 2003), threatening the economic viability of crop production.

Throughout evolution, plants have adapted to stresses by fine-tuning their molecular, cellular, and developmental activities. The complete knowledge of how plants cope with these stresses is still unknown.

Every organism in the ecosystem interacts with its physical and biological environment (Gupta et al., 2017). Despite being immobile, plants interact with their biological environment, and the plant-microbe interaction is one prominent form of interaction (Cheng et al., 2019). Microbial populations including plant growth promoting rhizobacteria (PGPR) interact with plants in many different ways and affect their growth and development directly or indirectly (Ortíz-Castro et al., 2009).

Endophytes are microorganisms that reside inside the plant. Microorganisms are also present on the surface of the above-ground part of the plant (phyllosphere) or in soil influenced by the roots of a plant (rhizosphere) (Mendes et al., 2013; Montañez et al., 2012; Wu et al., 2005). The potential of microbes to enhance plant growth can be harnessed to increase the growth and development of food crops, eventually increasing crop production. Therefore, it is essential to study the interactions of microorganisms with plant roots (Lugtenberg et al., 2002). The microorganisms associated with plant roots can dramatically increase plant resistance to abiotic and biotic stresses (Etesami and Beattie, 2017; V. S.

Meena et al., 2017). Microorganisms can ameliorate stress conditions for crop plants and may be promising options for sustainable agriculture (Etesami and Beattie, 2017; Shrivastava and Kumar, 2015).

Plant-microbe interactions are complex relationships (Schirawski and Perlin, 2018). They are usually located in the rhizosphere or at the surface of plant roots (Habibi et al., 2014b). Plant growth promoting rhizobacteria (PGPR) promote plant growth by various mechanisms such as siderophore production, nitrogen fixation, and phytohormone production, etc. (Etesami and Maheshwari, 2018a). Some of the PGPRs also help plants grow by sequestering heavy metals (Glick, 2010), the production of antibiotics (Compant et al., 2005a), hydrolyzing the cell wall of fungal pathogens (Compant et al., 2005a), and solubilizing nutrients and minerals (Etesami and Maheshwari, 2018a).

PGPR are found effective against salt stress in plants (Paul and Lade, 2014; Qin et al., 2016; Shrivastava and Kumar, 2015) by enhancing the uptake of potassium and accumulation of compatible solutes such as amino acids, polyols, ectoines, and betaines (Dimkpa et al., 2009; Lamosa et al., 1998). Several PGPRs have also been shown to increase tolerance to drought in plants (Kaushal and Wani, 2016; Kavamura et al., 2013). Drought tolerance induced by rhizospheric bacteria involves several biochemical and physiological changes (Kaushal and Wani, 2016).

Hence, PGPRs can help crops improve their health and productivity even under stress or nutrient-deficit environments. Bacteria-induced production of IAA can lead to root architecture alterations. They can improve water and nutrient uptake, increase the root

surface area, and increase overall plant health (Etesami et al., 2015; Glick, 2012; Pii et al., 2015).

Plants that thrive in a particular region and are well adapted to their edaphic and climate conditions are known as native plants. They are used in horticulture for their ability to maintain viability and integrity in their native environment. The current study focused on *Ceanothus velutinus*, commonly known as snowbrush ceanothus - a native plant of Utah in the United States. More than half of Utah's land consists of wildland areas filled with endemic species. Many of these native plant species are known for their aesthetic value and ability to adapt to various environments and are very useful in conservation including rehabilitation and restoration of disturbed and degraded lands (Hooper et al., 2008). The farmers and ranchers of Utah have used these native plants in conservation by constructing snow shelters and windbreaks to increase livestock survival and crop production in rural areas (Hooper et al., 2008). These native plants have been used to rehabilitate or refurbish lands degraded by mining, soil erosion, fires, and intense cattle grazing. Land managers have also used native plant species to restore wildlife habitats and essential wetlands (Hooper et al., 2008). Native plants such as silver buffaloberry and mountain mahogany are recommended for low water use landscaping (Centre for Water Efficient Landscaping (CWEL) (https://cwel.usu.edu/). *Ceanothus velutinus* is an evergreen plant of the *Rhamnaceae* family. It is a branched, stout plant found in dry areas with sticky and shiny evergreen leaves and can fix nitrogen (Rupp and Wheaton, 2014). They are usually located at 2133- 2743 m elevation and are hard to grow from seeds. However, if the seeds of this plant are collected and appropriately treated, almost 80-90% of them will germinate (Rupp and Wheaton, 2014). The roots of snowbrush ceanothus propagated from cuttings are

susceptible to rotting and hard to grow from cuttings in landscape media (Rupp and Wheaton, 2014).

The present investigation explored the rhizosphere and endophytic microbiome of *Ceanothus velutinus*. The effect of the soil from underneath snowbrush ceanothus plants from Logan Canyon on the growth and development of snowbrush ceanothus plants grown in the greenhouse was studied by inoculating plants with native soil. We also investigated the effect of soil from the rhizosphere and endosphere of snowbrush ceanothus plants from Logan Canyon on the propagation of *Ceanothus velutinus* cuttings in the greenhouse.

The metagenomic study of the rhizosphere and endophytic microbiome of the snowbrush ceanothus provided insight into the presence of PGPR and their potential role in the growth and development of the plant. This knowledge was further helped us isolate and identify the role of these bacteria in promoting plant growth under environmental stresses and nutrient deficit conditions.

2. Methodology

2.1 Metagenomic study of *Ceanothus velutinus*

2.1.1 *Sample collection*

Ceanothus velutinus (snowbrush ceanothus) roots and soil samples were collected from three different locations in the Tony Grove region of Logan, Utah (41^{o}52’56” N 111^{o}33’53” W, elevation 1920m; 41^{o}52’34” N 111^{o}34’20” W, elevation 1950m and 41^{o}53’15” N 111^{o}36’4” W, elevation 2289m) following the protocol given by McPherson, 2018. Samples are named 1920, 1950, and 2289 after the elevation at which the samples were collected. Three different types of samples - bulk soil, rhizosphere, and roots were

collected from each location. The bulk soil was collected from a depth of nearly 30 cm by shovel and stored at -20 ^{o}C. The roots and the rhizosphere soil samples were collected in a phosphate buffer with surfactant (8.5 g/L Na_2HPO_4 anhydrous, 6.33 g/L NaH_2PO_4, 200 µl/L Tween 20, pH 6.5) and stored at -20 ^{o}C before further processing. The rhizosphere soil was separated from the roots by shaking them in the buffer solution. The roots samples were then blotted dry and then surface-sterilized in a solution with 35 ml of bleach+ 0.01% of Tween-20. They were washed with 70% ethanol, followed by washing three times with sterile distilled water. After blot drying with paper towels, roots were cut into 5 mm pieces and stored at -80^{o}C until further use.

The rhizosphere soil in the phosphate buffer was collected by centrifugation (3000g for 5 minutes) and supernatant discarded. The pellet of rhizosphere soil was stored frozen at -20 ^{o}C.

Snowbrush ceanothus plants were grown via seedlings under greenhouse conditions and the temperatures were set at 25/20 ^{o}C (day/night). The daily light integral (DLI) of 29.4 ± 8.6 $mol \cdot m^{-2} \cdot s^{-1}$ (mean ± SD) was calculated using a SQ-500-SS: Full-Spectrum Quantum Sensor (Apogee) inside the greenhouse. 1000-W high pressure sodium lamps were used to provide supplemental light from 0600 to 2200 hours (Hydrofarm, Petaluma, CA). These lamps were turned on when the greenhouse light intensity was lower than 544 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and the light intensity on these lamps were around 130 ± 18 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (mean ± SD) at the plant canopy level. Ten plants each were inoculated with 200 ml of native bulk soil 1920 and 2289, and 20 plants were inoculated with 200 ml of native bulk soil 1950. Ten were considered as a control without any soil inoculum. Six months after inoculation, rhizosphere and endosphere samples were collected using the same

protocol used for the native sample collection. Three samples were collected from each inoculation treatment.

2.1.2 *Physiochemical parameters of soil*

Soil moisture, pH, and EC

The fresh weight of 40 grams of bulk soil from each sample was taken and dried in a hot air oven at 60 ^{o}C for 72 hours, and dry weight was measured. Soil moisture percentage was calculated using the equation- [(wet soil weight) - (dry soil weight-bag weight) / (dry soil weight)] *100. The pH and EC of the snowbrush ceanothus soil was determined from samples treated with and without native soil (Cavins et al., 2008). Five plants were randomly selected from control and each treatment. 500 ml of tap water was poured into each pot, and leachate was collected. A few drops of the leachate were taken to measure the pH and EC by an EC and pH meter (Thermo Scientific Orion Star A112 2011).

Total carbon and phosphorus

The total carbon (TC) and available phosphorus were measured in the soil samples collected from near the snowbrush ceanothus plants in Tony Grove and the soil samples of snowbrush ceanothus seedlings grown with and without native soil under the greenhouse conditions. Triplicates were used per sample for each test.

Five grams of each soil sample were air-dried for 72 hours. Dried samples were crushed using a mortar and pestle and sieved through a 250 µm sieve. Total carbon content was measured in dried soil samples using the SKALAR Carbon Analyzer 2008 (Atoloye, 2020). Amount of soil taken for measuring total carbon weighed between 60 to 230 mg. The amount of phosphorus was calculated using Olsen’s sodium bicarbonate extraction

method (Baxter, 2018). One gram of soil was added to 20ml of $NaHCO_3$ (0.5 mol/L), and ammonium molybdate ascorbic acid was added. The available P was measured calorimetrically at 880 nm (SpectraMax M2, Molecular Devices, Sunnyvale, CA). A blank was without soil. A standard curve for inorganic phosphate was plotted using KH_2PO_4 (potassium dihydrogen phosphate), and the amount of available phosphorus (in µg/g) was determined (Atoloye, 2020).

Micronutrients, nitrogen, and potassium content

The available potassium and nitrogen were measured in the native and greenhouse soil samples (control and treatment) at the Utah State University Analytical Laboratories (USUAL) using Olsen $NaHCO_3$ Method (Olsen, 1954) and the Nitrate-N analysis in $CA(OH)_2$ extract (Haby, 1989), respectively. The micronutrient test (Fe, Zn, Cu, Mn) for the same soil samples was also tested at the USUAL using the DTPA extractable elements technique (Lindsay and Norvell, 1978).

2.1.3 *DNA isolation, library construction, and sequencing*

Microbial DNA was isolated from the roots of snowbrush ceanothus using Qiagen DNeasy PowerPlant Pro Kit 2018 and from bulk and rhizosphere soil samples using Qiagen DNeasy PowerSoil Pro Kit, respectively. Soil samples were mechanically crushed using metal beads in a SpexSampleprep 2010 Geno/Grinder (SPEX, Metuchen, NJ, USA) with a speed of 1000 and 30-second cycles with 1-minute breaks to disrupt the cells. The tubes were centrifuged, and the supernatant was collected. The supernatant containing microbial cells was then lysed using a lysis buffer, vortexed, and centrifuged to get the supernatant containing the DNA. A neutralization buffer was added, and the lysate was transferred to

a spin column. This lysate was then passed through the spin column. The bound DNA in the column was washed with 70% ethanol and eluted with elution buffer. The isolated DNA was quantified using a Nanodrop 2000 (Thermo scientific). A total of 250 mg of the bulk soil was taken for the DNA extraction and the concentrations obtained from a Nanodrop spectrophotometer.

Roots were frozen in liquid nitrogen and mechanically crushed with metal beads in extraction buffer containing RNase A in the SpexSampleprep 2010 Geno/Grinder (SPEX, Metuchen, NJ, USA). The supernatant was collected by centrifugation. The microbial cells were lysed by lysis buffer. The lysate was passed through a column, and the DNA was attached to the column. The column containing microbial DNA was washed with 70 % ethanol. The DNA is eluted with eluting buffer and quantified using the Nanodrop 2000 spectrophotometer.

For 16S rRNA gene sequencing, the DNA from rhizosphere and bulk soil was amplified for the V4 variable region using the V4 variable region-specific primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVHHHTWTCTAAT-3') (Gkarmiri et al., 2017). The DNA from roots was amplified for the V5-V7 variable region with V5-V7 (for endophytic) specific primers 799F (5' AACMGGATTAGATACCCKG) and 1193R (3' ACGTCATCCCCACCTTCC) (https://en.novogene.com/16s-18s-its-amplicon-metagenomic-sequencing/#overview). The amplification reaction mixture (25 µl) contained 13 µl of water, 10 µl of Platinum Hot Start PCR Master Mix (MM) (ThermoFisher), 0.5 µl of 10 mM forward and reverse primer each, and 1 µl of 5 ng/µl of the DNA. The polymerase chain reaction (PCR) was as follows: an initial denaturation of 3 minutes at 94 ^{o}C followed by 35 cycles of denaturation at 94 ^{o}C

for 45 seconds, annealing at 50 ºC for 60 seconds, and extension at 72 ºC for 60 seconds and with a final extension at 72 ºC for 10 minutes, using a DNA Engine DYAD Peltier Thermal Cycler (BIO-RAD).

The PCR product was diluted 50 times, and indexes were attached by secondary PCR. The second reaction mixture consisted of 5 µl of mM, 2 µl of i5 index, 3 µl of i7 index, and 1 µl of the diluted PCR product. The PCR conditions followed: an initial denaturation step at 94 ºC for 1 minute followed by 15 cycles of denaturation at 94 ºC for 15 seconds, annealing at 64 ºC for 15 seconds, and an extension at 72 ºC for 1 minute, and a final extension at 72 ºC for 3 minutes using a DNA engine dyad Peltier thermal cycler (BIO-RAD).

Once the indexes were attached, the samples were cleaned with AMPureXP beads, using a 1:1 ratio. The PCR products were quantified by fluorometry, and ~10-15% of the samples were analyzed on the TapeStation. The samples were then pooled and sequenced on the MiSeq using a 2×250 paired-end sequencing (500 cycle Nano sequencing kit) (Illumina, San Diego, CA, USA).

2.1.4 *Data analysis*

The sequenced data were analyzed using QIIME2/2019.4 (Bolyen et al., 2019). Analysis of amplicon sequence variants (ASVs) was carried out by divisive amplicon denoising algorithm (DADA2), and the taxonomic classification was done using a feature-classifier. Alpha diversity was calculated using the Shannon index and beta diversity metrics were determined using the Bray-Curtis index. Shannon index calculates the species richness which is the number of species in a given region are combined with its relative

abundance (https://www.omnicalculator.com/ecology/shannon-index). The Bray-Curtis dissimilarity index calculates the dissimilarity between the community at the two different sites (https://www.statology.org/bray-curtis-dissimilarity/). Multiple plots were generated from these diversity indices.

Effect of native soil on the growth of snowbrush ceanothus

2.1.5 *Effect of native soil on the rooting and survival of snowbrush ceanothus*

Ceanothus velutinus (snowbrush ceanothus) shoots were collected from the Tony Grove region of Logan, Utah. Twelve-cm long shoot cuttings were collected in June and July 2020. The shoots were washed in a 1% Zerotol solution and wounded onto the lower part of the stems. The wounded side of the cuttings was then dipped in a rooting hormone with 3,000 mg/L indole butyric acid (Hormodin 2) and placed into a soil mixture of peat moss and perlite (1:4 v/v) (Paudel, 2020). In the second set, to observe the effect of native soil on the rooting, half of the volume of the peat moss and perlite (1:4 v/v) soil mixture was mixed with the native bulk soil sample from near the native plants in the Tony Grove area; this resulted in 50% by volume native soil inoculation. In the third set of experiments, cuttings were placed in 100% native soil. The cuttings were placed on a mist bench and covered with a white cloth. The bottom heat was set to 23 ^{o}C using a heating mat (Propagation Mat, Grower's Nursery Supply). The misting was set using a Water Plus Vapor Pressure Deficit (VPD) mist controller (Phytotronics, Earth City, Montana) at 60 VPD units. These cuttings were observed for rooting after eight weeks in the mist bench. Once rooting was observed, the cuttings were transplanted to 3.8-L pots containing a soil mixture of peat moss (75%), vermiculite (13%), and rice husks (12%), wetting agent

(AquaGro G Aquatrols Corporation of America, NJ, US), and hydrated lime- $CaCO_3$. The survival rate of these rooted cuttings after ten months of transplantation was recorded.

2.1.6 *Effect of native soil on snowbrush ceanothus seedlings*

Ceanothus velutinus seeds were sacrificed in water at 90 ^{o}C for 10-20 seconds, followed by cooling in ice water for one hour. Then the seeds were given a cold treatment (stratification) for two months at 4 ^{o}C, and after that, the seeds were kept on a Petri dish with a wet blotting paper to germinate at room temperature (Paudel et al., 2020). The germinated seeds were transferred into 3.8-L pots with a soil mixture of peat moss and perlite (1:3). A total of 50 four-month-old plants were included in this study. Ten were considered as a control without any inoculum. Ten plants each were inoculated with 200 ml of native soil labeled as 1920 and 2289, and 20 plants were inoculated with 200 ml of native soil labeled as 1950. This study was conducted for a year, and the plant's macro and micronutrient content, growth parameters, and nodule formation were measured. No fertilizers were applied in this study.

2.1.7 *Biomass assay*

The number of primary and secondary shoots along with plant height and stem canopy width were recorded after one year of treatment (Karakurt and Aslantas, 2010; Karlidag et al., 2007;).The number of leaves, leaf fresh and dry weight, growth index, chlorophyll content, and chlorophyll fluorescence (Chen et al., 2021) were also measured in the control and treated plants. The leaves were dried at 80 ^{o}C for seven days and then the dry weight was measured. The growth index was measured using the formula (Height + Width 1 +Width 2)/3.

2.1.8 *Statistical analysis*

The experimental design is a repeated measure one-factor ANOVA. Five biological replicates were used to collect data for both control and plants treated with native soils. An analysis of variance (ANOVA) was carried out to check the effect of native soil on plant growth and development. The mean separation was adjusted using the Tukey-Kramer method for multiplicity at $\alpha = 0.05$.

3. Results

3.1 Metagenomic analysis of *Ceanothus velutinus*

The 16S rRNA sequencing data was analyzed using QIIME2. The microbial populations in the bulk soil, rhizosphere, and endosphere of snowbrush ceanothus from the three locations 1920, 1950, and 2289 were analyzed. The taxonomic classification indicated the dominance of the phyla *Proteobacteria* followed by *Actinobacteria* and a strong presence of other phyla such as *Acidobacteria, Bacteroidetes, Firmicutes*, and *Verrucomicrobia* (Fig 1.a.). The population of the phyla *Gammatimonadetes* in all the bulk soil samples and *Elusimicrobia* in the Bulk_1920 was significantly higher than the other bulk, rhizosphere, and endosphere samples (Fig 1.a.).

The taxonomic classification at the genus level revealed the presence of several potential PGPR, such as *Bacillus, Bradyrhizobium, Frankia, Mesorhizobium, Mycobacterium, Nocardia, Rhizobium, Rhodoplanes*, and *Streptomyces* (Fig 1.b.). The population of the genus *Kaistobacter* in all the bulk soil samples was significantly higher than the other bulk, rhizosphere, and endosphere samples (Fig 1.b.).

The Shannon index showed higher bacterial diversity in the bulk soil and rhizosphere when compared to the endosphere (Fig 1.c.). The beta diversity showed a scattered bacterial diversity in all the samples (Fig 1.d.).

The bulk soil DNA concentrations obtained from the Nanodrop were 6 µg/g DNA per 198.9g soil dry weight in 1920, 10 µg/g DNA per 192 g soil dry weight in 1950, and 13 µg/g DNA per 189.3 g soil dry weight in 2289.

The phylum relative abundance in the endosphere samples revealed the dominance of the phyla *Proteobacteria* and *Actinobacteria* in both the native and greenhouse samples. Some other predominant phyla present were *Acidobacteria*, *Bacteroidetes, Firmicutes,* and *Spirochaetes* in the greenhouse samples while *Acidobacteria, Bacteroidetes, Elusimicrobia, Firmicutes,* and *Verrucomicrobia* in the native soil samples (Fig 2.a.). The relative abundance of the phylum *Actinobacteria* in 1920 was significantly higher than in the other endosphere samples (Fig 2.a.).

The taxonomic classification revealed the presence of several PGPR species. There were many genera of bacterial species present in the native endosphere samples and the native soil treated samples, but absent in the control samples including *Bradyrhizobium, Devosia, Flavobacterium, Frankia, Hyphomicrobium, Janthinobacterium, Mesorhizobium, Rhizobium,* and *Staphylococcus* (Fig 2.b.). The relative abundance of the genera *Frankia* in 1920, *Streptomyces* in 2289GR and 1920, and *Bradyrhizobium* in 2289 was significantly higher than in the other endosphere samples (Fig 2.b.).

Our observations may indicate that these bacteria species colonized due to the native soil inoculation. The alpha diversity analysis showed that the microbial diversity in

1950 and 2289 was higher than in any other sample, and control had one of the lowest diversities (Fig 2.c.). The beta diversity analysis showed a scattered bacterial diversity in all the samples (Fig 2.d.).

The metagenomic analysis of the rhizosphere samples from the native habitat and the plants treated with and without native soil in greenhouse conditions revealed the dominance of the bacteria belonging to the phyla *Proteobacteria* followed by *Actinobacteria.* Some of the other predominant phyla were *Acidobacteria, Bacteroidetes, Firmicutes, Planctomycetes,* and *Verrucomicrobia* (Fig 3.a.). The population of the phyla *Acidobacteria* in 1950 was significantly higher than in the other rhizosphere samples (Fig 3.a.).

The genus-level classification showed the abundance of multiple PGPR species in all the samples. Several well-known PGPR bacteria were found in the native soil samples and the treated greenhouse samples but were absent in control samples. These bacteria belonged to several genera including *Bradyrhizobium, Devosia, Hyphomicrobium, Mesorhizobium, Pseudomonas,* and *Rhizobium* (Fig 3.b.). Our observations are suggestive of the colonization of these bacterial species in the treated samples due to the native soil inoculation. The alpha diversity results showed that the native rhizosphere samples had a higher microbial diversity than the control (Fig 3.c.). The beta diversity analysis showed a scattered microbial diversity in all the samples (Fig 3.d.).

3.1.1 *Physiochemical parameters of soil*

The soil moisture level in 1920, 1950, and 2289 were 25.69±3.42, 30.23±2.18, and 30.07±6.04%, respectively. The results revealed no significant difference between each other (Table 1).

The total carbon (TC) for soil samples from three locations, 1920, 1950, and 2289 were 2.21±0.77, 24.80±1.75, and 12.78±1.82 %, respectively. There is a significant difference in the total carbon content seen between each of the samples. The location 1920 has the lowest total carbon content while 1950 has the highest (Table 1). The results also suggested that locations 1950 and 2289 have more organic matter compared to 1920. The TCs in the samples collected from greenhouse growing medium inoculated native soil were 64.17±3.92% in control and 6.98±1.54, 28.86±1.27, and 22.27±2.16 % in 1920GR, 1950GR, and 2289GR, respectively (Table 2). The total carbon content was significantly higher in the control samples than in the treated ones.

The amount of nitrogen in the soil samples 1920, 1950, and 2289 were 2.66±1.85, 16.85±15.06, and 1.85±0.44 mg/kg. There was no significant difference in the amounts of nitrogen in the three native soil samples. In the samples from greenhouse growing medium Control, 1920GR, 1950GR, and 2289GR, the amount of nitrogen was 5.28±0.17, 0.97±0.22, 3.07±1.12, and 4.43±1.24 mg/kg respectively. The amount of nitrogen in 1920GR was lower than in the other samples such as Control, 1950GR, and 2289GR.

The total available phosphorus content was also observed in the native soil samples, and the results revealed that the total available phosphorus in the native soil samples were 31.62±2, 6.00±0.2, and 34.80±0.32 mg/kg in 1920, 1950, and 2289, respectively (Table 1).

The amount of available phosphorus in the soil samples collected from snowbrush ceanothus plants propagated via seeds treated with and without native soil under the greenhouse conditions were 50.61±2.84 in control and 12.33±0.52, 15.83±0.72, and 12.24±0.54 mg/kg in the treated samples 1920GR, 1950GR, and 2289GR, respectively (Table 2). The total available phosphorus was significantly higher in the control soil samples than in the treated soils.

The amount of potassium in the native soil samples was 469.5±115.5, 600.5±57.23, and 485.5±7.5 mg/kg in 1920, 1950, and 2289, respectively (Table 1). The amount of potassium in the greenhouse samples was 968±84 mg/kg in the control sample and 481.33±23.02, 608.33±19.70, and 519.67±27.17 mg/kg in 1920GR, 1950GR, and 2289GR, respectively (Table 2). The amount of potassium in control was significantly higher than that of the treated samples.

The amount of zinc in the native soil samples was 1.79±0.3, 2.17±0.03, and 2.56±0.2 mg/kg in 1920, 1950, and 2289 respectively (Table 1). The amount of iron was 70.35±11.45, 83.875±4.7, and 74.05±5.65 mg/kg in 1920, 1950, and 2289, respectively (Table 1). The quantity of copper in these samples was 1.355±0.35, 0.8375±0.12, and 1.005±0.14 mg/kg in 1920, 1950, and 2289, respectively (Table 1). The amount of manganese in native soil samples was 19.9±1.2, 26.7±1.44, and 18.5±0.8 mg/kg in 1920, 1950, and 2289, respectively (Table 1). More amounts of zinc were observed in 2289 when compared to 1920 and 1950. The amount of manganese in 1950 was significantly higher than in 1920 and 2289.

The micronutrient content in the soil of greenhouse-grown plants (control and treatment) was also measured. The amount of zinc in control was 15.2±1.4 mg/kg, and in 1920GR, 1950GR, and 2289GR was 2.79±0.27, 5.82±1.32, and 5.23±0.36 mg/kg, respectively (Table 2). The iron content in these samples were 140±10mg/kg in control, 58.23±6.41 mg/kg in 1920GR, 96.7±14.85 mg/kg in 1950GR, and 75.7±3.51 mg/kg in 2289GR (Table 2). The amount of copper was 26±0.7 mg/kg in control, 6.43±1.16 mg/kg in 1920GR, 7.08±1.41 mg/kg in 1950GR, and 7.12±0.18 mg/kg in 2289GR (Table 2). The manganese content in these samples were 49±4.2, 18.77±0.77, 29.4±4.98, and 33.5±2.70 mg/kg in the control, 1920GR, 1950GR, and 2289GR, respectively (Table 2). The amounts of zinc, iron, copper, and manganese in the control soil samples were significantly higher than those of the treated soil samples (1920GR, 1950GR, and 2289GR) (Table 2). It may indicate a higher nutrient absorption of treated plants when compared to the control sample.

The pH of the native soil samples 1920, 1950, and 2289 were 6.58±0.26, 6.24±0.26, and 6.39±0.15, respectively (Table 1). The EC of the native soil samples were 0.19±0.01, 0.24±0.01, and 0.125±0.005 µS/cm in 1920, 1950, and 2289, respectively (Table 1). The pH and EC of the control plants propagated via seedlings were 8.38±0.06 and 366±13.32 µS/cm (Table 2). The pH and EC of the soil samples from the native soil-treated plants in the greenhouse were measured. It was 8.65±0.08 and 303.2±25.72 µS/cm for 1920GR, 8.50±0.11 and 348.2±34.36 µS/cm for 1950GR, and 8.76±0.08 and 340.25±37.95 µS/cm for 2289GR, respectively (Table 2).

3.2 Effect of native soil on the growth of snowbrush ceanothus

3.2.1 *Cutting propagation*

The snowbrush ceanothus cuttings were checked for callus formation and rooting after 6-8 weeks of incubation in the mist bench. Once rooting was established, the cuttings were transplanted into a potting soil that contained a mixture of peat moss (75%), vermiculite (13%), and rice husks (12%). The initial results of this study showed denser and longer roots in treatment than in control (Fig 4). The percentages of callus formation, rooting, and survival rate was recorded in both June and July (Fig 5). The results revealed that in the June experiment, the callus formation was significantly greater in control (31%) than in treatment (3%). The results revealed that in the June experiment, the callus formation was significantly greater in control (31%) than in treatment (3%). However, no significant difference was noted for the rooting between control (4%) and treatment (5%). But the survival of the treated cuttings (33%) was greater than control (20%) (Fig 5 a and c). It showed that even though the callusing was higher in the control cuttings, survival of these cuttings were lower than those treated with native soils.

In July, the callus formation was greater in control (15%) than in the treatment (10%). But the rooting and survival percentage was significantly greater in treatment (9.5 and 50%) than in the control cuttings (3 and 0%) (Fig 5 b and d). It indicates the effect of native soil on the rooting and survival of snowbrush ceanothus cuttings under greenhouse conditions. The increased rooting and survival percentage in treatment cuttings than in control may be due to beneficial bacterial populations in the native soil.

3.2.2 *Effect of native soil on snowbrush ceanothus seedlings*

To investigate the effect of microbes in the native soil on the growth and development of plants, snowbrush ceanothus seedings were treated with 200 ml of native soil from 1920, 1950, or 2289 sites. The control plants were not treated with native soil. The results from this study showed increased biomass of the treated plants compared to the control. The native soil-treated snowbrush ceanothus plants showed a 2.3-fold increase in the number of secondary shoots compared to control plants (Table 3). The results of the plant width revealed a significant increase of 1.3-fold in the plants inoculated with native soil compared to control. The number of leaves was also higher in the treated plants compared to the control (Table 3). Almost all plants treated with native soil showed nodule formation, and none of the control plants had any nodulation (Fig 6 and 7). The plants inoculated with soil collected from 1920 or 2289 sites showed 100% nodulation, and the plants treated with soil collected from 1950 site showed 83% nodulation (Table 4). Chi-square analysis revealed that the nodulation maybe due to the native soil inoculation. The average diameter of nodules was the highest in 1950GR (1.37 ± 0.16 cm), the diameter of the largest nodule was 1920GR (2.7 cm), and 2289GR had the highest average number of nodules per plant (Table 4). Rootball (when the root of a plant forms a mass with the surrounding soil) of the control and treated plants show a better representation of the results (Fig 6 and 7). A close-up microscopic image of the nodules showed the size and shapes of the nodules (Fig 8 and 9).

4. Discussion

The metagenomic analysis of the native soil samples showed the dominance of the bacteria belonging to the phyla *Proteobacteria* and *Actinobacteria* along with *Firmicutes*,

Acidobacteria, *Verrucomicrobia*, and *Bacteroidetes.* Over the years, various PGPRs have been identified in these phyla. *Actinobacteria* is a gram-positive bacterium known to produce antibiotics and other metabolites. They help in phytohormone production, nutrient acquisition, removal of contaminants, and pathogen suppression via induction or competition of plant defense responses. *Actinobacteria* are most effective when they are in an endophytic relationship with plants (Passari et al., 2015).

Firmicutes and *Proteobacteria* are also predominantly found in the rhizospheric bacterial population (Drogue et al., 2012; Lugtenberg and Kamilova, 2009). They enhance nutrition via nitrogen fixation, siderophore production, phosphate solubilization, and degradation of petroleum hydrocarbons (Richardson et al., 2009). *Proteobacteria* is a phylum containing gram-negative bacteria and consists of many examples of PGPR (Hou et al., 2015).

Bacteroidetes is another gram-negative phylum commonly found in the soil and contains several PGPR (Hou et al., 2015). They have been isolated from compost, decaying plant materials, freshwater, marine water, activated sludge, and dairy products (Bernardet and Nakagawa, 2006; Reichenbach, 2006). They have also been found in soil-associated microorganisms and detected in the greenhouse soil (Kim et al., 2006), cultivated fields (Borneman et al., 1996; Martínez-Alonso et al., 2010), and unexplored areas (Buckley and Schmidt, 2003; Zhou et al., 2009). *Bacteroidetes* are known to degrade complex organic matter, especially proteins and polysaccharides in the environment (Church, 2008). They can also act as biopolymers and serve as an energy or carbon source for plants, algae, and animal compounds.

The phyla *Acidobacteria* is widespread possessing in the soil environment in plethora. It has been coined “difficult to culture” and associated with plants. The bacteria belonging to *Acidobacteria* have successful plant interactions with *Arabidopsis thaliana* and improved the plant’s growth (Chaparro et al., 2014). The *Acidobacteria* diversity in the soil is associated with plant secretions (Mao et al., 2014) and the plant-root relationship (Chaparro et al., 2014; da Rocha et al., 2010).

The genus-level classification revealed the abundance of plant growth-promoting bacteria in the rhizosphere belonging to *Streptomyces, Agrobacterium, Bradyrhizobium, Mesorhizobium, Devosia, Pseudomonas, Rhizobium, Rhodoplanes,* and *Mycobacterium*. Various species from these genera promote plant growth in many instances. *Streptomyces* is an essential PGPR that majorly produces antibiotics, siderophores, acts as a biocontrol agent, and possesses antimicrobial activity (Charousová et al., 2015; David et al., 1980; Etesami and Glick, 2020a; Rothrock and Gottlieb, 1981). Another widely studied group of PGPR that belongs to the genus is *Pseudomonas*. The PGPRs of the *Pseudomonas* promote plant growth through various activities. These activities include nitrogen fixation, siderophore production, phosphate solubilization, heavy metal tolerance, psychrotolerant, and/or phytohormone production (Babu et al., 2015a; Egamberdieva, 2012; Gu et al., 2020c, 2020a; Strano et al., 2017; Zhang et al., 2021). The PGPR belonging to *Bradyrhizobium, Mesorhizobium, Devosia,* and *Rhizobium* genera are known to have plant growth-promoting activities, such as phosphate solubilization, siderophore production, and/or phytohormone production, and in particular to fix nitrogen by many studies (Muresu et al., 2019; Rai and Nabti, 2017; Wdowiak-Wróbel et al., 2017; Wolińska et al., 2017). *Rhodoplanes* and *Mycobacterium* also possess PGPR characteristics like IAA production,

biocontrol activity, nitrogen fixation, etc. (Ahemad and Kibret, 2014; Gkarmiri et al., 2017; Karmakar et al., 2021).

The genus-level classification revealed in the endosphere samples showed the abundance of plant growth-promoting bacteria such as *Frankia, Streptomyces, Flavobacterium, Bradyrhizobium, Janthinobacterium, Hyphomicrobium, Mesorhizobium, Rhizobium, Devosia*, and *Staphylococcus*. *Janthinobacterium* species impart cold tolerance and produce anti-fungal compounds (Baitchman and Herman, 2015a; Haack et al., 2016; Sharma et al., 2015). *Staphylococcus* species helps in IAA, ACC deaminase and ammonia production, N-fixation, and phosphate solubilization (Biswas et al., 2018; Orhan, 2016). *Frankia* is a well-known genus for PGPR. These bacteria have the ability for auxin and cytokinin biosynthesis, siderophore production, and biological nitrogen fixation and help in alleviating biotic and abiotic stresses (Nouioui et al., 2019). *Flavobacterium* is another well-known genus containing various PGPRs. These bacteria can solubilize phosphate, produce siderophores, salicylic acid, auxin, hydrogen cyanide, and chitinase production (Soltani et al., 2010). *Hyphomicrobium* helps in bioremediation and has known to be a disease repellant (Franke-Whittle et al., 2015; He et al., 2020).

PGPRs support plant growth by supplying nutrients and by augmenting their availability in soil (Vejan et al., 2016). Nitrogen is one of the most important nutrients in plants. It helps plants during seed germination, branching, and rooting (Fredes et al., 2019). The presence of PGPRs improves plant nutrient uptake, such as nitrogen (Lee 2020). The metagenomic study of the native soil of snowbrush ceanothus revealed the presence of several PGPRs. Hence, the native soil inoculation could be one of the reasons behind the improved growth in the plants treated with native soil compared to the control.

Phosphorus is the second most important nutrient (Oteino, 2015). Phosphorus is usually present in abundance in soils as inorganic phosphorus but in an insoluble form (Miller, 2009). The limited availability of phosphorus in the soil affects the growth of plants (Oteino, 2015). The presence of phosphate solubilizing bacteria in soil mineralizes these insoluble phosphates into available phosphorus. *Pseudomonas* sp. is one such bacteria that mineralizes enzyme-mediated inorganic phosphates (Miller, 2009). The results of our study indicated a higher percentage of inorganic phosphate in the control soil compared to the plants treated with native soil. It may indicate an abundance of phosphate solubilizing bacteria (PSB) in the soil of native soil-treated plants.

Ceanothus velutinus is a native plant that is hard to propagate (Rupp and Wheaton, 2014). The cuttings of snowbrush ceanothus were inoculated with the native soil to investigate the effect of PGPR present in the native soil on the cutting propagation (Habibi et al., 2014b; Príncipe et al., 2007). The metagenomic study showed the presence of several PGPRs in the native soil samples. So, the soil was collected from the snowbrush ceanothus plants from Tony Grove and directly mixed with the greenhouse soil mixture to check its effect on rooting and survival of snowbrush ceanothus cuttings. The native soil-treated cuttings showed longer and denser roots visually. The cuttings treated with native soil showed a higher survival rate (33% and 50% in June and July, respectively). These results indicate that the PGPRs found in the native soil in the metagenomic study colonized and helped in the rooting and survival in the treated cuttings. The control cuttings did not have any PGPRs. Previous studies showed that PGPR promotes root growth by indole acetic acid production (IAA) (Grover et al., 2021). IAA is an auxin that regulates root growth and architecture (Grover et al., 2021; Vacheron et al., 2013).

Native soil is in commercial use as a bio-fertilizer that provides all the necessary nutrients and promotes plant growth (https://www.upcycleandcompany.com/). Native soil not only contains PGPRs that increase nutrient uptake but also harbors many bacteria that stimulate plant growth and may act as bio-stimulants (Habibi et al., 2014b; Príncipe et al., 2007). Bio-stimulants are microorganisms that enhance existing processes, improve nutrient uptake, increase tolerance to abiotic stresses and improve plant growth (Calvo et al., 2014). Bio-fertilizers contain any living organisms that enhance plant growth by increasing nutrient availability. That might have been one of the prime reasons the native soil inoculated plants showed an improved overall growth over control (no native soil). Many PGPRs produce gibberellins and volatile organic compounds (VOCs) that promote plant growth by enhancing shoot growth and increasing shoot biomass (Backer et al., 2018), and may increase shoot length and diameter (Karakurt and Aslantas, 2010; Karlidag et al., 2007). In this study, the number of secondary shoots, number of leaves, and plant width were more in the plants treated with native soil compared to control. These results may indicate the overall improvement in the growth of treated plants is due to the presence of the PGPRs in the native soil inoculum.

Ceanothus velutinus is an actinorhizal plant that forms a symbiotic relationship with bacteria and can fix atmospheric nitrogen. This shrub is known to form root nodules containing *Frankia* (Baker and Mullin, 1994; Delwiche et al., 1965). In this study, *Frankia* was observed in the native soil, endosphere samples, and greenhouse samples but was absent in control soil. We observed nodulation in almost all the native soil-treated plants but not in the control plants. Apart from *Frankia*, many other nitrogen-fixing bacteria such as *Rhizobium*, *Mesorhizobium*, and *Bradyrhizobium* were present in the plants treated with

native soil (Fig 2.b. and 3.b.). These bacteria induce nodule formation in the roots of plants as a result of plant-microbe symbiosis (González et al., 2019; Jarvis et al., 1982a; Pallardy, 2008; Wang et al., 2018; Wdowiak-Wróbel et al., 2017). Thus, the nodule formation in the plants can be any of the bacteria that form a symbiosis with snowbrush ceanothus roots. To our knowledge, this is the first report of nodule formation in *Ceanothus velutinus* under greenhouse conditions. Further research on the isolation of bacteria from these nodules will shed more light on bacteria responsible for nodulation.

5. Conclusions

The metagenomic study of native soil and native soil-treated plants provided information on the presence of many essential PGPRs. The treatment of greenhouse-grown plants with native soil revealed the effect of the PGPRs in the native soil on the rooting, survival, growth, and. This is the first observation for snowbrush ceanothus plants under greenhouse conditions forming nodules with native soil treatment. The PGPRs from the snowbrush ceanothus can be isolated and tested for their role in plant growth promotion on model and crop plants and for use in snowbrush ceanothus propagation by cuttings. This study helps identify the beneficial microbes that may be used as biofertilizers/bio-stimulants in sustainable organic agriculture. This study also generated resources for further research in the microbiome of native plants for sustainable landscapes.

Table 1. Macro and micronutrient concentrations, pH, EC, and soil moisture in the native soil samples.

Sample	Carbon (%)	Nitrate nitrogen (mg/kg)	Phosphorus (µg/g)	Potassium (mg/kg)	Zinc (mg/kg)	Iron (mg/kg)	Copper (mg/kg)	Manganese (mg/kg)	Soil Moisture (%)	pH	EC (µS/cm)
1920	2.2a*	2.7a	31.6a	469.5a	1.8a	70.4a	1.4a	19.9a	25.7a	6.6a	0.2a
1950	24.8b	16.9a	6.0a	600.5a	2.2ab	83.9a	0.8a	26.7b	30.2a	6.2a	0.2a
2289	12.8c	1.9a	34.8a	485.5a	2.6b	74.1a	1.0a	18.5a	32.1a	6.4a	0.1b

*Same letters within a column denote no significance among different samples by Tukey's method for multiplicity at $\alpha = 0.05$.

Table 2. Macro and micronutrient concentrations, pH, and EC in the greenhouse growing medium from the plants propagated from seedlings and treated with and without native soil (treatment and control samples).

Sample	Carbon (%)	Nitrate nitrogen (mg/kg)	Phosphorus (mg/kg)	Potassium (mg/kg)	Zinc (mg/kg)	Iron (mg/kg)	Copper (mg/kg)	Manganese (mg/kg)	pH	EC (µS/cm)
Control	64.2a*	5.3a	50.6a	968.0a	15.2a	140.0a	26.0a	49.0a	8.4a	366.0a
1920GR	7.0b	1.0b	12.3b	481.3b	2.8b	58.2b	6.4b	18.8b	8.7a	303.2a
1950GR	28.9c	3.1a	15.8b	608.3b	5.8b	96.7b	7.1b	29.4b	8.5a	348.2a
2289GR	22.3c	4.4a	12.2b	519.7b	5.2b	75.7b	7.1b	33.5ab	8.8a	340.3a

*Same letters within a column denote no significance among different samples by Tukey's method for multiplicity at $\alpha = 0.05$

Table 3. Growth parameters measured from the seedlings treated with and without native soil (treatment and control samples)

Sample	Growth index	Number of leaves	Chlorophyll content	Chlorophyll fluorescence	Number of primary shoots	Number of secondary shoots
Control	5.2a*	32a	37.4a	0.7a	1a	**8b**
1920GR	6.1a	78a	43.6a	0.7a	1a	**18a**
1950GR	6.3a	84a	43.5a	0.8a	1a	**20a**
2289GR	6.5a	59a	36.9a	0.7a	1a	**20a**

*The same letters within a column denote no significance among different samples by Tukey's method for multiplicity at $\alpha = 0.05$.

Table 4. Nodulation percentage, average diameter of nodules, diameter of the largest nodule, and average number of nodules of snowbrush ceanothus seedlings and treated with and without native soil (treatment and control samples)

Sample	Nodulation%	Average diameter of nodules (cm)	Diameter of the largest nodule (cm)	Average number of nodules per plant
Control	0b*	0b	0b	0b
1920GR	100a	1.1a	2.7a	3b
1950GR	83.33a	1.4a	2.4a	2b
2289GR	100a	1.1a	2.4a	31a

*Same letters within a column denote no significance among different samples by Tukey's method for multiplicity at $\alpha = 0.05$.

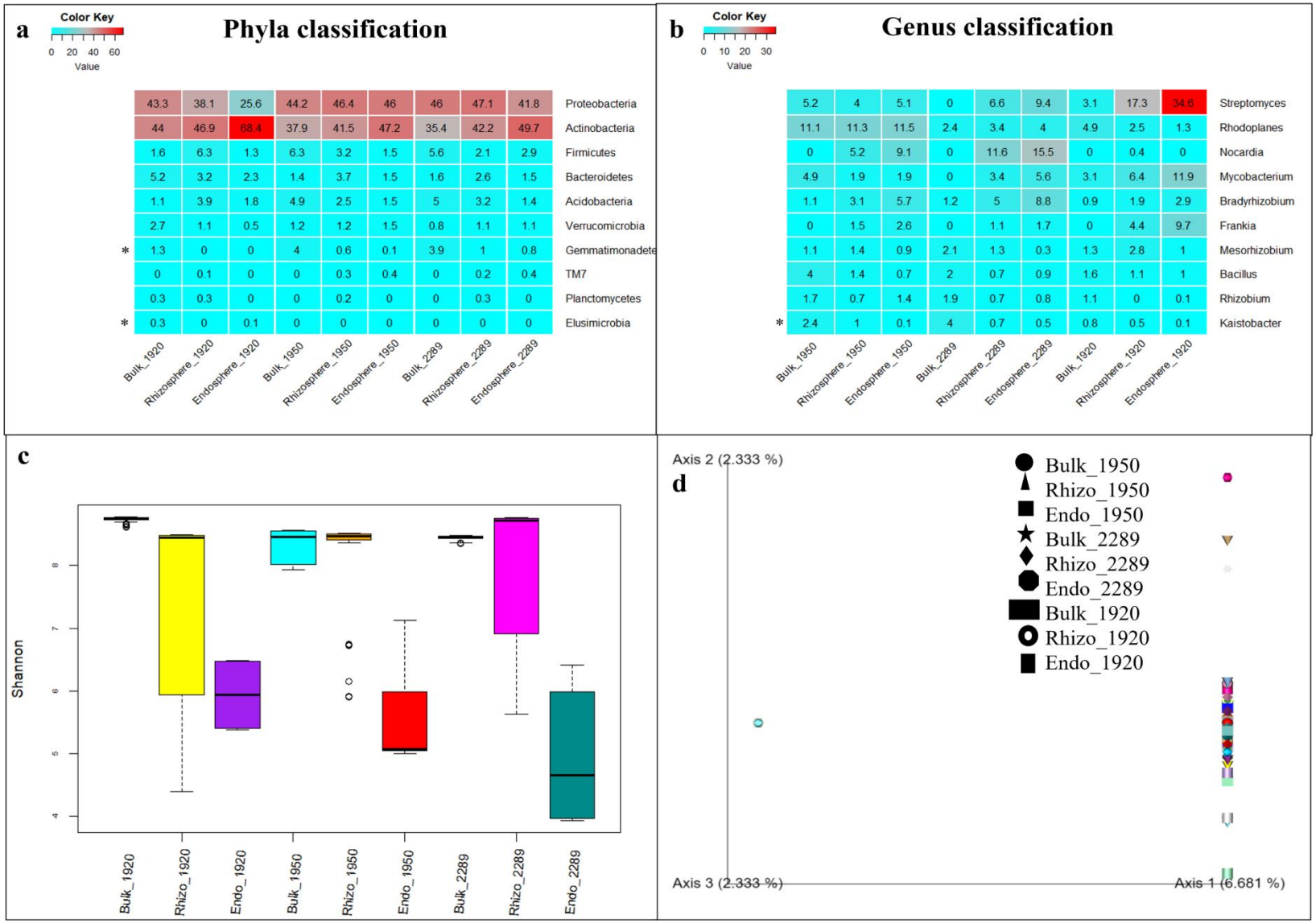
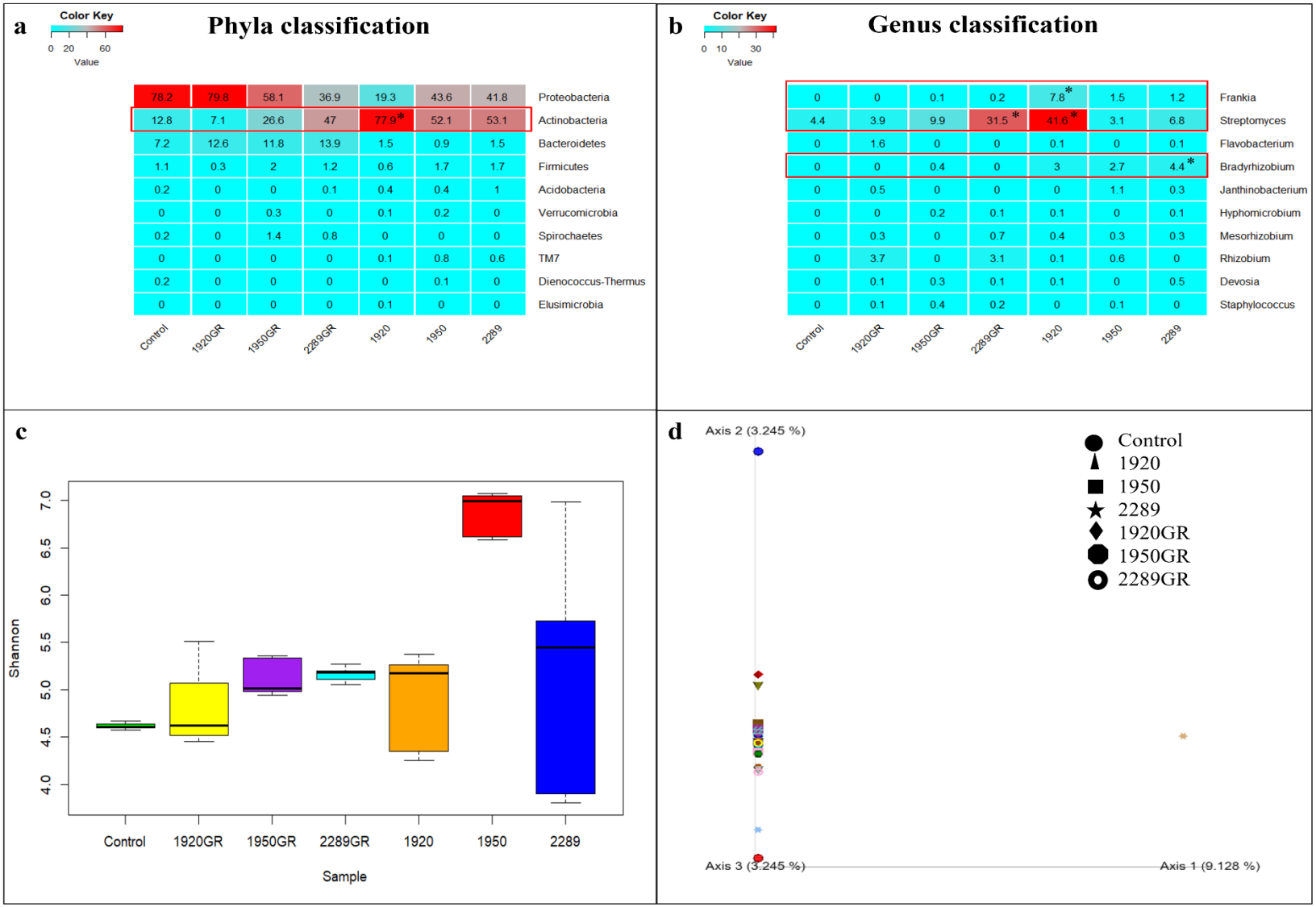

a
Color Key
0 20 40 60
Value
Phyla classification
43.3 38.1 25.6 44.2 46.4 46 46 47.1 41.8 Proteobacteria
44 46.9 68.4 37.9 41.5 47.2 35.4 42.2 49.7 Actinobacteria
1.6 6.3 1.3 6.3 3.2 1.5 5.6 2.1 2.9 Firmicutes
5.2 3.2 2.3 1.4 3.7 1.5 1.6 2.6 1.5 Bacteroidetes
1.1 3.9 1.8 4.9 2.5 1.5 5 3.2 1.4 Acidobacteria
2.7 1.1 0.5 1.2 1.2 1.5 0.8 1.1 1.1 Verrucomicrobia
* 1.3 0 0 4 0.6 0.1 3.9 1 0.8 Gemmatimonadete
0 0.1 0 0 0.3 0.4 0 0.2 0.4 TM7
0.3 0.3 0 0 0.2 0 0 0.3 0 Planctomycetes
* 0.3 0 0.1 0 0 0 0 0 0 Elusimicrobia
Bulk_1920 Rhizosphere_1920 Endosphere_1920 Bulk_1950 Rhizosphere_1950 Endosphere_1950 Bulk_2289 Rhizosphere_2289 Endosphere_2289
b
Color Key
0 10 20 30
Value
Genus classification
5.2 4 5.1 0 6.6 9.4 3.1 17.3 34.6 Streptomyces
11.1 11.3 11.5 2.4 3.4 4 4.9 2.5 1.3 Rhodoplanes
0 5.2 9.1 0 11.6 15.5 0 0.4 0 Nocardia
4.9 1.9 1.9 0 3.4 5.6 3.1 6.4 11.9 Mycobacterium
1.1 3.1 5.7 1.2 5 8.8 0.9 1.9 2.9 Bradyrhizobium
0 1.5 2.6 0 1.1 1.7 0 4.4 9.7 Frankia
1.1 1.4 0.9 2.1 1.3 0.3 1.3 2.8 1 Mesorhizobium
4 1.4 0.7 2 0.7 0.9 1.6 1.1 1 Bacillus
1.7 0.7 1.4 1.9 0.7 0.8 1.1 0 0.1 Rhizobium
* 2.4 1 0.1 4 0.7 0.5 0.8 0.5 0.1 Kaistobacter
Bulk_1950 Rhizosphere_1950 Endosphere_1950 Bulk_2289 Rhizosphere_2289 Endosphere_2289 Bulk_1920 Rhizosphere_1920 Endosphere_1920
c
Shannon
4 5 6 7 8
Bulk_1920 Rhizo_1920 Endo_1920 Bulk_1950 Rhizo_1950 Endo_1950 Bulk_2289 Rhizo_2289 Endo_2289
d
Axis 2 (2.333 %)
Axis 3 (2.333 %)
Axis 1 (6.681 %)
Bulk_1950
Rhizo_1950
Endo_1950
Bulk_2289
Rhizo_2289
Endo_2289
Bulk_1920
Rhizo_1920
Endo_1920

Fig 2. Percent read abundance at the phyla (a) and genus (b) level, and alpha (c) and beta (d) diversity of the bulk soil, rhizosphere, and endosphere samples of snowbrush ceanothus from the native habitat (1920, 1950, and 2289)

*Denotes significantly different bulk soil from rhizosphere or endosphere soils by Tukey's method for multiplicity at $\alpha = 0.05$.

Fig 1. Percent read abundance at the phyla (a) and genus (b) level, and alpha (c) and beta (d) diversity of the endosphere samples of snowbrush ceanothus from the native habitat (1920, 1950, and 2289) and the greenhouse (Control, 1920GR, 1950GR, and 2289GR).

*Denotes significance in the native sample from control by Tukey's method for multiplicity at $\alpha = 0.05$.


a
Color Key
0 20 60
Value
Phyla classification
78.2 79.8 58.1 36.9 19.3 43.6 41.8 Proteobacteria
12.8 7.1 26.6 47 77.9* 52.1 53.1 Actinobacteria
7.2 12.6 11.8 13.9 1.5 0.9 1.5 Bacteroidetes
1.1 0.3 2 1.2 0.6 1.7 1.7 Firmicutes
0.2 0 0 0.1 0.4 0.4 1 Acidobacteria
0 0 0.3 0 0.1 0.2 0 Verrucomicrobia
0.2 0 1.4 0.8 0 0 0 Spirochaetes
0 0 0 0 0.1 0.8 0.6 TM7
0.2 0 0 0 0 0.1 0 Dienococcus-Thermus
0 0 0 0 0.1 0 0 Elusimicrobia
Control 1920GR 1950GR 2289GR 1920 1950 2289
b
Color Key
0 10 30
Value
Genus classification
0 0 0.1 0.2 7.8* 1.5 1.2 Frankia
4.4 3.9 9.9 31.5* 41.6* 3.1 6.8 Streptomyces
0 1.6 0 0 0.1 0 0.1 Flavobacterium
0 0 0.4 0 3 2.7 4.4* Bradyrhizobium
0 0.5 0 0 0 1.1 0.3 Janthinobacterium
0 0 0.2 0.1 0.1 0 0.1 Hyphomicrobium
0 0.3 0 0.7 0.4 0.3 0.3 Mesorhizobium
0 3.7 0 3.1 0.1 0.6 0 Rhizobium
0 0.1 0.3 0.1 0.1 0 0.5 Devosia
0 0.1 0.4 0.2 0 0.1 0 Staphylococcus
Control 1920GR 1950GR 2289GR 1920 1950 2289
c
Shannon
4.0 4.5 5.0 5.5 6.0 6.5 7.0
Control 1920GR 1950GR 2289GR 1920 1950 2289
Sample
d
Axis 2 (3.245 %)
Control
1920
1950
2289
1920GR
1950GR
2289GR
Axis 3 (3.245 %)
Axis 1 (9.128 %)

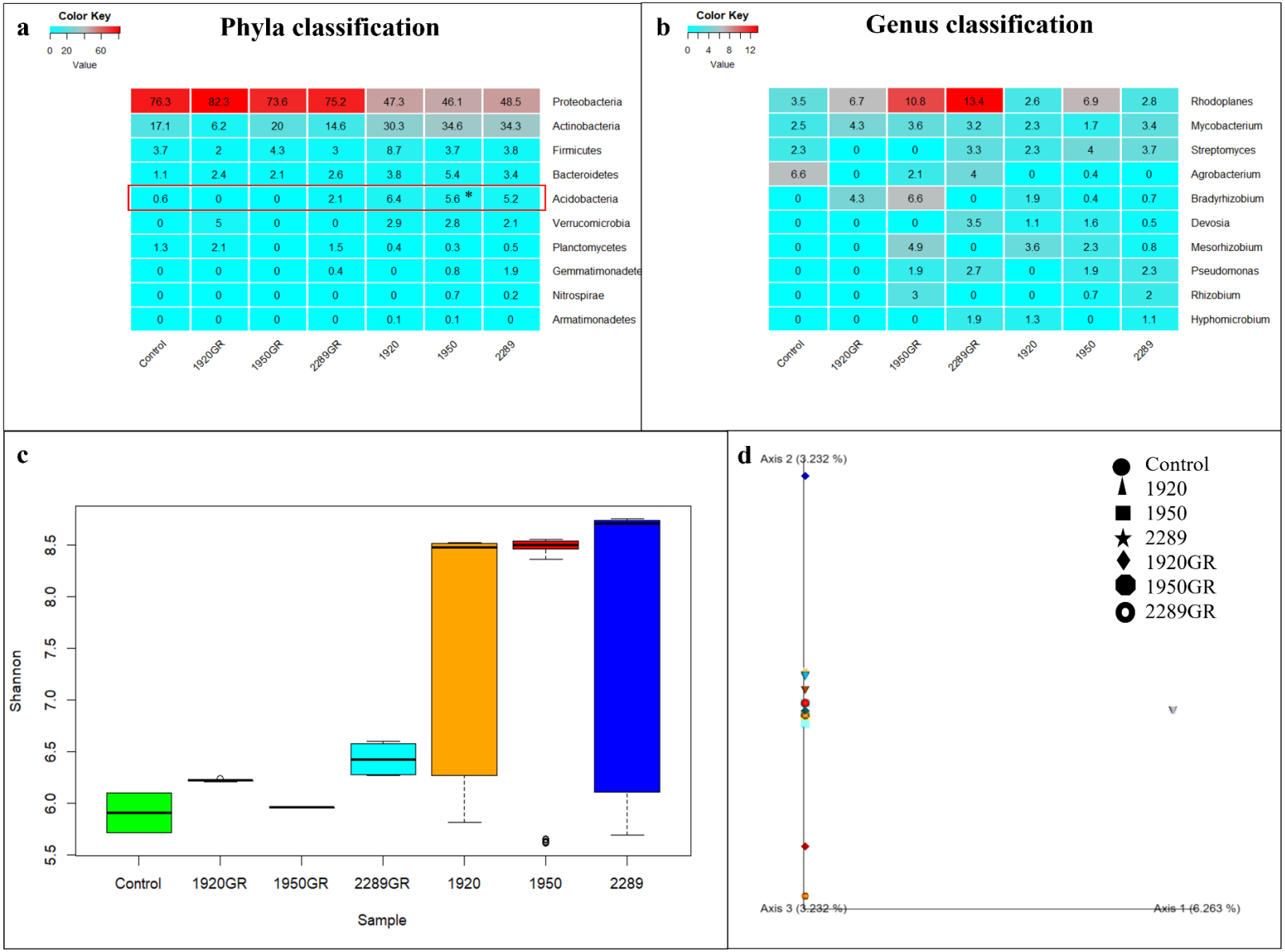

a
Color Key
0 20 60
Value
Phyla classification
76.3 82.3 73.6 75.2 47.3 46.1 48.5 Proteobacteria
17.1 6.2 20 14.6 30.3 34.6 34.3 Actinobacteria
3.7 2 4.3 3 8.7 3.7 3.8 Firmicutes
1.1 2.4 2.1 2.6 3.8 5.4 3.4 Bacteroidetes
0.6 0 0 2.1 6.4 5.6 * 5.2 Acidobacteria
0 5 0 0 2.9 2.8 2.1 Verrucomicrobia
1.3 2.1 0 1.5 0.4 0.3 0.5 Planctomycetes
0 0 0 0.4 0 0.8 1.9 Gemmatimonadete
0 0 0 0 0 0.7 0.2 Nitrospirae
0 0 0 0 0.1 0.1 0 Armatimonadetes
Control 1920GR 1950GR 2289GR 1920 1950 2289
b
Color Key
0 4 8 12
Value
Genus classification
3.5 6.7 10.8 13.4 2.6 6.9 2.8 Rhodoplanes
2.5 4.3 3.6 3.2 2.3 1.7 3.4 Mycobacterium
2.3 0 0 3.3 2.3 4 3.7 Streptomyces
6.6 0 2.1 4 0 0.4 0 Agrobacterium
0 4.3 6.6 0 1.9 0.4 0.7 Bradyrhizobium
0 0 0 3.5 1.1 1.6 0.5 Devosia
0 0 4.9 0 3.6 2.3 0.8 Mesorhizobium
0 0 1.9 2.7 0 1.9 2.3 Pseudomonas
0 0 3 0 0 0.7 2 Rhizobium
0 0 0 1.9 1.3 0 1.1 Hyphomicrobium
Control 1920GR 1950GR 2289GR 1920 1950 2289
c
Shannon
5.5 6.0 6.5 7.0 7.5 8.0 8.5
Control 1920GR 1950GR 2289GR 1920 1950 2289
Sample
d
Axis 2 (3.232 %)
Axis 3 (3.232 %)
Axis 1 (6.263 %)
Control
1920
1950
2289
1920GR
1950GR
2289GR

Fig 3. Percent read abundance at the phyla (a) and genus (b) level, and alpha (c) and beta (d) diversity of the rhizosphere samples of snowbrush ceanothus from the native habitat (1920, 1950, and 2289) as well as from the greenhouse (Control, 1920GR, 1950GR, and 2289GR)

*Denotes significance in native sample from control by Tukey's method for multiplicity at $\alpha = 0.05$.

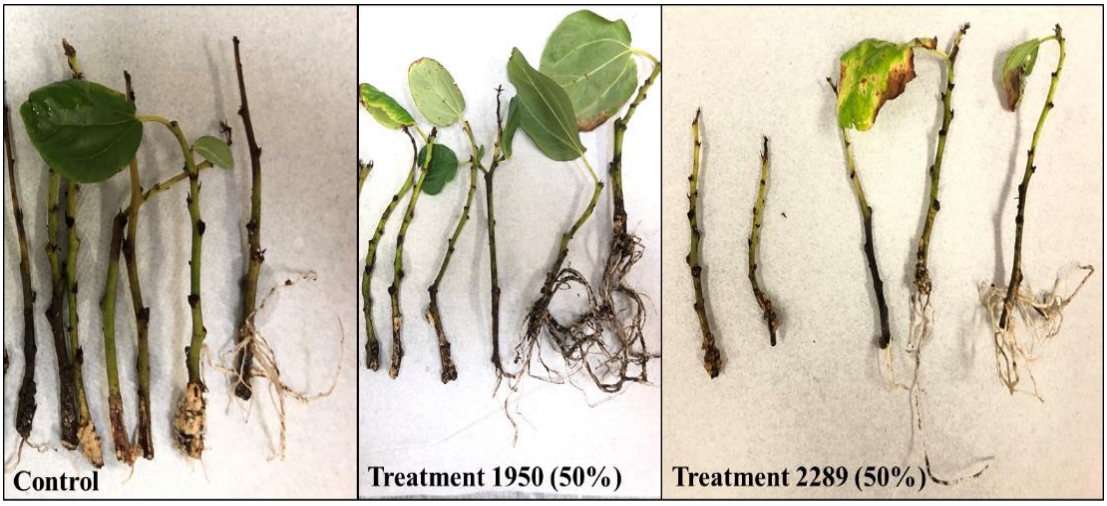


Fig 4. Callus formation and rooting in snowbrush ceanothus cuttings treated with and without native soil.

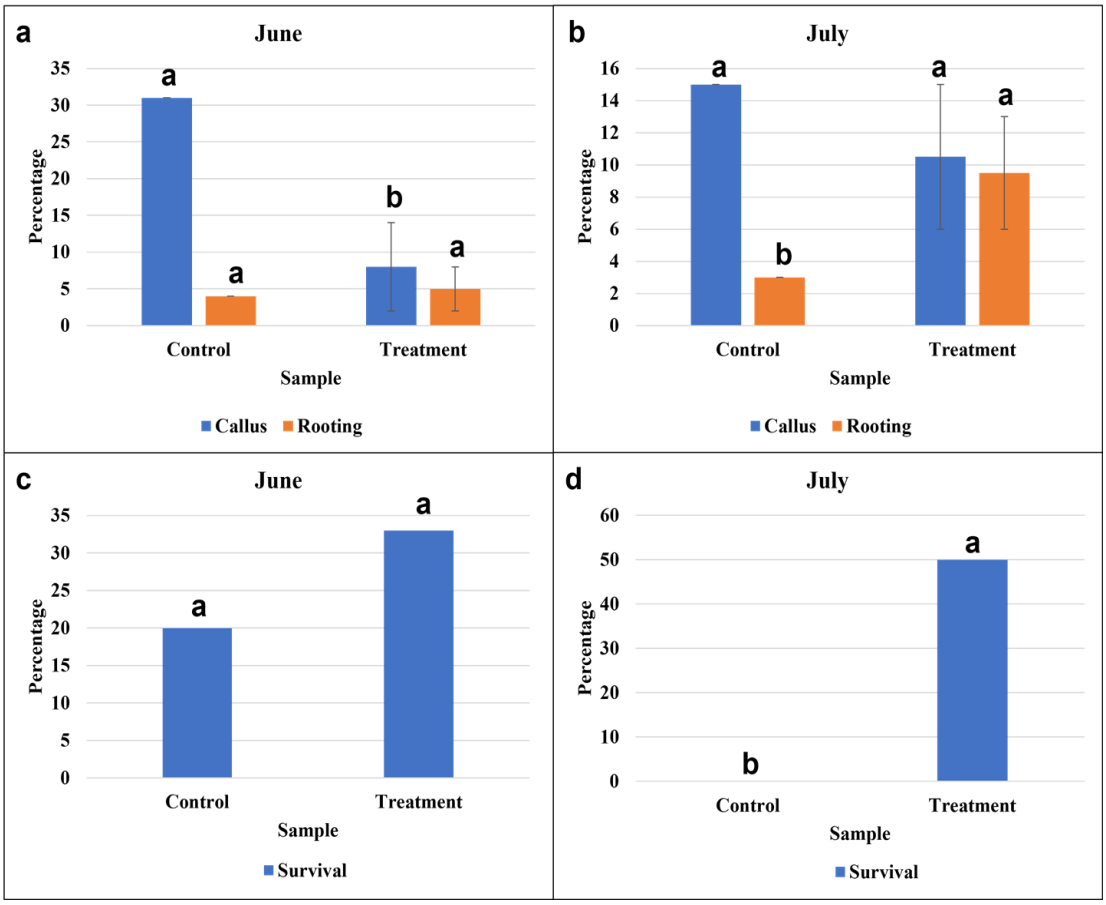


Fig 5. Callusing, rooting percentage, and survival of control and treatment snowbrush ceanothus cuttings in June (a and c) and July (b and d)

Same letters denote no significance among callus, rooting, and survival by Tukey's method for multiplicity at $\alpha = 0.05$.

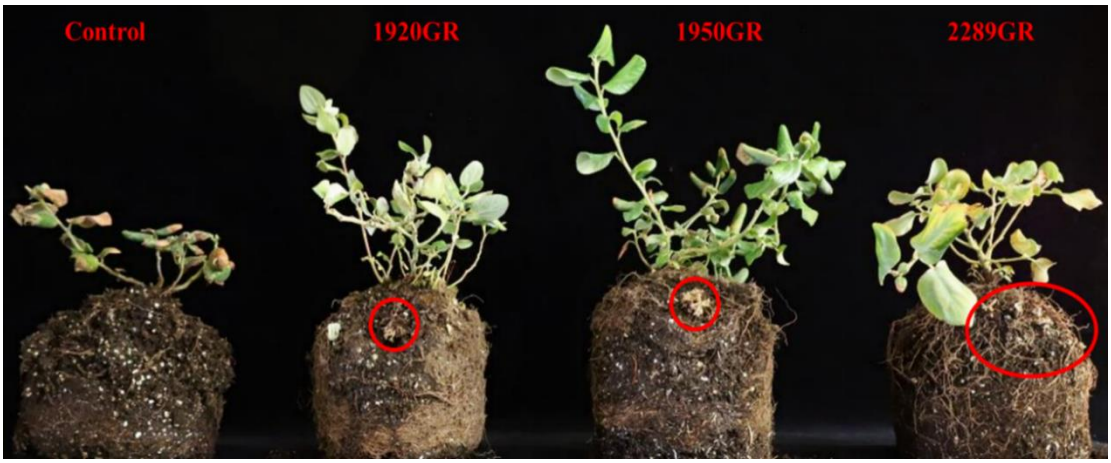


Fig 6. Rootball of snowbrush ceanothus seedling treated with and without native soil (1920GR, 1950GR, 2289GR and Control).

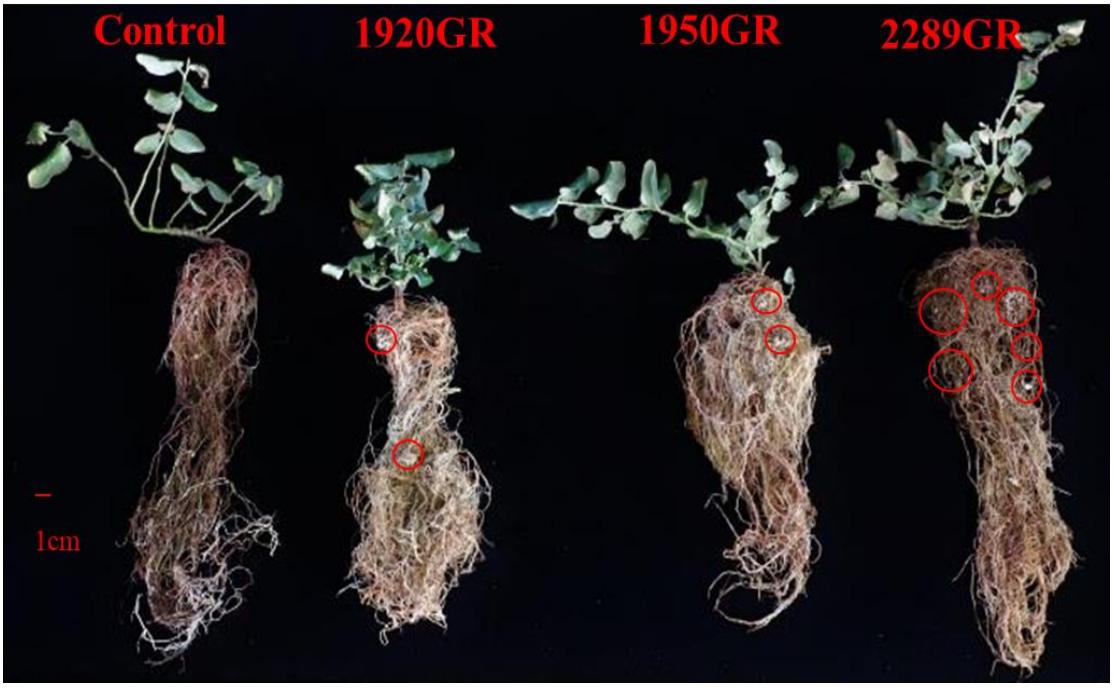


Fig 7. Visual representation of nodulation in the control and treated plants of snowbrush ceanothus seedlings under greenhouse conditions.

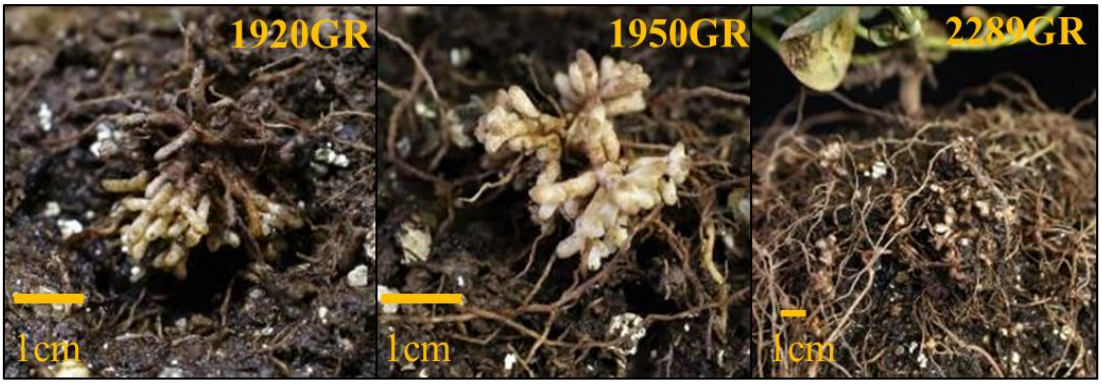


Fig 8. A close-up of the nodules observed in the snowbrush ceanothus plants treated with native soil.

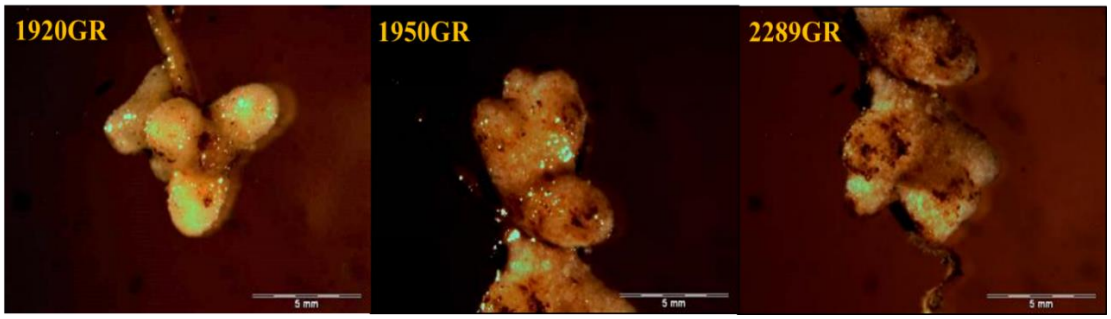


Fig 9. Microscopic view of the nodules found in the snowbrush ceanothus seedlings treated with native soil (1920GR, 1950GR, and 2289GR).

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CHAPTER III

EXPLORING THE PLANT GROWTH PROMOTING POTENTIAL OF THE RHIZOSPHERE BACTERIA FROM *CEANOTHUS VELUTINUS*- NATIVE TO INTERMOUNTAIN WEST

Abstract

The rhizosphere microbiome of a plant plays a significant role in the plant's growth and development. They promote the plant's overall health by nutrient uptake/availability, stress tolerance, bio-control activity, etc. In this study, we have isolated 64 bacterial colonies from the rhizosphere of snowbrush ceanothus plants from the native location and the native soil-treated plants in the greenhouse study. We also conducted a metagenomic study in snowbrush ceanothus plants treated with native soil under greenhouse conditions. Out of the 64 isolates, 27 were from the native habitat, and 37 were from the greenhouse conditions. These isolates were characterized based on their morphology and gram stain. They were also tested for plant growth-promoting activities, such as catalase, siderophore, and indole acetic acid production, to fix nitrogen and, solubilize phosphate. Fourteen bacterial isolates tested positive for all plant growth-promoting abilities tested in this study. The isolates belonging to the genera *Pantoea*, *Pseudomonas,* and *Ancylobacter* tested positive for all the PGPR traits tested in this study. These isolates can be tested for their plant growth-promoting ability in other modal and crop plants. Most of the bacterial isolates in this study have a great potential to become biofertilizers and bio-stimulants.

Highlights

- Rhizosphere microbiome promote plant growth and development.
- Metagenomic analysis of snowbrush ceanothus plants treated with and without native soil under greenhouse conditions
- Sixty-four bacterial colonies were isolated from the rhizosphere of snowbrush ceanothus plants and characterized for PGPR traits.
- Isolates identified to the genera *Pantoea*, *Pseudomonas*, and *Ancylobacter* have the potential for use as biofertilizers and bio-stimulants.

Keywords: PGPR (Plant Growth Promoting Rhizobacteria), Rhizosphere, Snowbrush ceanothus

1. Introduction

Microorganisms present in the rhizosphere communicate with plant roots and influence their processes. They play a significant role in plant health, nutrition, and yield (Habibi et al., 2014b). The roots secrete several substances into the rhizosphere that trigger microbial activity. Vitamins, hormones, enzymes, carbohydrates, flavonoids, volatile compounds, and organic acid are a few examples of these root exudates (Habibi et al., 2014a; Prescott et al., 2007). The diversity and amount of the rhizosphere microorganisms in a plant depend on the amount and type of root exudate secreted by plants (Söderberg et al., 2002). The soil type , soil depth, plant species, and environmental factors alter the rhizosphere microbial communities (Grayston et al., 1998; Kuske et al., 2002).

The rhizosphere soil shelters various beneficial microbes, such as plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi (AMF), microparasitic fungi, and protozoa (Meena et al., 2017b, 2017a; Meena et al., 2017). Plant growth-

promoting rhizobacteria are beneficial microorganisms that colonize around the roots and reside in the rhizosphere region of plants (Kumari et al., 2018). They enhance tolerance towards biotic and abiotic stresses such as salinity, heavy metals, drought, etc. (Selvakumar et al., 2012; Zolla et al., 2013). They also protect plants from phytopathogens, acting as bio-control agents (Mendes et al., 2011), and improve plant growth and development (Huang et al., 2014). PGPRs promote plant growth in two ways – direct and indirect mechanisms (Kumari et al., 2018). These direct mechanisms to promote plant growth are either the secretions of plant hormones such as auxins (indole acetic acid-IAA), cytokinins, gibberellins (GA_3), and ethylene (Bent et al., 2001; Chabot et al., 1996) or enhancing nutrient availability by nitrogen fixation, phosphate solubilization, other mineral solubilization such as potassium and zinc, production of ACC, (1-aminocyclopropane-1-carboxylate) deaminase enzyme, and siderophore production (Ahmad et al., 2008; Habibi et al., 2014a; Reyes et al., 2002). The indirect mechanism involves the suppression of infection by pathogenic bacteria, fungi, nematodes, and viruses (Barea et al., 2005) by acting as bio-control agents (Romero et al., 2007).

As mentioned, PGPR produces siderophores that promote plant growth. Siderophores are low molecular weight iron chelators. This secondary metabolite solubilizes iron and forms an iron-siderophore complex in its surrounding environment (Andrews et al., 2003). It chelates ferric ions with a high affinity and can be extracted and solubilized from most organic complexes (Wandersman and Delepelaire, 2004). Siderophore production by microbes in the soil can increase iron uptake in plants when a plant recognizes the iron-siderophore complex in the bacteria (C. O. Dimkpa et al., 2009). Siderophore production by PGPR gives an additional advantage to them to colonize around the roots over other microorganisms present in the same ecological

area (Haas and Défago, 2005). Siderophores produced by pseudomonads have a higher affinity to ferric ions (Sujatha and Ammani, 2013). A dominant siderophore-pyoverdine inhibited the growth of other bacteria and fungi that produce less potent siderophores in an iron-deficient media *in vitro* (Kloepper et al., 1980). Another study reported that siderophores produced by fluorescent pseudomonas suppress fungal pathogen growth (Beneduzi et al., 2012).

Plant growth-promoting rhizobacteria also helps in biological nitrogen fixation (BNF) that is an essential process for life. Biological nitrogen fixation helps by reducing atmospheric nitrogen into ammonia which is then biologically available for plants (Navarro-Noya et al., 2012). The nitrogenase enzyme drives the nitrogen fixation process. Nitrogen-fixing bacteria form a symbiotic relationship with the roots of the plant and form root nodules (Tamás et al., 2010). This symbiosis is a subset of plant growth-promoting rhizobacteria (PGPR). Plant growth-promoting rhizobacteria that can fix atmospheric nitrogen is commonly found in the phyla *Proteobacteria* and *Actinobacteria*. Some common nitrogen-fixing bacteria include *Azospirillum, Rhizobium, Paenibacillus*, and *Bacillus* (Ding et al., 2005; Mus et al., 2016a). These nitrogen-fixing bacteria can be isolated and characterized *in vitro* by growing them in a nitrogen-free medium (Reis et al., 2015). N-fixing bacteria such as *Rhizobium* promote plant growth by stimulating plant biomass and increasing nitrogen content and have been used as inoculants in biofertilizers (Bhattacharjee et al., 2008).

Another common PGPR trait is phosphate solubilization. Phosphorus in the soil can be present in the organic or inorganic form. Most of the phosphorus present in the soil are insoluble and hence are not available for the plant. The inorganic phosphorus in soil is frequently an insoluble mineral, and the organic forms are usually immobilized, and plants cannot absorb them as nutrition. The plants can take up the

only accessible form of phosphate (both organic and inorganic), such as orthophosphate (Rodríguez et al., 2006). Several studies have reported the ability of bacteria to solubilize inorganic phosphates. The most common genera of bacterial species that can solubilize phosphate are *Agrobacterium, Bacillus, Burkholderia, Flavobacterium, Micrococcus, Pseudomonas,* and *Rhizobium*. The rhizosphere soil contains a large population of phosphate solubilizing anaerobic and aerobic bacteria. The detection of these bacteria can be carried out *in vitro* using a plate screening technique. The bacterial species are grown in a media containing insoluble phosphorus, and those species that can solubilize it will show a clear halo around the colony. It is one way to characterize phosphate solubilizing microorganisms (Rodríguez and Fraga, 1999).

Some plant growth-promoting bacteria also act as bio-stimulants and help the plants deal with biotic and abiotic stresses and increase crop productivity. They also help in nitrogen fixation, humic substance production, and nutrient availability in soil, so plants have heightened access to nutrients (Silva et al., 2017). One of the ways is by indole acetic acid production. IAA is a dominant form of plant growth hormone auxin and is a secondary metabolite of L-tryptophan metabolism. Many PGPR species can also produce IAA that helps in root production by increasing lateral roots and the number of root hair. IAA also promotes cell elongation by tweaking certain conditions such as increasing cell permeability of water, decreasing the pressure in the cell wall, increasing cell wall synthesis and osmotic contents in the cell (Mohite, 2013). Previous studies have shown a higher potential to produce IAA by the microorganisms isolated from the rhizosphere than the surrounding soil (Bal et al., 2013).

According to previous literature, the majority of the PGPR species belonged to the genera- *Arthrobacter, Azospirillum, Bacillus, Burkholderia, Erwinia, Flavobacterium, Pseudomonas, Rhizobium,* and *Serratia*, etc. (Orhan et al., 2006).

In this study, we have isolated the potential plant growth-promoting bacteria from the rhizosphere of *Ceanothus velutinus*- a native plant, commonly known as snowbrush ceanothus. Snowbrush ceanothus is an evergreen plant native to western North America from British Colombia and Alberta to Utah, Colorado, and California. It is an ornamental and medicinal plant as Native Americans used it to treat pain, flu, and gonorrhea (Francis, 2009). Snowbrush ceanothus is also capable of nitrogen fixation and heat resistance (Stein et al., 2010; Zavitkovski, 1966). The native plants of Intermountain West have great potential as a resource for plant growth-promoting bacteria. We attempted to isolate PGPR and further use them as bio-fertilizers/biostimulants as chemical fertilizers have adverse effects on the environment. Bio-fertilizers/biostimulants are becoming very popular, especially in organic farming, to maintain soil health, plants growth and development, and sustainability. Using PGPR in bio-fertilizer or biostimulants can be eco-friendly, economical, and productive. Bacterial cultures of PGPR can be used as inoculants and lead to a sustainable agricultural technique (Kumari et al., 2018).

2. Methodology

2.1 Sample collection of rhizosphere soil from the native habitat of *Ceanothus velutinus*

The rhizosphere soil samples from *Ceanothus velutinus* (snowbrush ceanothus) were collected at three locations in the Tony Grove, Logan, Utah (41^{o}52’56” N-111^{o}33’53” W, 41^{o}52’34” N- 111^{o}34'20" W, and 41^{o}53’15” N - 111^{o}36’4” W). The methodology is described elsewhere with slight modifications (McPherson et al., 2018). The snowbrush ceanothus roots were collected, stored in conical centrifuge tubes with a phosphate buffer with surfactant, and immediately placed on ice for transportation. In the laboratory, the root samples were shaken on a rotary shaker to separate the

rhizosphere soil. The roots were removed, and the rhizosphere soil was collected by centrifuging at 3000g for 5 minutes. The pellets containing the rhizosphere soil were washed with phosphate buffer without surfactant and stored at 4 ^{o}C.

2.2 Sample collection of rhizospheres soils from the greenhouse grown plants

Snowbrush ceanothus plants propagated from cuttings were treated with 200 mL of native soil to see the effect of native soil on the growth and development. Two months old rooted cuttings were placed in 3.8 L pots with a soil mixture of 75% peat moss (Canadian Sphagnum peat moss, SunGro Horticulture Canada, Agawam, MA), 25% vermiculite (Therm-O-Rock West, Chandler, AZ), 0.89 kg.m^{-3} gypsum (92% Calcium Sulfate Dihydrate, 21% Calcium, 17% Sulfur, Athletic White Sports Field Marking Gypsum, Western Mining and Minerals, Bakersfield, CA), 1.57 kg m^{-3} dolomitic lime (Lhoist North America, Salinas, CA), and 0.65 kg m^{-3} wetting agent (AquaGro G; Aquatrols®, Paulsboro, NJ) (Chen et al., 2020). The control plants were not inoculated with native soil. This study was conducted for six months, and the difference in growth was observed.

The number of secondary shoots, plant width, and the amount of available nitrogen/total nitrate content (Chen et al., 2020) were measured after six months of inoculation. The rhizosphere soil was collected from the treated plants for PGPR isolation after six months of native soil treatments. The sample collection was similar to the one followed for the native samples (McPherson et al., 2018). Root and rhizosphere samples were also collected for metagenomics analysis.

2.3 Metagenomic analysis of the rhizosphere of snowbrush ceanothus plants

Microbial DNA was isolated from the rhizosphere soil of snowbrush ceanothus plants (control and treatment) grown in the greenhouse using Qiagen DNeasy

PowerSoil Pro Kit. The isolated DNA was quantified using a nanodrop 2000 (Thermo scientific). The V4 variable region of 16S rRNA was sequenced.

The V4 variable region of the 16S rRNA gene was amplified using the V4 variable region-specific primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVHHHTWTCTAAT-3') (https://en.novogene.com/16s-18s-its-amplicon-metagenomic-sequencing/#overview). The amplification reaction mixture (25 µl) contained 13 µl of water, 10 µl of Platinum Hot Start PCR Master Mix (MM) (Thermo Fisher), 0.5 µl of 10 mM forward and reverse primer each, and 1 µl of 5 ng/µl of the DNA. The PCR conditions were as follows: an initial denaturation of 3 minutes at 94 ^{o}C followed by 35 cycles of denaturation at 94 ^{o}C for 45 seconds, annealing at 50 ^{o}C for 60 seconds and extension at 72 ^{o}C for 60 seconds, and with a final extension at 72 ^{o}C for 10 minutes, and ending with an infinite hold at 4 ^{o}C using the DNA engine dyad Peltier thermal cycler.

The PCR products were diluted 50 times, and the indexes were attached by a secondary PCR reaction. The second reaction mixture consisted of 5 µl of mM, 2 µl of i5 index, 3 µl of i7 index, and 1 µl of the diluted PCR product. The PCR conditions are as follows: an initial denaturation step at 94 ^{o}C for 1 minute followed by 15 cycles of denaturation at 94 ^{o}C for 15 seconds, annealing at 64 ^{o}C for 15 seconds, and an extension at 72 ^{o}C for 1 minute, and a final extension at 72 ^{o}C for 3 minutes using a DNA engine dyad Peltier thermal cycler (BIO-RAD).

Once the indexes were attached, the samples were cleaned up with AMPureXP beads, using a 1:1 ratio. The PCR products were quantified by fluorometry, and quality was analyzed on the TapeStation. The samples were then pooled and sequenced on the MiSeq using a 2×250 paired-end sequencing chip size (Illumina).

The sequenced data were analyzed using QIIME2/2019.4 (Bolyen et al., 2019). Analysis of amplicon sequence variants (ASVs) was carried out by divisive amplicon denoising algorithm (DADA2), and the taxonomic classification was obtained using a feature-classifier. Multiple plots were then generated, such as alpha and beta diversity.

2.4 Growth media selection and preparation

Five different media compositions were used for the isolation of bacteria from the rhizosphere. They included $1/4^{th}$ nutrient agar (NA), $1/4^{th}$ tryptic soy agar (TSA; SIGMA-ALDRICH), yeast mannitol agar (YMA; SIGMA-Life Science), minimal M9 media (MM9; BD Difco), and actinomycete isolation agar (AIA; SIGMA-ALDRICH) (Table 1 and 2). The Yeast Mannitol Agar helps isolate *Rhizobium* strains (Jarvis et al., 1982b), and the Actinomycete Isolation Agar is used to isolate *Actinomycetes* (Nanjwade et al., 2010).

2.5 Isolation and purification of PGPR

The rhizosphere pellets were diluted to a 10:95 ratio with water where 10 grams of soil was resuspended in 95 ml of sterilized water. It was then serially diluted in the ratio of 1:10. 100 µl of the last three dilutions 10^{-3}, 10^{-4}, and 10^{-5} were plated onto the five media compositions using a spread plate method. The plates were incubated at 28 ^{o}C for 24 hours. Once growth appeared, single colony was selected based on their visual characteristics such as color, texture, transparency, size, consistency, and any other distinct morphological trait. The selected colonies were purified by the streak plate method (Woeste, 1996). The screening process was repeated three times, and the purified colonies were preserved as glycerol stocks at -80 ^{o}C.

2.6 PCR amplification, 16S rRNA sequencing, and BLAST

The isolated bacterial colonies were subjected to colony PCR to amplify the 16S rRNA region. Almost the whole length of the 16S rRNA variable region was amplified using the 27F (V1 region- 5′-AGAGTTTGATCCTGGCTCAG-3′) as the forward primer and 1492R (V9 region- 5′-TACGGYTACCTTGTTACGACTT-3′) as the reverse primer. A 20 µl PCR reaction containing 250 nM of each forward and reverse primer, 1X DreamTaq buffer, 200 nM dNTP mix, 0.5 U of DreamTaq DNA polymerase. The PCR program was as follows: 95 ^{o}C initial denaturation for 2 minutes, 35 cycles of 95 ^{o}C denaturation for 30 seconds, 54.3 ^{o}C annealing for 30 seconds, and 72 ^{o}C extension for 1 and a half minutes, followed by a final extension at 72 ^{o}C for 10 minutes (Applied Biosystems- ProFlex PCR system). The amplicons were run on agarose gel electrophoresis to confirm the PCR products. The PCR products were sequenced. The sequencing results were run on a BLAST (Basic Local Alignment Search Tool) against a 16S rRNA database on NCBI to identify the bacterial species.

2.7 Bacterial characterization

2.7.1 Morphological

The identified bacterial species were then characterized based on morphological traits and their ability to produce siderophores, indole acetic acid (IAA), and catalase, solubilize phosphate and fix atmospheric nitrogen. They were also characterized based on gram stain. The bacterial colonies isolated were characterized based on color (such as white, yellow, orange, etc.), texture (matte, glossy, and 50/50 glossy & matte), transparency (transparent, translucent, and opaque), size (dot, normal, spread, and widespread), consistency (dry, viscous, and mucus) and other morphological traits (flat, raised, normal, pigment secretion and cloudy/chalky, etc.) (Reynolds, 2018) (Fig 12.a.).

BD BBL™ Gram Stain Kits was used to carry out gram staining. Gram-positive species-stained violet color and gram-negative species-stained pink color.

2.7.2 Biochemical

2.7.2.1 Catalase test

On a glass slide, a single colony was picked and placed. 1-2 drops of hydrogen peroxide were mixed. Bubbling shows a positive result for catalase activity (Pakpour and Horgan, 2021).

2.7.3 Phosphate solubilization

The bacterial isolates were screened for phosphate solubilization on Pikovskaya medium (Pikovskaya, 1948) (HiMedia). The bacterial colonies were streaked onto this medium and incubated at 28 ^{o}C for four days or until a clear halo was observed (Chung et al., 2005) (Fig 12.b.). The colonies were screened three times.

2.7.4 Siderophore production

The siderophore-producing bacteria screened on CAS (chrome azurol S) agar (Millipore SIGMA) (Schwyn and Neilands, 1987). The bacterial samples were streaked onto it and incubated at 28 ^{o}C for four days or until a yellow-orange halo was observed on blue-colored media (Gamit and Tank, 2014) (Fig 12.c.). Three rounds of screening were done to confirm the positive results.

2.7.5 Nitrogen fixation

Nitrogen-fixing bacteria were screened on Norris-glucose nitrogen free agar (HiMedia) for their ability to fix atmospheric nitrogen (Nur Amira Ezaty Mohd Tarmizi, 2016). The bacterial isolates were streaked on the plates and incubated at 28 ^{o}C for three days or until a clear zone around the colony appeared. The appearance of a

clear zone indicates a positive result for nitrogen fixation (Wafula and Murunga, 2020) (Fig 12.d.). The experiment was repeated three times to confirm the results.

2.7.6 Indole acetic acid (IAA) production

A colorimetric method of detecting the amount of IAA produced by the isolated bacteria was carried out (Gordon and Weber, 1951). The protocol described elsewhere (Sarker and Al-Rashid, 2013) was followed to characterize the bacterial isolates for IAA production. The bacterial colonies were cultured in 5 ml LB broth (Fisher Scientific) supplemented with 0.1% Tryptophan (EMD Millipore Corporation) at 28 ^{o}C for 72 hours. A non-inoculated culture broth was a control. The supernatant was separated by centrifugation at 10000rpm for 10 minutes. One ml of the supernatant was mixed with 1 ml of Salkowski reagent and incubated for 25 minutes. The development of pink color indicated the presence of IAA (Fig 12.f.) and read at 530 nm in the Spectramax Microplate reader (Molecular Devices). An IAA (SIGMA) standard curve was prepared (Fig 13) and used to calculate the amount of IAA (Fig 12.e.).

3. Results

3.1 Effect of native soil on the growth and developments of snowbrush ceanothus plants

The native soil-treated snowbrush ceanothus plants showed a visual difference in the growth of plants compared to control plants (Fig 10.a.). An increase of 7-fold was seen in the change in secondary shoot number from 0 days to 6 months in the snowbrush ceanothus plants treated with native soil compared to control (Fig 10.b.). The plants treated with native soil showed a significant increase of about 11% in the nitrate content (mg/L) when compared to control (no inoculation) after six months of treatment (Fig 10.c.).

3.2 Bacterial population in the rhizosphere of snowbrush ceanothus plants grown in the greenhouse

The 16S rRNA sequencing data were analyzed using QIIME2 to study the microbial populations in the rhizosphere samples of control and treatment plants. The taxonomic classification at the phyla level revealed the dominance of the phyla *Actinobacteriota, Acidobacteriota, Bacteroidota, Firmicutes, Gammatimonadota,* and *Verrucomicrobiota* in the treated rhizosphere samples compared to the control (Fig 11.a.). The genus-level classification revealed the dominance of *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium, Bradyrhizobium, Massilia, Nitrosospira,* and *Sphingobium* in the treated sample compared to the control (Fig 11.b.). It also revealed the presence of *Bacillus* and *Pseudoarthrobacter* in the treatment samples but absent in control (Fig 11.b.). *Nitrosospira* is a genus belonging to ammonia oxidizing bacteria (AOB) and found abundantly in the treatment rhizosphere samples. *Massilia* is another genus present abundantly in the rhizosphere of treated plants (Fig 11.b.).

The alpha diversity analysis revealed a higher diversity in the treated samples when compared with the control (Fig 11.c.). The beta diversity analysis showed a similar microbial diversity within all the rhizosphere samples (Fig 11.d.).

3.3 Isolation and identification of PGPR from rhizosphere

A total of 69 bacterial colonies were isolated from the native soil. Nine colonies were isolated from MM9 media, 22 from TSA media, 21 from ¼ NA media, six from AIA media, and 11 were from YMA media (Table 10). Twenty- seven out of the 69 colonies were selected for 16s rRNA sequencing, followed by a BLAST search for identification. Out of these 27, 9 were from ¼ TSA media, 12 from ¼ NA media, two from MM9 media, one from AIA media, and three from YMA media (Table 10). The BLAST results of the 27 colonies from the native soil revealed the presence of bacterial

species from the genus *Streptomyces, Nocardia, Neorhizobium, Pseudomonas, Xenophilus, Promicromonospora, Pedobacter*, and *Pantoea* are amongst a few others. *Streptomyces* was the most abundant genus as 44 % of the identified bacterial samples belonged to this genus (Table 10).

Seventy-eight bacterial colonies were isolated from the rhizosphere of snowbrush ceanothus grown in the greenhouse. Out of all the bacterial colonies isolated, 13 colonies were from MM9 media, 33 from ¼ NA media, 14 from ¼ TSA media, nine from AIA media, and nine from YMA media (Table 11). The colonies were isolated and purified by the streak plate method. The variable region of 16s rRNA was sequenced and identified in 36 purified bacterial colonies against the 16s rRNA database on NCBI. Out of 34 colonies, eight were from MM9 media, 14 forms ¼ NA media, four from AIA media, six from ¼ TSA media, and four from YMA media. BLAST search revealed the presence of the genus *Streptomyces, Pseudomonas, Variovorax, Priestia, Bacillus, Xenophilus, Acidovorax, Ancylobacter*, and *Pedobacter*. Most isolates belonged to *Pseudomonas* (41.7%) and *Streptomyces* (19 %) (Table 11).

3.4 Bacterial characterization

The bacterial isolates from the rhizosphere of plants in the native habitat and plants grown in the greenhouse and treated with native soil were characterized for four PGPR associated traits: nitrogen fixation, phosphate solubilization, catalase, siderophore, and IAA production. Out of the 27 identified bacterial isolates, three showed two or more PGPR traits from the native soil samples (Table 10). Twelve of 37 bacterial isolates from greenhouse samples showed a positive result for all the PGPR traits. Apart from the 12 isolates, 11 isolated bacteria showed two or more PGPR traits tested in this study (Table 11).

3.4.1 *Morphological characteristics*

The bacterial isolates were characterized based on color, texture, transparency, size, consistency, and morphology of the colonies (Table 10 and 11) (Fig 12.a.).

3.4.2 *Gram stain*

The gram stain results revealed that 74 % of the bacterial colonies was gram-positive, and 26% gram-negative in the isolates from the native soil (Table 10). The greenhouse samples have 23 gram-negative bacteria (64%) and 13 gram-positive bacteria (36 %) (Table 11).

3.4.3 *Catalase test*

Out of the total isolates from the native soil, 97% of them were catalase-positive (Table 10). In the bacterial species isolated from the greenhouse sample, 78% were catalase-positive (Table 11).

3.4.4 *Phosphate solubilization*

Out of the 27 bacterial isolates from the native soil, five of them, GK_NR_133, GK_NR_145, GK_NR_162, GK_NR_182, and GK_NR_186, showed a clear halo around the colony indicating a 19% positive result for phosphate solubilization (Fig 12.b.) (Table 5). Fifteen isolates showed clear halo from the snowbrush ceanothus rhizosphere in the greenhouse. About 42% of the total isolates showed a positive result for phosphate solubilization (Table 5).

3.4.5 *Siderophore production*

Seven bacterial (GK_NR_129, GK_NR_133, GK_NR_136, GK_NR_144, GK_NR_149, GK_NR_150, and GK_NR_194) species out of 27 bacterial isolates from the native soil showed a yellow-orange halo around the bacterial colony, indicating that 26% of the bacterial isolates can produce siderophores (Fig 12.c.) (Table 6). Nineteen

out of 36 (53%) bacterial isolates from the rhizosphere of snowbrush ceanothus grown in greenhouse showed siderophore production (Table 6). GK_GR_52, GK_GR_55, GK_GR_60, GK_GR_90, and GK_GR_115 are a few isolates that showed a bigger and brighter halo than the others (Table 6).

3.4.6 *Nitrogen fixation*

Two of the bacterial colonies (GK_NR_133 and GK_NR_194) isolated from the rhizosphere of snowbrush ceanothus grown in the native habitat revealed a clear halo around it, indicating that 7% of them can fix atmospheric nitrogen (Fig 12.d.) (Table 7). Eighteen bacterial isolates or 50% of the greenhouse samples showed a positive result for bacterial nitrogen fixation. (Table 7).

3.4.7 *IAA production*

The IAA test in the bacterial samples revealed that GK_NR_133 (*Pantoea* sp.) and GK_NR_149 (*Brevibacterium* sp.) isolated from the rhizosphere of the native soil produced the highest amount of IAA 33.52±0.15 µg/ml and 14.88±0.11 µg/ml, respectively. The IAA production observed in the samples ranged from approximately 1 to 33 µg/ml. Seven isolates out of 27 or about 26% of the total isolates produced more than 1 µg/ml of IAA (Table 8).

Out of all the bacteria isolated from the rhizosphere of plants grown in the greenhouse GK_GR_41 (*Pseudomonas* sp.), GK_GR_60 (Pseudomonas sp.), GK_GR_61 (*Agrobacterium* sp.), GK_GR_98 (*Pseudomonas* sp.), GK_GR_52 (*Pseudomonas* sp.), and GK_GR_64 (*Pseudomonas* sp.) produced 14, 12, 12, 12, 11, and 11 µg/ml IAA, respectively, which were higher than the other bacterial isolates . The IAA production in these samples ranged from approximately 1 to 14 µg/ml (Table 8). Thirty-two isolates out of 36 or about 89% of the total isolates from the rhizosphere of plants grown in the greenhouse produced more than 1 µg/ml of IAA (Table 8).

Twelve isolates out of all bacteria isolated in this study tested positive for all the PGPR traits. One of these isolates was from the rhizosphere of snowbrush ceanothus grown in the native habitat. The other 11 were from the rhizosphere of snowbrush ceanothus treated with the native soil under greenhouse conditions. These isolates belonged to the genera *Pseudomonas, Pantoea, Bacillus,* and *Ancylobacter* (Table 9).

4. Discussion

The metagenomic analysis of the rhizosphere samples from the plants treated with and without native soil revealed the presence of bacteria from the phyla *Proteobacteria, Actinobacteriota, Acidobacteriota, Bacteroidota, Firmicutes, Gammatimonadota,* and *Verrucomicrobiota.* These are common phyla found in the rhizosphere soil of other crop plants such as maize and sugarcane (Correa-Galeote et al., 2016; Pisa et al., 2011; Susilawati et al., 2010). The genus-level classification revealed the presence of several PGPR such as *Streptomyces, Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium, Pseudomonas, Acidovorax Bradyrhizobium,* and *Sphingobium*. The PGPRs widely belong to the genus *Streptomyces* and *Pseudomonas* and help in plant growth and development by various means such as antibiotic production, phosphate solubilization, nitrogen fixation, siderophore production acting as bio-control agents (Charousová et al., 2018; Etesami and Glick, 2020b; Gu et al., 2020c; Li et al., 2017a; Mehnaz and Lazarovits, 2006; Nandimath et al., 2017). The genera *Acidovorax Allorhizobium, Bradyrhizobium, Neorhizobium, Rhizobium,* and *Sphingobium* have also been shown to promote plant growth in – legumes and beachgrass (Li et al., 2012; Mousavi et al., 2014; Wanees et al., 2018). The genera *Bacillus* and *Pseudoarthrobacter* were present in the treated samples but absent in control. *Bacillus* is a well-known genus containing PGPR that produces siderophores, fixes nitrogen, is a bio-control agent, and can help in biotic and

abiotic stress (Ghazy and El-Nahrawy, 2021; Hashem et al., 2019). *Pseudarthrobacter* has also been found to produce high amounts of IAA when isolated from shooting range soil in Korea and from the rhizosphere of *Curcuma longa* L. (Ham et al., 2021; Kharshandi et al., 2021). The abundance of *Massilia* and *Nitrosospira* was more in the treated samples when compared to the control. According to previous studies, *Massilia* has shown plant growth-promoting activity, for example, *Massilia phosphatilytica* could solubilize phosphate when isolated from agricultural soil in China and *Massilia rhizosphaerae* showed antibacterial activity against *Ralstonia solanacearum* (Li et al., 2021; Zheng et al., 2017). *Nitrosospira* is an ammonia-oxidizing bacteria (AOB) that can produce nitrous oxide (N_2O) through nitrification in soil (Shaw et al., 2006).

The prime objective of this study was to isolate, purify, identify, and characterize bacteria colonies from the rhizosphere of *Ceanothus velutinus* to find potential PGPRs. The PGPRs are adapted to the native conditions of the Intermountain West region of North America and are potential candidates for biofertilizers and biostimulants to be used in a similar ecosystem. In this study, 27 bacterial isolates were shortlisted from the native soil and 36 from the greenhouse identified. The bacteria belonging to the genus *Streptomyces* (41%) dominated the isolates from the rhizosphere of the native habitat. *Streptomyces* is known to have several bacterial species which possess PGPR traits. *Streptomyces longisporoflavus* can solubilize phosphate (Nandimath et al., 2017). *Streptomyces umbrinus*, a halotolerant bacteria, can produce siderophores (Etesami and Glick, 2020a). *Streptomyces ederensis* and *Streptomyces rishiriensis* bacteria produce antibiotics (Charousová et al., 2018, 2015), *Streptomyces canus* helps in zinc biosorption (Timková et al., 2018).

Other genera isolated in this study included *Nocardia* sp. which helps in siderophore production and iron uptake in *Nocardia fluminea* (Aloo et al., 2020).

Neorhizobium sp. carries out nitrogen fixation. *Neorhizobium galegae* induce nodulation in *Galega orientalis,* and *Neorhizobium alkalisoli* was isolated from the nodules of *Caragana intermedia* (Mousavi et al., 2014; Yang et al., 2009). *Promicromonospora* sp. produces GA_3 and solubilizes phosphate in tomatoes (Kang et al., 2012) and reduces salinity and drought stress in cucumber (Kang et al., 2014a). *Janthinobacterium* sp. produces anti-fungal compounds in *Janthinobacterium lividum* and *Janthinobacterium agaricidamnosum* (Baitchman and Herman, 2015b; Haack et al., 2016) and is psychrophilic (Sharma et al., 2015). *Pedobacter* sp. is a PGPR psychrotolerant in tomatoes (Ho et al., 2017).

Many *Pseudomonades* sp. was isolated in this study and carry out essential processes. For example, *Pseudomonas koreensis* can execute nitrogen fixation in sugarcane (Li et al., 2017b) and act as a bio-control agent (Gu et al., 2020b). *Pseudomonas chlororaphis* can act as a bio-control agent and solubilize zinc, and is salt-tolerant(Egamberdieva, 2012; Shahid et al., 2017). *Pseudomonas mediterranea* can produce siderophores, ammonia, protease, and solubilizes phosphate (Gu et al., 2020c). *Pseudomonas putida* produces IAA and anti-fungal compounds, fixes atmospheric nitrogen, and solubilizes phosphate (Mehnaz and Lazarovits, 2006). *Pseudomonas monteilii, Pseudomonas plecoglossicida, Pseudomonas koreensis,* and *Pseudomonas moraviensis* are resistant to heavy metals (Babu et al., 2015b; Dharni et al., 2014; Hassan et al., 2017).

The identified isolates from the greenhouse samples showed more *Pseudomonas* species followed by *Streptomyces* sp. Other identified bacterial isolates with PGPR traits are *Variovorax* sp., *Priestia* sp., and *Brevundimonas* sp. *Variovorax ginsengisoli* helps denitrification (Im et al., 2010). *Priestia aryabhattai*- a plant growth-promoting bacteria, promotes plant growth in soybean by increasing the levels of

phytohormones, such as ABA and GA, and is also tolerant to oxidative stress (Park et al., 2017). Bacterium *Priestia megaterium* promotes growth in mustard by phosphate solubilization and in capsicum by zinc solubilization (Bhatt and Maheshwari, 2020; Kang et al., 2014b). Bacterium *Brevundimonas vesicularis* can produce IAA, solubilize phosphate and promote maize growth (Breedt et al., 2017), and can potentially help bioremediation (Chasanah et al., 2018).

The native soil isolates showed higher catalase-positive bacteria (96 %) than the greenhouse isolates (78%). In this study, some of the bacterial isolates showed a positive result in the hydrogen peroxide test and belonged to the genera *Streptomyces, Nocardia, Pantoea,* and *Pseudomonas*. The species in these genera have been known to be catalase positive according to prior studies (Goveas et al., 2020; Kekuda, 2012; Palleroni, 2015; Rathish and Zito, 2021).

Catalase is an antioxidant enzyme (Saravanakumar et al., 2011) that can prevent DNA and membrane damage (Bowler et al., 1992). They also remove free radicals, help in drought stress, and reduce toxic ROS (reactive oxygen species) (Bowler et al., 1992; Diaz-Albiter et al., 2011).

Out of total bacterial isolates from the native soil and the greenhouse samples, 19% and 42% of the isolates could solubilize phosphate, respectively. The ability to solubilize phosphate is an important characteristic of a bacterial species for the selection to increase available phosphorus content in the rhizosphere (Kalayu, 2019). Soil consists of both organic and inorganic phosphate, and mineralization of phosphate from insoluble form to soluble form can be carried out by enzymes such as phosphatase and phytase or by secreting oxalic acid, gluconic acid, or organic acids (Yavarian et al., 2021). In our study, the bacterial isolates that showed phosphate solubilization belonged to the genus *Pantoea* and *Streptomyces*. *Pantoea ananatis* has been shown to

solubilize phosphate in previous studies and can potentially promote plant growth in the rhizosphere (Bakhshandeh et al., 2014; da Silva et al., 2015). Other *Pantoea* species, such as *Pantoea agglomerans,* isolated from the rhizosphere of *Araucaria*, have also been shown to solubilize phosphate (Li et al., 2020). Streptomyces is a known PGPR that can solubilize phosphate in the rhizosphere and has been shown to promote plant growth (Balakrishnan et al., 2021). The species *Streptomyces longisporoflavus* is one such example that can solubilize phosphate when isolated from compost in Maharashtra, India (Nandimath et al., 2017).

In this study, 26% and 53% showed a positive result for siderophore production in the bacterial isolates from native soil and greenhouse samples. The siderophore-producing isolates belonged to the genus *Bacillus*, *Brevibacterium, Leifsonia, Pantoea, Peribacillus*, *Pseudomonas, Streptomyces, Variovorax,* and *Xenophilus*. Siderophores promote plant growth in two different ways. The direct method is supplying iron to the plants, and the plants can directly uptake the iron-siderophore complex (Glick et al., 1999). The indirect way is to deprive pathogenic fungal species of iron (Ahmad et al., 2008). *Pseudomonas* is a known PGPR that produces siderophores, especially in *Pseudomonas koreensis* (Ghazy and El-Nahrawy, 2021) and *Pseudomonas putida* (Pahlavan Yali and Hajmalek, 2021). *Streptomyces* is another known PGPR that produces siderophores as a potential antibiotic (Terra et al., 2020). *Variovorax* and *Peribacillus* have also been shown to produce siderophores in other studies (Gaete et al., 2020; Hofmann et al., 2021). *Bacillus subtilis* is a common PGPR (Santoyo et al., 2021) that produces siderophores (Ghazy and El-Nahrawy, 2021). Studies have shown that different species from the genera *Pantoea*, *Brevibacterium,* and *Leifsonia* produce siderophores (Burbank et al., 2015; Dastager et al., 2009; Kang et al., 2016; Meena et al., 2017; Noordman et al., 2006; Passari et al., 2015).

The percentage of bacterial species that showed nitrogen fix ability were 7% and 50% in the native soil and greenhouse isolates (Table 1 and 2). The bacterial isolates that showed nitrogen fixation potential were *Pantoea, Pseudomonas, Bacillus*, and *Ancylobacter*. Biological nitrogen fixation is an essential process where atmospheric nitrogen is converted into inorganic compounds such as ammonia for the plants to uptake (https://www.britannica.com/science/nitrogen-fixing-bacteria). Nitrogen-fixing bacteria form a symbiotic relationship with the plant and commonly colonize in the plant rhizosphere. Nitrogen-fixing bacteria are found in several phyla and are a subset of PGPR (Mus et al., 2016b). Several studies have shown that genus *Pantoea* can fix nitrogen (Suleimanova et al., 2021) in both free-living and symbiotic states (Nadarasah and Stavrinides, 2014), and *Ancylobacter* can potentially fix nitrogen in wild rice (Banik et al., 2016). *Pseudomonas fluorescens* (both free-living and in symbiosis) and *Pseudomonas koreensis* are a few examples of nitrogen-fixing bacteria in the *Pseudomonas* genus (Chitra and Jijeesh, 2021; Li et al., 2017b; Lucy et al., 2004). *Bacillus subtilis* is an essential PGPR that promotes plant growth and enhance nitrogen fixation in both free-living and symbiosis (Hashem et al., 2019; Kloepper et al., 1989).

IAA production by bacterial species present in the rhizosphere has been shown to promote plant growth and improve root growth (both initiation and elongation) (Olanrewaju et al., 2017). In this study, the bacterial isolates from the native soil showed an increased amount of IAA and belonged to the genera *Pantoea* (33.52±0.15 µg/ml) and *Brevibacterium* (14.88±0.11 µg/ml) (Table 1). Many species from the genus *Pantoea* have been shown to produce IAA and promote plant growth. For example, *Pantoea ananatis* can promote rice- *Oryza sativa* growth under saline stress (Lu et al., 2021). *Pantoea agglomerans* isolated from the rhizosphere of legumes (lentils- *Lens culinaris*, pea- *Pisum sativum*, and canola- *Brassica napus*) promotes plant growth by

IAA production (Sergeeva et al., 2007); *Pantoea stewartii* synthesizes IAA when isolated from maize- *Zea mays* (Rahma et al., 2014). Studies have shown that several *Brevibacterium* species can produce IAA, such as *Brevibacterium sediminis,* a potential PGPR, isolated from the rhizosphere of tea- *Camellia sinensis* (Chopra et al., 2020). *Brevibacterium frigrotolerans* isolated from the rhizosphere of Aloe- *Aloe vera* produce IAA (Meena et al., 2017). *Brevibacterium casei* has been isolated from heavy metal contaminated rhizosphere soil of white mustard- *Sinapis alba* (Płociniczak et al., 2016). Increased IAA production observed in the bacterial isolates from the greenhouse soil and most of them belonged to the genera *Pseudomonas* followed by *Agrobacterium*. Several species have been shown to produce IAA, such as *Pseudomonas syringae, Pseudomonas savastanoi,* and *Agrobacterium tumefaciens* (Spaepen and Vanderleyden, 2011). A study conducted in 2013 revealed IAA production by *Pseudomonas fluorescens* (Sivasakthi et al., 2013). Other species from the *Pseudomonas* genera- *Pseudomonas koreensis, Pseudomonas chlororaphis,* and *Pseudomonas mandelii* also promote plant growth by IAA production (Guo et al., 2020; Habibi et al., 2014b; Liu et al., 2007).

Hence the isolates GK_NR_133 (*Pantoea* sp.), GK_GR_41 (*Pseudomonas* sp.), GK_GR_52 (*Pseudomonas* sp.), GK_GR_55 (*Pseudomonas* sp.), GK_GR_60 (*Pseudomonas* sp.), GK_GR_64 (*Pseudomonas* sp.), and GK_GR_98 (*Pseudomonas* sp.) has a great potential to improve plant growth as they tested positive for all the PGPR traits. They belonged to the genera *Pantoea* and *Pseudomonas*. *Pseudomonas* has been proven to improve plant growth in many studies including, lettuce- *Lactuca sativa* and garlic- *Allium sativum* (Cipriano et al., 2016; Jiménez et al., 2020). Another study conducted on maize- *Zea mays* and chickpea- *Cicer arietinum* revealed the plant growth-promoting ability of *Pantoea agglomerans* (Mishra et al., 2011).

5. Conclusions

Amongst the bacterial colonies isolated from the rhizosphere of the native soil and the growing medium, GK_NR_133 (*Pantoea* sp.) showed the highest IAA production and nitrogen fixation. This isolate produced siderophores and solubilized phosphates in addition to nitrogen fixation and IAA production. *Pantoea* sp. has been known for its plant growth-promoting abilities. IAA induces root growth and elongation and is an important PGPR trait to increase plant growth. Snowbrush ceanothus is an ornamental plant recommended for low-water-use landscaping but difficult to propagate by cuttings. The PGPR-producing IAA isolated from this study can be tested to enhance rooting in snowbrush ceanothus cutting propagation. In the future, these microbes can be used to grow snowbrush ceanothus in the landscape.

The isolates belonging to the genus *Pseudomonas* showed the highest siderophore production and phosphate solubilization. These isolates were tested positive for all the other PGPR traits in this study. Many species of *Pseudomonas* are well-known PGPRs that are tested for their plant growth-promoting ability. Therefore, these isolates will be tested on various crops in the future and identified by whole-genome sequencing. They have the potential for a bio-fertilizer and bio-stimulant in sustainable organic agriculture. They can also improve plant growth in native conditions such as in Utah.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 5. Bacterial characterization from the rhizosphere of snowbrush ceanothus grown in the native habitat or greenhouse based on phosphate solubilization

SN	Code	Phosphate Solubilization	BLAST
1	GK_GR_42	+++	*Pseudomonas* sp.
2	GK_GR_45	+++	*Pseudomonas* sp.
3	GK_GR_52	+++	*Pseudomonas* sp.
4	GK_GR_60	+++	*Pseudomonas* sp.
5	GK_GR_94	+++	*Pseudomonas* sp.
6	GK_GR_119	+++	*Pseudomonas* sp.
7	GK_NR_133	+++	*Pantoea sp.*
8	GK_GR_41	++	*Pseudomonas* sp.
9	GK_GR_55	++	*Pseudomonas* sp.
10	GK_GR_90	++	*Pseudomonas* sp.
11	GK_GR_109	++	*Pseudomonas* sp.
12	GK_NR_182	++	*Streptomyces sp.*
13	GK_GR_64	+	*Pseudomonas* sp.
14	GK_GR_73	+	*Bacillus* sp.
15	GK_GR_98	+	*Pseudomonas* sp.
16	GK_GR_104	+	*Pseudomonas* sp.
17	GK_GR_106	+	*Ancylobacter* sp.
18	GK_NR_145	+	*Streptomyces sp.*
19	GK_NR_162	+	*Streptomyces sp.*
20	GK_NR_186	+	*Streptomyces sp.*

‘+’ mild positive, ‘++’ moderately positive, ‘+++’ strongly positive

Table 6. Bacterial characterization from the rhizosphere of snowbrush ceanothus grown in the native habitat or greenhouse based on siderophore production

SN	Code	Siderophore production	BLAST
1	GK_GR_52	++++	*Pseudomonas* sp.
2	GK_GR_55	++++	*Pseudomonas* sp.
3	GK_GR_60	++++	*Pseudomonas* sp.
4	GK_GR_90	++++	*Pseudomonas* sp.
5	GK_GR_115	++++	*Pseudomonas* sp.
6	GK_GR_41	+++	*Pseudomonas* sp.
7	GK_GR_64	+++	*Pseudomonas* sp.
8	GK_GR_66	+++	*Pseudomonas* sp.
9	GK_GR_104	+++	*Pseudomonas* sp.
10	GK_GR_109	+++	*Pseudomonas* sp.
11	GK_GR_112	+++	*Pseudomonas* sp.
12	GK_NR_129	+++	*Streptomyces sp.*
13	GK_NR_144	+++	*Xenophilus sp.*
14	GK_GR_51	++	*Variovorax* sp.
15	GK_GR_72	++	*Xenophilus* sp.
16	GK_GR_73	++	*Bacillus* sp.
17	GK_GR_97	++	*Peribacillus* ap.
18	GK_GR_98	++	*Pseudomonas* sp.
19	GK_GR_119	++	*Pseudomonas* sp.
20	GK_NR_136	++	*Streptomyces sp.*
21	GK_GR_42	+	*Pseudomonas* sp.

22	GK_GR_45	+	*Pseudomonas* sp.
23	GK_GR_70	+	*Xenophilus* sp.
24	GK_GR_74	+	*Priestia* sp.
25	GK_GR_79	+	*Acidovorax* sp.
26	GK_GR_81	+	*Pedobacter* sp.
27	GK_GR_94	+	*Pseudomonas* sp.
28	GK_GR_99	+	*Brevundimonas* sp.
29	GK_GR_106	+	*Ancylobacter* sp.
30	GK_NR_133	+	*Pantoea sp.*
31	GK_NR_149	+	*Brevibacterium sp.*
32	GK_NR_150	+	*Leifsonia sp.*
33	GK_NR_194	+	*Pseudomonas sp.*

'+' mild positive, '++' moderately positive, '+++' strongly positive, '++++' highly positive

Table 7. Bacterial characterization from the rhizosphere of snowbrush ceanothus grown in the native habitat or greenhouse based on nitrogen fixation

S. No.	**Code**	**Nitrogen Fixation**	**BLAST**
1	GK_NR_133	++++	*Pantoea sp.*
2	GK_GR_42	+++	*Pseudomonas* sp.
3	GK_GR_45	+++	*Pseudomonas* sp.
4	GK_GR_52	+++	*Pseudomonas* sp.
5	GK_GR_55	+++	*Pseudomonas* sp.
6	GK_GR_64	+++	*Pseudomonas* sp.
7	GK_GR_98	+++	*Pseudomonas* sp.

8	GK_GR_104	+++	*Pseudomonas* sp.
9	GK_GR_112	+++	*Pseudomonas* sp.
10	GK_GR_115	+++	*Pseudomonas* sp.
11	GK_GR_119	+++	*Pseudomonas* sp.
12	GK_NR_194	+++	*Pseudomonas sp.*
13	GK_GR_41	++	*Pseudomonas* sp.
14	GK_GR_60	++	*Pseudomonas* sp.
15	GK_GR_66	++	*Pseudomonas* sp.
16	GK_GR_90	++	*Pseudomonas* sp.
17	GK_GR_94	++	*Pseudomonas* sp.
18	GK_GR_109	++	*Pseudomonas* sp.
19	GK_GR_73	+	*Bacillus* sp.
20	GK_GR_106	+	*Ancylobacter* sp.

'+' mild positive, '++' moderately positive, '+++' strongly positive, '++++' highly positive

Table 8. Bacterial characterization from the rhizosphere of snowbrush ceanothus grown in the native habitat or greenhouse based on IAA production

S N	**Code**	**IAA (ug/ml) production**	**BLAST**
1	GK_NR_133	33.52±0.15	*Pantoea sp.*
2	GK_NR_149	14.88±0.11	*Brevibacterium sp.*
3	GK_GR_41	14.08±0.58	*Pseudomonas* sp.
4	GK_GR_60	12.27±0.04	*Pseudomonas* sp.
5	GK_GR_61	12.02±0.40	*Agrobacterium* sp.
6	GK_GR_98	11.79±0.08	*Pseudomonas* sp.

7	GK_GR_52	11.33±1.23	*Pseudomonas* sp.
8	GK_GR_64	10.60±0.17	*Pseudomonas* sp.
9	GK_GR_55	9.82±0.17	*Pseudomonas* sp.
10	GK_NR_194	8.97±0.45	*Pseudomonas sp.*
11	GK_GR_75	8.96±0.33	*Priestia* sp.
12	GK_GR_45	7.35±0.04	*Pseudomonas* sp.
13	GK_GR_79	7.00±0.57	*Acidovorax* sp.
14	GK_GR_72	6.65±0.50	*Xenophilus* sp.
15	GK_GR_122	6.29±0.09	*Streptomyces* sp.
16	GK_GR_70	6.25±0.43	*Xenophilus* sp.
17	GK_GR_42	6.05±0.27	*Pseudomonas* sp.
18	GK_GR_106	5.82±0.23	*Ancylobacter* sp.
19	GK_GR_104	5.49±0.09	*Pseudomonas* sp.
20	GK_GR_68	5.27±0.05	*Streptomyces* sp.
21	GK_GR_90	5.19±0.16	*Pseudomonas* sp.
22	GK_GR_115	4.68±0.06	*Pseudomonas* sp.
23	GK_GR_94	4.16±0.02	*Pseudomonas* sp.
24	GK_GR_112	3.86±0.06	*Pseudomonas* sp.
25	GK_GR_66	3.82±0.03	*Pseudomonas* sp.
26	GK_NR_150	3.57±0.04	*Leifsonia sp.*
27	GK_GR_73	3.46±0.06	*Bacillus* sp.
28	GK_GR_111	3.28±0.07	*Streptomyces* sp.
29	GK_GR_88	3.18±0.10	*Priestia* sp.
30	GK_GR_74	3.09±0.17	*Priestia* sp.
31	GK_NR_143	2.41±0.04	*Streptomyces sp.*

32	GK_GR_99	2.39±0.07	*Brevundimonas* sp.
33	GK_GR_59	2.19±0.06	*Priestia* sp.
34	GK_GR_81	2.01±0.09	*Pedobacter* sp.
35	GK_NR_197	1.88±0.06	*Janthinobacterium sp.*
36	GK_GR_51	1.69±0.05	*Variovorax* sp.
37	GK_GR_44	1.49±0.02	*Streptomyces* sp.
38	GK_NR_162	1.22±0.06	*Streptomyces sp.*
39	GK_GR_58	1.08±0.04	*Streptomyces* sp.

Table 9. List of bacterial isolates that tested positive for the PGPR traits.

S N	Code	Morphology	Catalase production	Siderophore production	Phosphate solubilization	Nitrogen Fixation	IAA (µg/ml) production	BLAST
1	GK_NR_133	Yellow, glossy, translucent, widespread, viscous, and raised	+	+	+++	++++	33.52±0.15	*Pantoea* sp.
2	GK_GR_41	Off-white, glossy, transparent, normal, and mucus	++	+++	++	++	14.08±0.58	*Pseudomonas* sp.
3	GK_GR_42	White, glossy, translucent, normal, and viscous	++	+	+++	+++	6.05±0.27	*Pseudomonas* sp.

4	GK_GR_55	Off-white, glossy, translucent, normal, and mucus	++	++++	++	+++	9.82±0.17	*Pseudomonas* sp.
5	GK_GR_60	Tan glossy, translucent, normal, and viscous	+	++++	+++	++	12.27±0.04	*Pseudomonas* sp.
6	GK_GR_64	Yellow (light)/white, glossy, translucent, normal, and viscous	+	+++	+	+++	10.60±0.17	*Pseudomonas* sp.
7	GK_GR_73	White/yellow, 50/50 glossy matte, opaque, normal, viscous, and blooming effect	+	++	+	+	3.46±0.06	*Bacillus* sp.
8	GK_GR_90	Yellow (pastel), glossy, opaque, normal, and viscous	+	++++	++	++	5.19±0.16	*Pseudomonas* sp.

9	GK_GR_94	Tan/ white, glossy, translucent, spread, viscous, and normal	+	+	+++	++	4.16±0.02	*Pseudomonas* sp.
10	GK_GR_98	White (tinge), glossy, translucent, widespread, mucus, and normal	++	++	+	+++	11.79±0.08	*Pseudomonas* sp.
11	GK_GR_104	White, glossy, translucent, spread, viscous, and normal	+	+++	+	+++	5.49±0.09	*Pseudomonas* sp.
12	GK_GR_106	White, glossy, translucent, spread, viscous, and normal	+	+	+	+	5.82±0.23	*Ancylobacter* sp.

‘+’ mild positive, ‘++’ moderately positive, ‘+++’ strongly positive, ‘++++’ highly positive

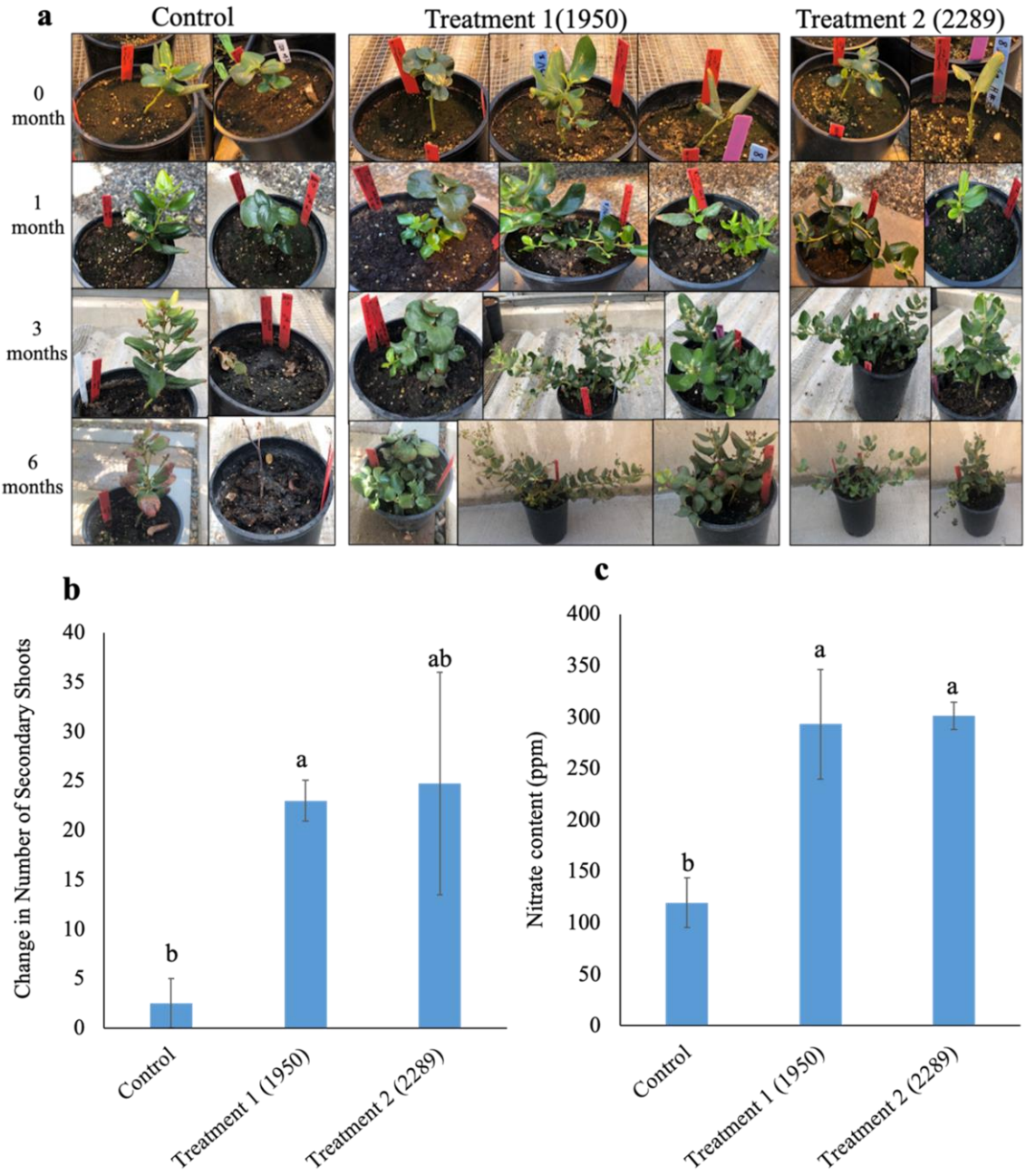

Fig 10. a. Change in the growth of snowbrush ceanothus plants (with and without native soil) in six months. **b.** Change in the number of secondary shoots of snowbrush ceanothus plants from rooted cuttings treated with and without native soil after six months of treatment. **c.** The nitrogen content of snowbrush ceanothus plants after six months of treatment.

Same letters denote no significance among different treatments by Tukey's method for

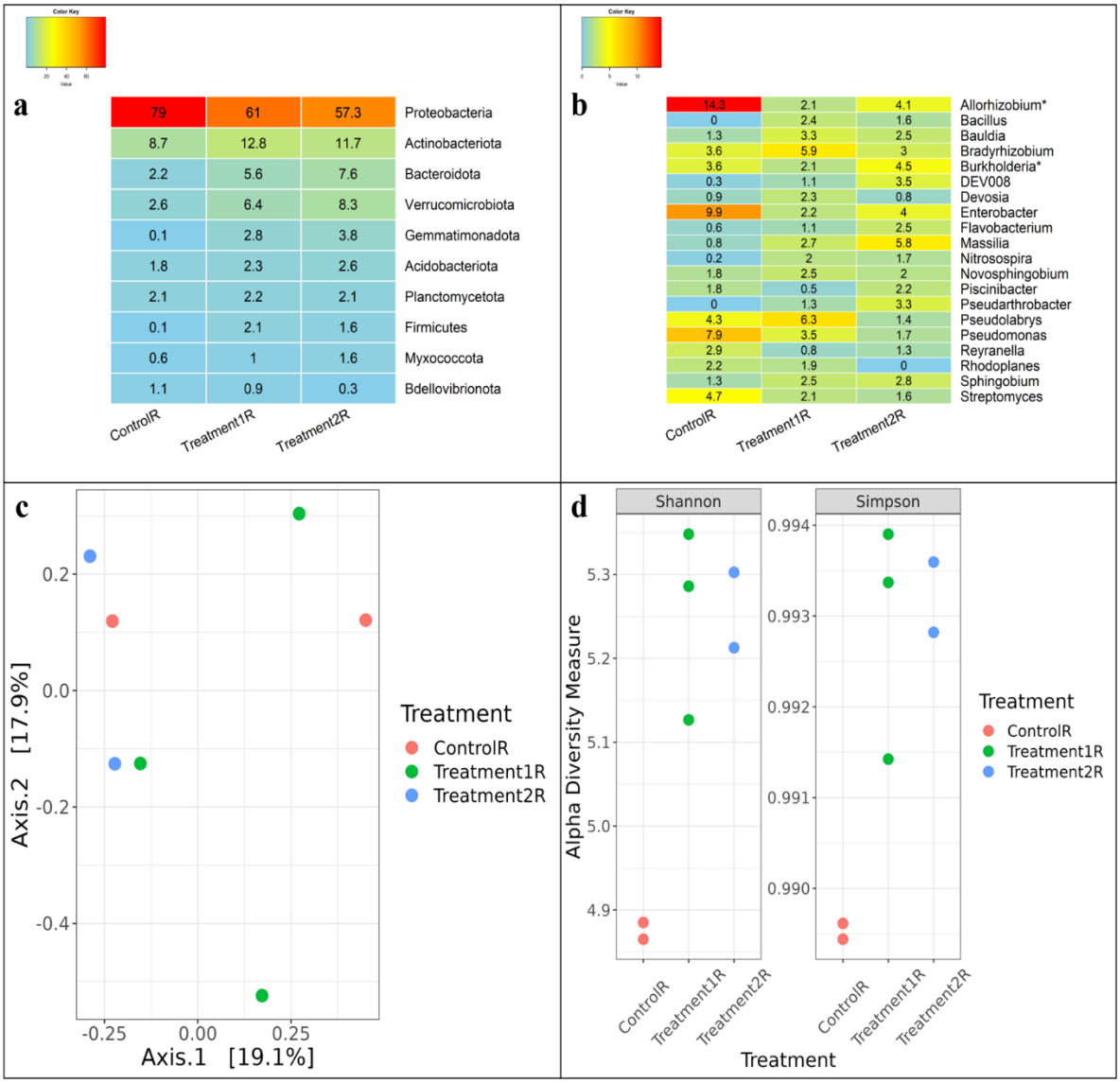

Fig 11. a. Percent read abundance at the phyla (a) and genus (b) level, and alpha (c) and beta (d) diversity of the rhizosphere samples from control and treatment of snowbrush ceanothus grown in the greenhouse.

**Allorhizobium- Allorhizobium, Neorhizobium, Pararhizobium,* and *Rhizobium*

**Burkholderia- Burkholderia, Caballeronia,* and *Paraburkholderia*

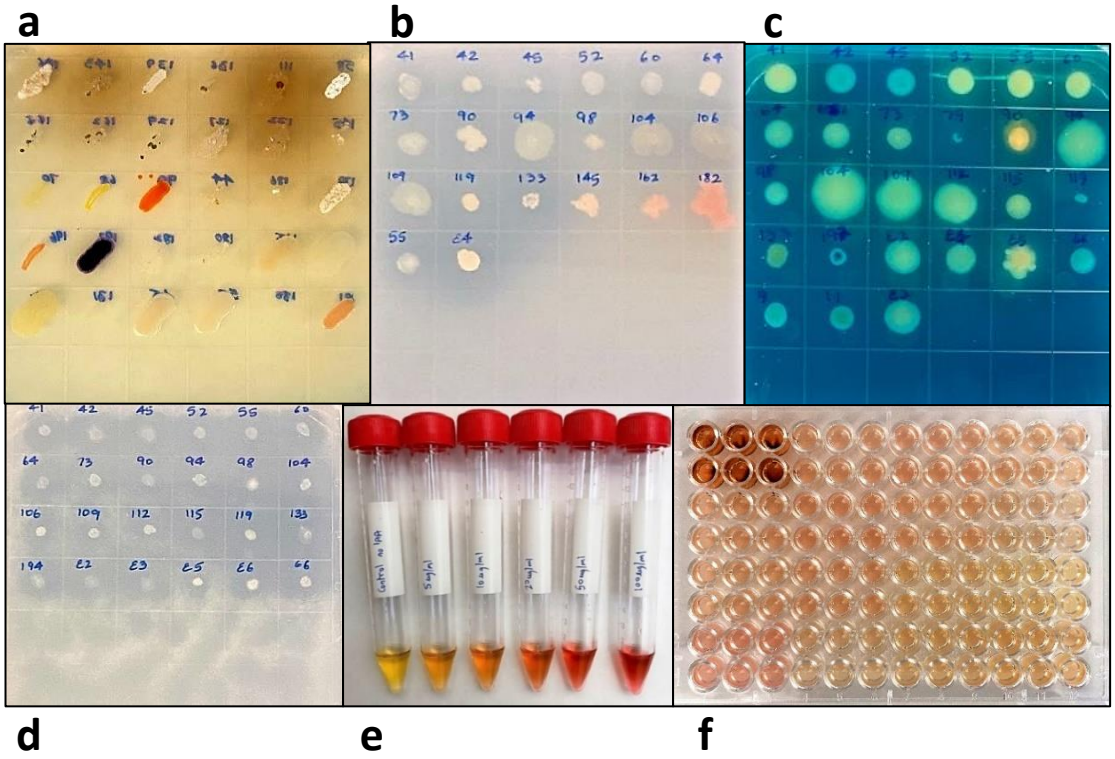

Fig 12. a. Bacterial characterization of rhizosphere isolates based on morphology, **b.** phosphate solubilization in Pikovakaya's agar media, **c.** siderophore production in CAS agar media, **d.** nitrogen fixation in Norris Glucose Nitrogen-free media, **e.** IAA standard, and **f.** IAA production

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CHAPTER IV

CHARACTERIZATION OF POTENTIAL PLANT GROWTH-PROMOTING ENDOPHYTES FROM THE NATIVE PLANT *CEANOTHUS VELUTINUS*

Abstract

Plant growth promoting bacteria help plant nutrient uptake, biocontrol activity, phytohormone and secondary metabolite production, etc. These bacteria can be a great alternative to chemical fertilizers. They are commonly found in the endosphere of a plant and share a symbiotic relationship with the plant. In this study, we isolated 22 endophytes from the roots of the native plant *Ceanothus velutinus*. These bacterial isolates were isolated, purified, and identified by 16S rRNA sequencing and further characterized based on morphology and plant growth-promoting traits. They were tested for catalase, siderophores, and indole acetic acid production, phosphate solubilization, and nitrogen fixation. Seventeen out of the 22 isolates showed a positive result for at least one of the PGPR traits tested. Out of the 22 isolates, three isolates belonging to the genera *Serratia* and *Arthrobacter* tested positive for all the PGPR traits. These isolates can further be tested on plants for their role in plant growth promotion. These isolates have a great potential to become biofertilizers and bio-stimulants.

Highlights:

- Twenty-two bacteria were isolated from the roots of *Ceanothus velutinus*.
- These isolates were identified and characterized for plant growth promoting traits.

- Three isolates belonging to the genera *Serratia* and *Arthrobacter* tested positive for siderophores, indole acetic acid production, phosphate solubilization, and nitrogen fixation.
- These isolates have potential as bio-fertilizers and bio-stimulants for further investigation.

Keywords: endosphere, endophytes, plant growth-promoting rhizobacteria (PGPR)

1. Introduction

Chemical fertilizers are the most added to crops as a source of nutrients. But this process has been shown to have several flaws including, soil sanitization, high cost, and water pollution (Olanrewaju et al., 2017; Sánchez-Cruz et al., 2019). Plant growth-promoting rhizobacteria (PGPR) can be used as biofertilizers to replace chemical fertilizers. They promote phytohormone production in plants such as auxins, cytokinins, and gibberellins. Some of them release metabolites such as siderophores and hydrogen cyanide (HCN). They help to enhance nutrient uptake by solubilizing phosphate and fixing atmospheric nitrogen. They also act as biocontrol agents and protect the plants from pests and diseases by producing antibiotic and antifungal metabolites. Plant growth-promoting rhizobacteria also helps to supply nitrogen by carrying out nitrogen fixation.

Plant growth-promoting rhizobacteria can also act as bio-stimulants and promote plant growth by increasing nutrient availability in soil by expanding root area for plants to access nutrients. Bio-stimulants are defined as microorganisms that promote plant growth by improving crop productivity, help plants deal with biotic and abiotic stresses, and increase nutrient uptake by plants (Silva et al., 2017). Endophytic bio-stimulants can promote plant growth by interacting with the plant's signaling

cascades (Brown and Saa, 2015). Bio-stimulants are being used in agriculture for promoting plant growth for years and have been exponentially increasing in the past decade (Silva et al., 2017). Hence, they can be used as bio-fertilizers and bio-stimulants as they are eco-friendly, and a great replacement for chemical fertilizers (Etesami and Maheshwari, 2018b; Sánchez-Cruz et al., 2019; Sukul et al., 2021).

Previous studies have shown that PGPR found in the endophyte region possess many plant growth-promoting activities such as nitrogen fixation, phosphate solubilization, siderophore production, phytohormone production, and nutrient availability (Compant et al., 2005a; Lee et al., 2004; Lodewyckx et al., 2002; Puente et al., 2009; Wakelin et al., 2007). Bacterial endophytes share a symbiotic relationship with the plant and reside or colonize inside the plant (like roots) (Sukul et al., 2021). Bacterial endophytes promote plant growth by regulating osmotic potential, altering the root morphology and physiology, modifying stomatal responses, and increasing nutrient uptake (Compant et al., 2005b, 2005c). They also act as biocontrol agents by producing antibiotics, enzymes such as chitinases, hydrolases, and glucanases (Compant et al., 2005b). Endophytic bacteria can also prompt plant growth by triggering induced systemic resistance (Barka et al., 2002). In addition to these, many endophytes have also been reported to carry out phytoremediation (Puente et al., 2009).

The present study centers on the isolation, identification, and characterization of the endophytic population of a native plant, *Ceanothus velutinus,* commonly known as snowbrush ceanothus. A metagenomic study of the microbial population of snowbrush ceanothus roots revealed the presence of several plant growth-promoting bacteria. This observation prompted us to isolate the endophytic bacteria from the roots of the snowbrush ceanothus. Snowbrush ceanothus is an evergreen shrub that is native to western North America. It distributes from British Colombia to California, east to

Colorado. Snowbrush ceanothus is an aromatic plant that grows in dry areas and is cold tolerant. This plant can also fix atmospheric nitrogen and is of great use in restoration sites (Stein et al., 2010; Zavitkovski, 1966).

2. Methodology

2.1 Sample collection

The bacterial population was isolated from the roots of the native plant *Ceanothus velutinus* (snowbrush ceanothus). The root samples were collected from the Tony Grove region of Logan, Utah (41°52’56” N-111°33’53” W, 41°52’34” N-111°34'20" W, and 41°53’15” N - 111°36’4” W). Sample collection was carried out by following the method described by McPherson et al. (2018). The snowbrush ceanothus roots were collected in conical centrifuge tubes with phosphate buffer and surfactant (6.33 g/L NaH_2PO_4, 8.5 g/L Na_2HPO_4 anhydrous, pH = 6.5, 200 µl/L Tween 20). The roots were removed from the solution after rhizosphere soil was removed by shaking. The root samples were blot dried and placed into new tubes. Then the root samples were washed with 35 ml of 50% bleach (5.5% sodium hypochlorite) and 0.01% Tween 20 for 60 seconds. The bleach solution was poured off, and the roots were washed with 70% ethanol for 60 seconds. Then the root samples were washed with 70% ethanol for 60 seconds, followed by five washes of autoclaved distilled water for about 1 minute each. The root samples were blot dried in sterile tissue paper, cut into 5 mm pieces, and stored at 4 °C.

2.2 Isolation of endophyte from snowbrush ceanothus roots

The root samples were crushed in 1 ml PBS buffer with metal beads in a SpexSampleprep 2010 Geno/Grinder (SPEX, Metuchen, NJ, USA) and centrifuged at 5000 rpm for 10 minutes. The supernatant was carefully transferred into fresh tubes. The root extract was then serially diluted in the ratio of 1:10 that means 1 ml of the

bacterial extract with 9 ml of autoclaved distilled water (ddH_2O) and serially diluted five times with sterile distilled water. The last three dilutions were spread plated onto two media compositions- ¼ nutrient agar (NA) and ¼ tryptic soy agar (TSA) (SIGMA-ALDRICH). The plates were incubated at 28 ^oC for four days or until growth was observed. Distinct colonies were picked and streaked separately to isolate single colonies. The pure cultures were obtained by streaking bacterial colonies three times.

2.3 Identification of isolated bacterial strains

Once the colonies were isolated, a colony PCR was carried out using 16S primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) as the forward and reverse primers, respectively. A 20 µl of PCR reaction was prepared, containing forward primer and reverse primer (250 nM each), DreamTaq buffer (1 X), dNTP mix (200 nM), and Dream Taq DNA polymerase (0.5 U). The PCR program was started by heating the PCR mixture at 98 ^oC for 10 minutes to break down the bacterial cells. The initial denaturation was done at 95 ^oC for 2 minutes, followed by 35 cycles of denaturation at 95 ^oC for 30 seconds, annealing at 54.3 ^oC for 30 seconds, and extension at 72 ^oC for 1½ minute. A final extension was done at 72 ^oC for 10 minutes (Applied Biosystems-ProFlex PCR system). The purified PCR samples were sequenced and searched against the 16S rRNA database in NCBI by using BLAST (Basic Local Alignment Search Tool)

2.4 Characterization of bacterial strains

2.4.1 *Morphological characterization*

The bacterial isolates identified were primarily characterized based on their texture, color, transparency, consistency, size, and other morphology traits. The texture was glossy, matte, or 50/50 glossy and matte. The bacterial colonies were either

transparent, translucent, or opaque. The size of the bacterial colony was described as a dot, normal, spread, or widespread; the consistency was categorized into dry, viscous, or 50/50 dry and viscous. Other morphological traits included additional visible characteristics of the bacterial colony, such as pigmentation (Reynolds, 2018) (Fig 14.a.). All the bacterial isolates were tested for gram stain using a gram stain kit- BD BBL™ Gram Stain Kits (Becton, Dickinson and Company, NJ, USA).

2.4.2 *Catalase production*

The isolated bacterial colonies were tested for their abilities to produce catalase using the hydrogen peroxide (H_2O_2) test. Two drops of 30% H_2O_2 were mixed with a single colony on a glass slide. If the solution starts to bubble, there is catalase production. No bubbling indicates a negative result for catalase production (Pakpour and Horgan, 2021).

2.4.3 *Siderophore production*

The isolated bacterial colonies were tested for the production of siderophore by inoculating bacteria on Chrome Azurol S (CAS) agar. CAS agar was prepared by initially preparing the CAS blue dye. Three solutions were prepared. The Solution I consisted of 0.06 g CAS dye (Millipore SIGMA) in 50 ml of ddH_2O, Solution II consisted of 0.0027 g $FeCl_3 \cdot 6\ H_2O$ (SIGMA-ALDRICH) in 10 ml of 10 mM HCl, and Solution III contained 0.073 g HDTMA (Hexadecyltrimethylammonium bromide) (SIGMA-Life Science) in 40 ml of ddH_2O. CAS blue dye was prepared by mixing 1 ml of Solution I with 9 ml of Solution II and then adding this mixture to Solution III. LB (Luria-Bertini Broth) (Fisher Scientific) and CAS dye were autoclaved separately. Once they cooled down to under 50 ^oC, 100 ml of CAS dye was added to 900 ml of LB agar (pH-6.8). The isolated bacteria were streaked on these plates and incubated at 28

^{o}C. The siderophore-producing bacteria developed a yellow-orange halo on CAS agar (Gamit and Tank, 2014) (Fig 14.b.).

2.4.4 *Indole acetic acid (IAA) production*

The amount of IAA produced by the bacterial isolated in this study was determined using a colorimetric method (Gordon and Weber, 1951). The procedure (Sarker and Al-Rashid, 2013) begins with growing the bacterial isolates in LB broth (Fisher Scientific) supplemented with 0.1% tryptophan (EMD millipore corporation) at 28 ^{o}C for 72 hours. A control had no inoculation. After 72 hours, the bacterial culture was centrifuged at 13,000 rpm for 10 minutes, and 1 ml of supernatant was transferred into a fresh tube. To this, 1 ml of Salkowski's reagent was added (Salkowski's reagent was prepared by mixing 2 ml of 0.5 mM $FeCl_3$ in 49 ml of water and then carefully adding 49 ml of 70% perchloric acid (SIGMA-ALDRICH). The bacterial supernatant along with Salkowski's reagent was incubated at RT for 25 minutes. The pink color was developed in the samples producing IAA (Fig 14.c.). After the incubation time, absorbance was read at 530 nm (Molecular Devices Spectramax). The amount of IAA (µg/ml) was determined by generating a standard curve and plotting the absorbance of the bacterial isolates against it. The standard curve was made with IAA (SIGMA) in the culture medium at 0, 5, 10, 20, 50, and 100 µg/ml (Fig 14.d.).

2.4.5 *Phosphate solubilization*

The isolated bacterial colonies from the roots of snowbrush ceanothus were tested for their ability to solubilize phosphate by streaking them on Pikovskaya's agar (HiMedia) (Pikovskaya and Ri, 1948) 28 ^{o}C for four days or until a clear halo was observed. The formation of a transparent halo around the bacterial colony indicated a positive result for phosphate solubilization on this medium (Chung et al., 2005) (Fig

14.e.). No halo formation indicated that the bacterial isolate did not solubilize phosphate. The experiment was repeated three times.

2.4.6 *Nitrogen fixation*

The ability to fix atmospheric nitrogen by the bacterial isolates was tested by growing bacteria on Norris Glucose Nitrogen-Free Medium (HiMedia) (Tarmizi, 2016) at 28 ^{o}C for four days or until a clear halo was observed. A clear halo was observed around the colony, indicating a positive result for nitrogen fixation (Wafula and Murunga, 2020) (Fig 14.f.). The isolates were screened three times.

3. Results

3.1 Isolation and identification of endophytes from snowbrush ceanothus roots

A total of 22 bacterial species were isolated from the roots of snowbrush ceanothus. Five bacterial species were isolated from ¼ NA medium (Table 14), and 17 bacterial colonies were isolated from ¼ TSA medium (Table 15) at 28 ^{o}C. The bacterial isolates sequenced and identified using a BLAST revealed the presence of the genus *Serratia* and *Pseudomonas* from ¼ NA (Table 14) and *Micrococcus, Rhodococcus, Arthrobacter, Stenotrophomonas, Microbacterium, Pedobacter, Streptomyces,* and *Paenibacillus* from ¼ TSA (Table 15). Out of the five bacterial isolates from ¼ NA medium, 3 of them (60%) belonged to the genus *Pseudomonas,* and 2 of them (40%) were from the genus *Serratia* (Table 14). Out of the 22 isolates from ¼ TSA medium, 5 of the bacterial isolates (23%) belonged to the genus *Rhodococcus*, 2 of them (about 9%) each belonged to the genus Micrococcus, *Stenotrophomonas*, and *Streptomyces* (Table 15).

3.2 Characterization of bacterial strains

3.2.1 *Morphological characterization*

The morphological characterization included color, texture, size, transparency, consistency, and other distinctive traits. They have a color range from orange, yellow, tan, brown, clear, cream, pink and white. Fifteen out of the 22 isolated colonies were glossy in texture, four were matte, and 18 were glossy. One out of 22 bacterial colonies were transparent, 15 were translucent, and six were opaque. The size of the bacterial isolates was as follows- four were dot-sized, eight had spread, two were widespread, and the rest 8 were normal-sized. The consistency of the bacterial isolates varied as follows: 4 were dry, and 18 were viscous consistency. Some other morphological traits included chalky or fuzzy (Table 14 and 15) (Fig 14.a.).

3.2.2 *Gram stain*

The gram stain results revealed that 68.2% (15 out of 22) of the total bacterial isolates were gram-positive, and 31.8% (7 out of 22) were gram-negative. All the isolates from the ¼ NA medium were gram-negative, whereas 88.2% (15 out of 17) of the bacterial isolates from ¼ TSA medium were gram-positive, and 11.8% (2 out of 17) were gram-negative.

3.2.3 *Catalase production*

All the bacterial isolates were catalase-positive from both growth mediums (Table 14 and 15).

3.2.4 *Siderophore production*

Fourteen out of the 22 bacterial colonies (64%) isolated from the roots of the snowbrush ceanothus revealed a yellow halo around the colony indicated siderophores production (Fig 14.b.) (Table 16). Four out of five or 80% of the bacterial colonies

isolated from ¼ NA medium showed siderophore production and belonged to *Serratia* and *Pseudomonas* (JG_EA_22, JG_EA_23, JG_EA_24, and JG_EA_25). The colony JG_EA_25 produced the largest halo around its colony than any other isolate, and it belonged to the genus *Pseudomonas*. Ten out of the 17 colonies (59%) isolated from ¼ TSA medium showed siderophore production by producing a yellow halo around the colony (Table 16). When identified by 16S rRNA sequencing, they belonged to the genus *Micrococcus, Rhodococcus, Pedobacter, Microbacterium, Arthrobacter,* and *Stenotrophomonas*.

3.2.5 *IAA production*

The isolates JG_EA_3 and JG_EA_5 showed the highest IAA production of 11 and 11 µg/ml, respectively (Table 17). Both isolates were isolated from ¼ TSA medium and belonged to the genus *Rhodococcus*. JG_EA_25 and JG_EA_1 showed some IAA production, 6 and 5 µg/ml, respectively. They were isolated from the ¼ NA media and ¼ TSA media, respectively. Identification revealed that JG_EA_25 belonged to the genus *Pseudomonas* and JG_EA_1 was an actinobacterium (Table 17).

3.2.6 *Phosphate solubilization*

Out of the total bacterial colonies isolated, seven isolates (32% of the total) revealed a clear halo when streaked onto Pikovskaya's agar, indicating a positive result for phosphate solubilization (Fig 14.e.) (Table 18). Three isolates were from the ¼ NA media (JG_EA_22, JG_EA_23, and JG_EA_24) and belonged to the genera *Pseudomonas* and *Serratia*.

Four isolates were from ¼ TSA media (JG_EA_4, JG_EA_7, JG_EA_9, and JG_EA_10) and belonged to the genera *Rhodococcus, Arthrobacter, Stenotrophomonas,* and *Bacillus* (Table 18).

3.2.7 *Nitrogen fixation*

About 27% of the total bacterial isolates (6 out of 22) revealed a clear halo around the bacterial colony (Fig 1.f.) (Table 19). They were JG_EA_22, JG_EA_23, JG_EA_25, JG_EA_26, JG_EA_4, and JG_EA_7. Four of the isolates were isolated from ¼ NA media and belonged to the genera *Pseudomonas* and *Serratia.* Two isolates were isolated from ¼ TSA media and belonged to the genera *Rhodococcus* and *Arthrobacter* (Table 19).

4. Discussion

The 22 endophytic bacterial strains were isolated from the roots of the snowbrush ceanothus and identified by 16S rRNA sequencing. The BLAST search showed that the isolates belong to the phylum *Actinobacteria* (12), *Proteobacteria* (7 isolates), *Firmicutes* (2 isolates), and *Bacteroidetes* (1 isolate). They belong to genera *Arthrobacter* (1 isolate), *Bacillus* (1 isolate), *Microbacterium* (1 isolate), *Micrococcus* (2 isolates), *Paenibacillus* (1 isolate), *Pedobacter* (1 isolate), *Pseudomonas* (3 isolates), *Rhodococcus* (5 isolates), *Serratia* (2 isolates), *Stenotrophomonas* (2 isolates), and *Streptomyces* (2 isolates). Bacterial endophytes from these genera have been isolated and reported in previous studies, which have promoted plant growth (Cueva-Yesquén et al., 2021; Müller et al., 2013; Oliveira Silva et al., 2020; Sabu et al., 2019; Santoyo et al., 2016; Shishido et al., 2006; Vandana et al., 2021).

Catalase is an important antioxidant enzyme that helps to protect from ROS and also breaks down hydrogen peroxide into oxygen and water to convert the toxic compounds into something non-toxic (Gerna et al., 2020). All the bacterial endophytes isolated from the roots of the snowbrush ceanothus exhibited catalase production. They belonged to the genera *Arthrobacter, Bacillus, Microbacterium, Micrococcus, Paenibacillus, Pedobacter, Pseudomonas, Rhodococcus, Serratia, Stenotrophomonas,*

and *Streptomyces.* A study conducted in the endophytic isolates from black pepper revealed catalase production by several strains of *Arthrobacter, Bacillus, Micrococcus, Pseudomonas,* and *Serratia* (Aravind et al., 2009). Some *Arthrobacter* species, such as *Arthrobacter deserti* and *Arthrobacter mobilis*, showed catalase-positive when isolated from desert soils (Hu et al., 2016; Ye et al., 2020). *Bacillus* species are a well-known catalase-producing genus, and some examples of endophytic catalase-producing *Bacillus* species include *Bacillus megaterium* in *Medicago sativa* and *Bacillus drentensis* (Anjum and Chandra, 2015; Chopra and Kumar, 2020; Khalifa and Almalki, 2015). Studies have shown catalase producing strains are present in the genera *Microbacterium, Paenibacillus, Micrococcus, Serratia, Stenotrophomonas,* and *Streptomyces* (Kaparullina et al., 2009; Mukhopadhyay et al., 1996; Nimal Christhudas et al., 2012; Padda et al., 2017; Pinski et al., 2020; Vcs, 2013; Walitang et al., 2017; Wang et al., 2013). The genus *Pedobacter* is another catalase-positive endophyte, and *Pedobacter chitinilyticus* and *Pedobacter zeae* have been isolated from wheat (Gao et al., 2017; Zhang et al., 2018). Various *Rhodococcus* species have shown catalase production such as *Rhodococcus cercidiphylli, R. artemisiae, R. pyridinivorans, R. rhodochrous,* and *R. gordoniae* (Li et al., 2008; Zhao et al., 2012).

Bacterial siderophore production chelates ferric ions that help the plant in iron uptake. Siderophore-producing bacteria also act as a bio-control agent that protects the host plants from pathogenic fungi by depriving them of iron (Ghosh et al., 2021). In this study, siderophore-producing bacterial strains belonged to the genera *Micrococcus, Pseudomonas, Rhodococcus, Serratia, Stenotrophomonas, Arthrobacter, Microbacterium, Pedobacter*, and *Bacillus*. Bacterial strains in these genera have previously been shown to produce siderophores in other studies, such as *Pseudomonas simiae* and *Pseudomonas koreensis* from the genus *Pseudomonas* (Ghazy and El-

Nahrawy, 2021; Montes-Osuna et al., 2021). Bacteria belonging to the genera *Micrococcus, Rhodococcus, Serratia,* and *Stenotrophomonas* have also shown siderophore production in earlier studies (Ghazy and El-Nahrawy, 2021; Montes-Osuna et al., 2021; Schmidt et al., 2021; Ulrich et al., 2021). Some siderophore-producing strains in the genera *Arthrobacter, Microbacterium, Pedobacter,* and *Bacillus* have also been noticed in previous studies (Borah et al., 2018; Garg et al., 2021; Koul and Adlakha, 2021; Liu et al., 2021; Orozco-Mosqueda and Santoyo, 2021).

Indole acetic acid (IAA) is an important plant growth hormone that helps in root development, elongation, and apical dominance. This auxin is a common by-product in tryptophan metabolism amongst several endophytes (Ghosh et al., 2021). The maximum amount of IAA production was observed in the bacterial isolates JG_EA_3 and JG_EA_5. These bacterial strains belonged to the genus *Micrococcus*. *Micrococcus* has been shown to promote plant growth and produce IAA in several studies (Dastager et al., 2010; Shahzad et al., 2017). A study showed IAA production by *Micrococcus luteus* in association with orchid roots (Tsavkelova et al., 2007). Another study conducted in rice seeds showed the IAA production in *Micrococcus yunnanensis* and *Micrococcus luteus* (Shahzad et al., 2017). These two species are known as IAA producing endophytic bacteria that are also potential PGPR for several plants, including Gray Mangrove- *Avicennia marina* and cucumber- *Cucumis sativus,* and sorghum- *Sorghum bicolor* (Eid et al., 2021).

Phosphate solubilization is defined as the solubilization of precipitated and insoluble forms of phosphates that are not available for plants uptake. It can be done either, by chelation, ion exchange, or acidification, into available phosphorus so the plant uptake. Many endophytic bacteria can solubilize phosphate into the available form of phosphorus for plant uptake (Ghosh et al., 2021). In this study, the endophytic

isolates JG_EA_22, JG_EA_23, JG_EA_24, JG_EA_4, JG_EA_7, JG_EA_9, and JG_E_A_10 showed the ability to solubilize phosphate. They belonged to the genera *Pseudomonas, Serratia Rhodococcus, Arthrobacter, Stenotrophomonas,* and *Bacillus*. The endophytic bacteria that belonged to the same genera were also previously reported to show the ability to solubilize phosphate and promote plant growth and development. (Alori et al., 2017; Banerjee et al., 2010; Chen et al., 2006; Mahdi et al., 2021; Oteino et al., 2015; Shah et al., 2021; Shulse et al., 2019; Ulrich et al., 2021).

Plant growth promotion by endophytic bacteria includes macro and micronutrient availability. One of the most important mechanisms of this is nitrogen fixation (Ghosh et al., 2021). Out of the 22 bacterial species isolated from the roots of the snowbrush ceanothus, seven of them formed a transparent halo around them in a nitrogen-free medium, indicating that they potentially fix atmospheric nitrogen. The positive bacterial isolates belonged to the genera *Pseudomonas, Rhodococcus, Arthrobacter,* and *Serratia*. Several studies have shown that endophytic bacteria of these genera can promote plant growth by nitrogen fixation and some even promote nodulation (Cueva-Yesquén et al., 2021; Maheshwari and Annapurna, 2017; Nyambura Ngamau et al., 2012). Nitrogen fixation by endophytes and the amount of nitrogen still need to be explored more. Some endophytic bacterial species contain both denitrification and nitrification genes (Ghosh et al., 2021).

The three bacterial isolates JG_EA_22, JG_EA_23, and JG_EA_7 showed all the PGPR traits tested in this study and belonged to the genus *Serratia* and *Arthrobacter*. Several species from *Serratia* have been tested for their plant growth-promoting abilities on common bean- *Phaseolus vulgaris* and ginger- *Zingiber officinale* (Sabu et al., 2019; Tavares et al., 2018). *Arthrobacter* has also promoted plant growth in various instances, such as helping with weed management when isolated from

Korean turf grass- *Zoysia japonica* and inoculated into the radish- *Raphanus sativus*, possessing nitrogen-fixing ability when isolated from corn- *Zea mays*, and surviving difficult conditions, such as drought (Maheshwari and Annapurna, 2017). The two bacterial isolates JG_EA_24 and JG_EA_25 belonging to the genus *Pseudomonas* also showed multiple plant growth-promoting abilities. Several endophytic *Pseudomonas* sp. have been tested for their plant growth-promoting abilities on plants such as *Sedum alfredii*, rice- *Oryza sativa*, and Pea- *Pisum sativum* L. (Chen et al., 2017; Oteino et al., 2015b; Pham et al., 2017)

5. Conclusions

Twenty-two bacterial strains were isolated from the roots of the snowbrush ceanothus in this study. Three of the bacterial isolates JG_EA_22, JG_EA_23, and JG_EA_7 belonged to the genus *Serratia* and *Arthrobacter* showed all the PGPR traits, such as nitrogen fixation, phosphate solubilization, IAA, and siderophore production. The plant growth-promoting abilities in these three isolates were more intense than the others. These isolates can be tested on model plants such as *Arabidopsis thaliana* and *Medicago truncatula* and eventually on crop plants such as maize- *Zea mays*, alfalfa- *Medicago sativa*, rice- *Oryza sativa*, and wheat- *Triticum aestivum* to promote plant growth. They are capable of being a potential bio-fertilizer and bio-stimulants to plants.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 14. Identification and morphological characterization of the endophytic strains isolated from the roots of snowbrush ceanothus in ¼ nutrient agar.

SN	Code	Color	Texture	Transparency	Size	Consistency	Morphology	Gram stain	Catalase test	BLAST results
1	JG_EA_22	Orange/Tan	Glossy	Translucent	Spread	Viscous	Faded edge	-	+++	*Serratia* sp.
2	JG_EA_23	Tan/Clear	Glossy	Translucent	Spread	Viscous	Normal	-	+++	*Serratia* sp.
3	JG_EA_24	Brown	Glossy	Translucent	Spread	Viscous	Translucent outer cloud	-	+++	*Pseudomonas* sp.
4	JG_EA_25	Clear/Cream	Glossy	Translucent	Spread	Viscous	Normal	-	+++	*Pseudomonas* sp.
5	JG_EA_26	Clear/Cream	Glossy	Translucent	Widespread	Viscous	Normal	-	++	*Pseudomonas* sp.

'-' negative, '+' mild positive, '++' moderately positive, '+++' strongly positive, '++++' highly positive

Table 15. Identification and morphological characterization of the endophytic strains isolated from the roots of snowbrush ceanothus in ¼ tryptic soy agar.

SN	Code	Color	Texture	Transparency	Size	Consistency	Morphology	Gram stain	Catalase test	BLAST results
1	JG_EA_1	Yellow	Glossy	Translucent	Normal	Viscous	Normal	+	++	Actinobacterium
2	JG_EA_3	Yellow	Matte	Opaque	Dot	Dry	Normal	+	+++	*Micrococcus* sp.
3	JG_EA_4	White	Glossy	Translucent	Widespread	Viscous	Normal	+	++++	*Rhodococcus* sp.
4	JG_EA_5	White	Matte	Opaque	Dot	Dry	chalky	+	++	*Micrococcus* sp.
5	JG_EA_7	White	Glossy	Translucent	Normal	Viscous	Normal	+	++	*Arthrobacter* sp.
6	JG_EA_8	Pink	Glossy	Opaque	Spread	Viscous	Normal	+	++	*Rhodococcus* sp.
7	JG_EA_9	Yellow/ White	Glossy	Translucent	Spread	Viscous	fuzzy	-	+++	*Stenotrophomonas* sp.
8	JG_EA_10	White	Glossy	Transparent	Spread	Viscous	Normal	+	+++	*Bacillus* sp.
9	JG_EA_11	Orange/ Yellow	Glossy	Translucent	Normal	Viscous	Normal	+	+	*Stenotrophomonas* sp.

10	JG_EA_12	Orange/ Yellow	Matte	Opaque	Dot	Dry	Normal	+	++	*Rhodococcus* sp.
11	JG_EA_13	Tan	Glossy	Translucent	Spread	Viscous	Normal	+	++++	*Rhodococcus* sp.
12	JG_EA_14	Yellow	Glossy	Translucent	Normal	Viscous	Normal	+	++	*Microbacterium* sp.
13	JG_EA_17	Orange (neon)	Glossy	Opaque	Normal	Viscous	Normal	+	+	*Rhodococcus* sp.
14	JG_EA_18	Pink	Glossy	Translucent	Normal	Viscous	Transparent halo	-	++++	*Pedobacter* sp.
15	JG_EA_19	Yellow (bright)	Glossy	Translucent	Normal	Viscous	Normal	+	+	*Streptomyces* sp.
16	JG_EA_20	Brown and White	Matte	Opaque	Dot	Dry	Brown color generation	+	+	*Streptomyces* sp.
17	JG_EA_21	Tan	Glossy	Translucent	Normal	Viscous	Forms a halo	+	++	*Paenibacillus* sp.

‘-’ negative, ‘+’ mild positive, ‘++’ moderately positive, ‘+++’ strongly positive, ‘++++’ highly positive

Table 16. Characterization of bacterial isolates from the endosphere of snowbrush ceanothus from the native habitat based on their ability to produce siderophores.

SN	Code	Siderophore production	BLAST results
1	JG_EA_25	++++	*Pseudomonas* sp.
2	JG_EA_18	+++	*Pedobacter* sp.
3	JG_EA_22	+++	*Serratia* sp.
4	JG_EA_24	+++	*Pseudomonas* sp.
5	JG_EA_7	++	*Arthrobacter* sp.
6	JG_EA_9	++	*Stenotrophomonas* sp.
7	JG_EA_10	++	*Bacillus* sp.
8	JG_EA_11	++	*Stenotrophomonas* sp.
9	JG_EA_23	++	*Serratia* sp.
10	JG_EA_1	+	Actinobacterium
11	JG_EA_5	+	*Micrococcus* sp.
12	JG_EA_12	+	*Rhodococcus* sp.
13	JG_EA_14	+	*Microbacterium* sp.
14	JG_EA_17	+	*Rhodococcus* sp.

‘+’ mild positive, ‘++’ moderately positive, ‘+++’ strongly positive, ‘++++’ highly positive

Table 17. Characterization of bacterial isolates from the endosphere of snowbrush ceanothus from the native habitat based on their ability to produce indole acetic acid (IAA).

SN	Code	IAA production	BLAST results
1	JG_EA_5	10.86±0.07	*Micrococcus* sp.
2	JG_EA_3	10.83±0.15	*Micrococcus* sp.
3	JG_EA_25	5.94±0.01	*Pseudomonas* sp.
4	JG_EA_1	4.82±0.46	Actinobacterium
5	JG_EA_24	3.75±0.05	*Pseudomonas* sp.
6	JG_EA_7	3.71±0.23	*Arthrobacter* sp.
7	JG_EA_26	3.59±0.04	*Pseudomonas* sp.
8	JG_EA_14	3.54±0.03	*Microbacterium* sp.
9	JG_EA_17	3.05±0.06	*Rhodococcus* sp.
10	JG_EA_22	2.97±0.06	*Serratia* sp.
11	JG_EA_23	2.56±0.06	*Serratia* sp.
12	JG_EA_11	2.42±0.08	*Stenotrophomonas* sp.
13	JG_EA_9	1.35±0.05	*Stenotrophomonas* sp.
14	JG_EA_12	1.25±0.06	*Rhodococcus* sp.
15	JG_EA_10	1.19±0.37	*Bacillus* sp.

Table 18. Characterization of bacterial isolates from the endosphere of snowbrush ceanothus from the native habitat based on their ability to solubilize phosphate.

SN	Code	Phosphate solubilization	BLAST results
1	JG_EA_24	++++	*Pseudomonas* sp.
2	JG_EA_7	+++	*Arthrobacter* sp.
3	JG_EA_23	+++	*Serratia* sp.
4	JG_EA_4	++	*Rhodococcus* sp.
5	JG_EA_22	++	*Serratia* sp.
6	JG_EA_9	+	*Stenotrophomonas* sp.
7	JG_EA_10	+	*Bacillus* sp.

'+' mild positive, '++' moderately positive, '+++' strongly positive, '++++' highly positive

Table 19. Characterization of bacterial isolates from the endosphere of snowbrush ceanothus from the native habitat based on their ability to fix nitrogen.

SN	Code	Nitrogen fixation	BLAST results
1	JG_EA_4	++++	*Rhodococcus* sp.
2	JG_EA_22	++++	*Serratia* sp.
3	JG_EA_23	++++	*Serratia* sp.
4	JG_EA_25	++	*Pseudomonas* sp.
5	JG_EA_7	+	*Arthrobacter* sp.
6	JG_EA_26	+	*Pseudomonas* sp.

'+' mild positive, '++' moderately positive, '+++' strongly positive, '++++' highly positive

Table 20. Bacterial isolates that tested positive for all the PGPR traits tested in this study.

SN	Code	Color	Gram stain	Catalase test	Siderophore production	Phosphate Solubilization	Nitrogen Fixation	IAA (ug/ml)	BLAST results
1	JG_EA_22	Orange/Tan, glossy, translucent, spread, viscous, and faded edge	-	+++	+++	++	++++	2.97±0.06	*Serratia* sp.
2	JG_EA_23	Tan/Clear, glossy, translucent, spread, viscous, and normal	-	+++	++	+++	++++	2.56±0.06	*Serratia* sp.
3	JG_EA_7	White, glossy, translucent, normal, and viscous	+	++	++	+++	+	3.71±0.23	*Arthrobacter* sp.

'-' negative, '+' mild positive, '++' moderately positive, '+++' strongly positive, '++++' highly positive

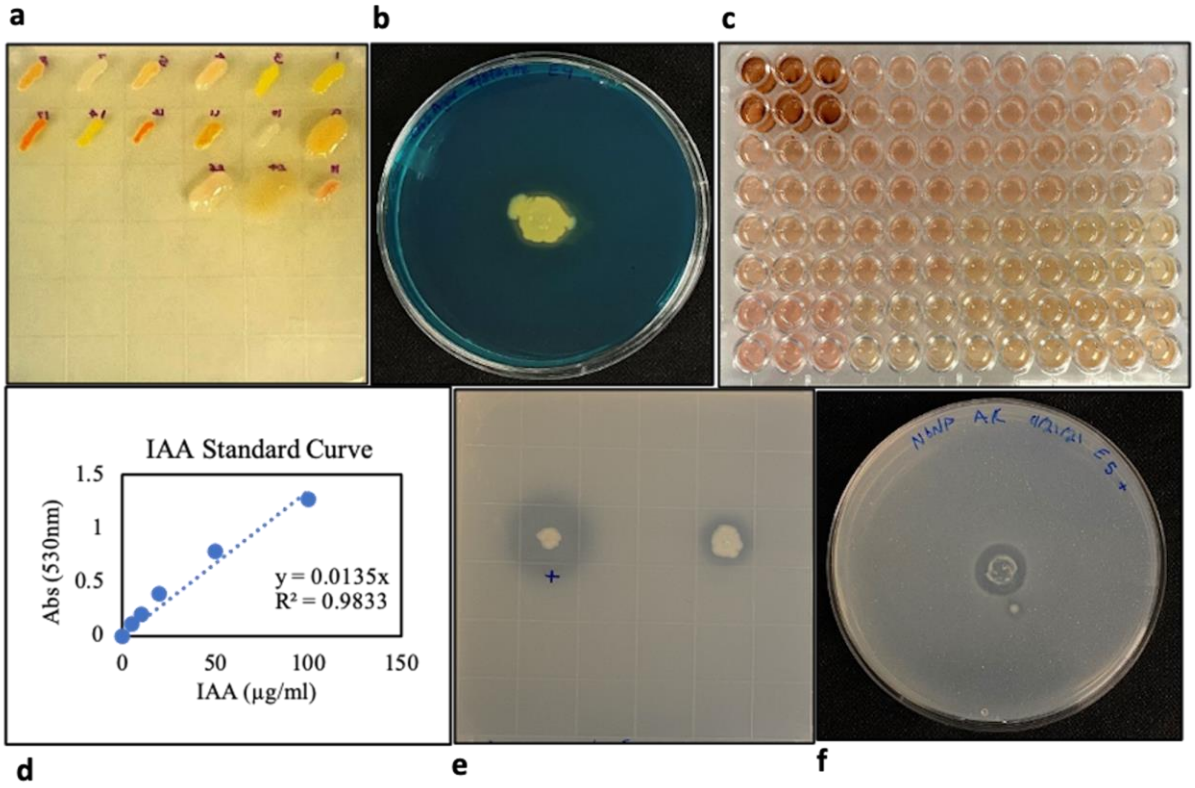

Fig 14. Bacterial characterization of isolates from the endosphere of snowbrush ceanothus **a.** Representative figure for morphological characteristics, **b.** siderophore production on CAS agar media, **c.** IAA production **d.** IAA standard curve, **e.** phosphate solubilization on Pikovskaya's agar media and **f.** nitrogen fixation on Norris Glucose nitrogen-free agar media.

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CHAPTER V

CONCLUSIONS

The metagenomic study of native soil and native soil-treated plants provided information on the presence of many essential PGPRs. The treatment of greenhouse-grown plants with native soil revealed the effect of the PGPRs in the native soil on the rooting, survival, growth, and development of snowbrush ceanothus plants. Nodulation was observed for the first time on snowbrush ceanothus plants under greenhouse conditions on native soil treatment. The PGPRs from the snowbrush ceanothus can be isolated and tested for their role in plant growth promotion on model and crop plants and snowbrush ceanothus propagation by cuttings. In the next step, we isolated identified and characterized bacteria from the rhizosphere and roots of snowbrush ceanothus.

Amongst the bacterial colonies isolated from the rhizosphere of the native soil and the greenhouse, GK_NR_133 (*Pantoea* sp.) showed the highest IAA production and nitrogen fixation. This isolate produced siderophores and solubilized phosphates in addition to nitrogen fixation and IAA production. *Pantoea* sp. has been known for its plant growth-promoting abilities. IAA induced root growth and elongation and is an important PGPR trait to increase plant growth. The isolates belonging to the genus *Pseudomonas* showed the highest siderophore production and phosphate solubilization. These isolates also tested positive for all the other PGPR traits in this study. Many species of *Pseudomonas* are well-known PGPRs showing their plant growth-promoting ability on many plants.

Twenty-two bacterial strains were isolated from the roots of the snowbrush ceanothus in this study. Three of the bacterial isolates JG_EA_22, JG_EA_23, and JG_EA_7 belonged to

the genus *Serratia* and *Arthrobacter* and showed all the PGPR traits, such as nitrogen fixation, phosphate solubilization, IAA, and siderophore production. The plant growth-promoting abilities in these three isolates were more intense than the others.

Snowbrush ceanothus is an ornamental plant recommended for low-water-use landscaping but difficult to propagate by cuttings. The PGPR-producing IAA isolated from this study was tested to enhance rooting in snowbrush ceanothus cutting propagation. In the future, these microbes can be used to grow snowbrush ceanothus in the landscape.

These isolates from the rhizosphere and roots of snowbrush ceanothus can be tested on model plants such as *Arabidopsis thaliana* and *Medicago truncatula* and eventually on crop plants such as maize- *Zea mays*, alfalfa- *Medicago sativa*, rice- *Oryza sativa*, and wheat- *Triticum aestivum* to promote plant growth. They can also be identified by whole-genome sequencing. Many of these isolates are potential bio-fertilizer and bio-stimulants to plants.

APPENDIX

Table 10. Bacterial isolation from the rhizosphere of snowbrush ceanothus grown in the native habitat and morphological characterization

SN	Code	Media	Color	Texture	Transparency	Size	Consistency	Morphology	Gram stain	Catalase production	BLAST
1	GK_NR_127	M9	White (Bullseye)	Matte	Opaque	Dot	Dry	Raised	+	+	*Streptomyces* sp.
2	GK_NR_188	M9	Yellow	Glossy	Translucent	Normal	Viscous	Flat	+	+	*Janthinobacterium* sp.
3	GK_NR_129	1/4 TSA	White/ Brown (Bullseye)	Matte	Opaque	Dot	Dry	Raised	+	+	*Streptomyces* sp.
4	GK_NR_130	1/4 TSA	Cream	50/50 Glossy Matte	Translucent	Normal	Viscous	Normal	-	-	*Peribacillus* sp.
5	GK_NR_131	1/4 TSA	Cream/ Pink	Glossy	Translucent	Widespread	Mucus	Normal	-	+	*Neorhizobium* sp.

6	GK_NR_133	1/4 TSA	Yellow	Glossy	Translucent	Widespread	Viscous	Raised	+	+	*Pantoea* sp.
7	GK_NR_136	1/4 TSA	Off-White/ Brown (bullseye)	Matte	Opaque	Dot	Dry	Normal	-	++	*Streptomyces* sp.
8	GK_NR_139	1/4 TSA	White	Matte	Opaque	Dot	Dry	Chalky	+	+	*Nocardia* sp.
9	GK_NR_177	1/4 TSA	Yellow (ish)/ White	Glossy	Opaque	Dot	Viscous	Flat	-	+	*Promicromonospora* sp.
10	GK_NR_195	1/4 TSA	Yellow	50/50 Glossy Matte	Translucent	Normal	Viscous	Cloudy	+	+	*Janthinobacterium* sp.
11	GK_NR_196	1/4 TSA	Pink (ish)	Glossy	Translucent	Normal	Viscous	Flat	+	+	*Pedobacter* sp.
12	GK_NR_143	1/4 NA	Brown (dark) (Bullseye)	Matte	Opaque	Dot	Dry	Raised	+	+	*Streptomyces* sp.

13	GK_NR_144	1/4 NA	White (bullseye)	Matte	Opaque	Dot	Dry	Chalky	+	+	*Xenophilus* sp.
14	GK_NR_145	1/4 NA	Yellow/ White	Matte	Opaque	Dot	Dry	Raised	+	+	*Streptomyces* sp.
15	GK_NR_146	1/4 NA	Yellow (Bullseye)	Matte	Opaque	Dot	Dry	Normal	+	++	*Streptomyces* sp.
16	GK_NR_149	1/4 NA	White	50/50 Glossy Matte	Opaque	Dot	Viscous	Flat	+	+	*Brevibacterium* sp.
17	GK_NR_150	1/4 NA	Clear	Glossy	Transparent	Spread	Viscous	Flat	+	++	*Leifsonia* sp.
18	GK_NR_154	1/4 NA	Yellow (ish)	Glossy	Translucent	Spread	Viscous	Flat	+	++	*Agromyces* sp.
19	GK_NR_179	1/4 NA	White/ Dark (bullseye)	Matte	Opaque	Dot	Dry	Chalky, raised	+	+	*Streptomyces* sp.
20	GK_NR_180	1/4 NA	Brown (bullseye)	Matte	Opaque	Dot	Dry	Normal	+	+	*Streptomyces* sp.

21	GK_NR_182	1/4 NA	White (bullseye)/ Purple (center)	Matte	Translucent	Dot	Dry	Flat	+	+	*Streptomyces* sp.
22	GK_NR_194	1/4 NA	Clear	Matte	Transparent	Dot	Dry	Flat	+	+	*Pseudomonas* sp.
23	GK_NR_197	1/4 NA	Indigo/ Purple (deep)	50/50 Glossy Matte	Translucent	Dot	Dry	Flat	-	+	*Janthinobacterium* sp.
24	GK_NR_156	AIA	Yellow (pale)	Matte	Translucent	Dot	Dry	Normal	-	+	*Staphylococcus* sp.
25	GK_NR_162	YMA	Off-White/ Mustard/ Red (bullseye)	Matte	Opaque	Dot	Dry	Normal	-	++	*Streptomyces* sp.
26	GK_NR_166	YMA	White	Matte	Opaque	Dot	Dry	Raised	-	+	*Streptomyces* sp.
27	GK_NR_186	YMA	Off-White/	Matte	Opaque	Dot	Dry	Raised	-	+	*Streptomyces* sp.

			Yellow (center) (dark)								

‘-’ negative, ‘+’ mild positive, ‘++’ moderately positive

Table 11. Bacterial isolation from the rhizosphere of snowbrush ceanothus under greenhouse conditions and morphological characterization

SN	**Code**	**Media**	**Color**	**Texture**	**Transparency**	**Size**	**Consistency**	**Morphology**	**Gram stain**	**Catalase production**	**BLAST**
1	GK_GR_41	M9	Off-white	Glossy	Transparent	Normal	Mucus	Normal	-	++	*Pseudomonas* sp.
2	GK_GR_58	M9	White/ Dark brown (bullseye)	Matte	Opaque	Normal	Dry	Normal	-	++	*Streptomyces* sp.
3	GK_GR_59	M9	Tan (light)	50/50 Glossy matte	Translucent	Spread	Dry	Wrinkly	+	-	*Priestia* sp.

4	GK_GR_74	M9	Mustard (dull)	50/50 Glossy Matte	Translucent	Normal	Viscous	Small-matte/Big-Glossy	+	-	*Priestia* sp.
5	GK_GR_75	M9	White (off)/ Yellow	Matte	Opaque	Dot	Dry	Normal	-	-	*Priestia* sp.
6	GK_GR_94	M9	Tan/ White	Glossy	Translucent	Spread	Viscous	Normal	-	+	*Pseudomonas* sp.
7	GK_GR_106	M9	White	Glossy	Translucent	Spread	Viscous	Normal	-	++	*Ancylobacter* sp.
8	GK_GR_109	M9	Off yellow	Glossy	Translucent	Spread	Viscous	Normal	-	++	*Pseudomonas* sp.
9	GK_GR_55	1/4 TSA	White (off)	Glossy	Translucent	Normal	Mucus	Normal	+	+	*Pseudomonas* sp.
10	GK_GR_88	1/4 TSA	White	Matte	Translucent	Spread	Dry	Normal	+	+	*Priestia* sp.
11	GK_GR_90	1/4 TSA	Yellow (pastel)	Glossy	Opaque	Normal	Viscous	Normal	-	+	*Pseudomonas* sp.

12	GK_GR_119	1/4 TSA	Off-white	Glossy	Translucent	Spread	Viscous	Normal	-	+	*Pseudomonas* sp.
13	GK_GR_122	1/4 TSA	Yellow (pale)	Matte	Opaque	Dot	Dry	Raised	-	+	*Streptomyces* sp.
14	GK_GR_124	1/4 TSA	White	Glossy	Translucent	Normal	Viscous	Normal	-	-	*Streptomyces* sp.
15	GK_GR_42	1/4 NA	White	Glossy	Translucent	Normal	Viscous	Normal	+	+	*Pseudomonas* sp.
16	GK_GR_43	1/4 NA	White/ Brown (bullseye)	Matte	Opaque	Normal	Dry	Raised	-	++	*Streptomyces* sp.
17	GK_GR_44	1/4 NA	Yellow/ Cream (bullseye)	Matte	Opaque	Dot	Dry	Raised	-	+	*Streptomyces* sp.
18	GK_GR_45	1/4 NA	Off-white/ Tan	Glossy	Translucent	Spread	Mucus	Normal	-	+	*Pseudomonas* sp.
19	GK_GR_60	1/4 NA	Tan	Glossy	Translucent	Normal	Viscous	Normal	+	++	*Pseudomonas* sp.

20	GK_GR_61	1/4 NA	White, Grey (bullseye)	Glossy	Translucent	Normal	Viscous	Normal	+	-	*Agrobacterium* sp.
21	GK_GR_64	1/4 NA	Yellow (light)/ White	Glossy	Translucent	Normal	Viscous	Normal	-	-	*Pseudomonas* sp.
22	GK_GR_79	1/4 NA	Clear	Matte	Transparent	Normal	Dry	Foggy	-	-	*Acidovorax* sp.
23	GK_GR_81	1/4 NA	Orange (pale)/ Pink	Glossy	Translucent	Normal	Viscous	Normal	+	+	*Pedobacter* sp.
24	GK_GR_97	1/4 NA	White/ Tan	Glossy	Translucent	Normal	Viscous	Normal	-	+	*Peribacillus* ap.
25	GK_GR_98	1/4 NA	White (tinge)	Glossy	Translucent	Widespread	Mucus	Normal	-	+	*Pseudomonas* sp.
26	GK_GR_99	1/4 NA	Orange (bright)	50/50 Glossy Matte	Translucent	Dot	Dry	Normal	+	++	*Brevundimonas* sp.
27	GK_GR_111	1/4 NA	Tan/ Mustard	Matte	Opaque	Dot	Dry	Normal	-	++	*Streptomyces* sp.

28	GK_GR_112	1/4 NA	White, Tan, Pink (bullseye)	Glossy	Translucent	Spread	Viscous	Normal	-	++	*Pseudomonas* sp.
29	GK_GR_51	AIA	Yellow	Matte	Transparent	Dot	Dry	Normal	-	+	*Variovorax* sp.
30	GK_GR_52	AIA	White (off)	Glossy	Transparent	Normal	Mucus	Normal	-	+	*Pseudomonas* sp.
31	GK_GR_66	AIA	Yellow/ White	Glossy	Opaque	Normal	Viscous	Normal	-	-	*Pseudomonas* sp.
32	GK_GR_68	AIA	Orange/ Yellow	Matte	Opaque	Spread	Dry	Secretes yellow/orange pigment	+	+	*Streptomyces* sp.
33	GK_GR_115	AIA	Yellow/ White	Glossy	Opaque	Normal	Viscous	Fuzzy	-	+	*Pseudomonas* sp.
34	GK_GR_70	YMA	Yellow (mustard)	Matte	Translucent	Dot	Dry	Secretes mustard yellow pigment	-	+	*Xenophilus* sp.

35	GK_GR_72	YMA	Yellow (light)/ White	Glossy	Transparent	Spread	Viscous	Normal	-	+	*Xenophilus* sp.
36	GK_GR_73	YMA	White/ Yellow	50/50 Glossy Matte	Opaque	Normal	Viscous	Blooming effect	+	+	*Bacillus* sp.
37	GK_GR_104	YMA	White	Glossy	Translucent	Spread	Viscous	Normal	+	++	*Pseudomonas* sp.

'-' negative, '+' mild positive, '++' moderately positive

Table 12. Bacterial characterization from the rhizosphere of snowbrush ceanothus from the native habitat based on gram stain, nitrogen fixation, phosphate solubilization, and catalase, siderophore, and IAA production

S N	Code	Gram Stain	Catalase test	Siderophore production	Phosphate solubilization	Nitrogen fixation	IAA (µg/ml)	BLAST
1	GK_NR_127	+	+	-	-	-	0	*Streptomyces* sp.
2	GK_NR_129	+	+	+++	-	-	0.02±0.06	*Streptomyces* sp.
3	GK_NR_130	+	+	-	-	-	0.17±0.02	*Peribacillus* sp.
4	GK_NR_131	-	-	-	-	-	0.23±0.09	*Neorhizobium* sp.
5	GK_NR_133	-	+	+	+++	++++	**33.52±0.15**	*Pantoea* sp.
6	GK_NR_136	+	+	++	-	-	0	*Streptomyces* sp.
7	GK_NR_139	-	++	-	-	-	0	*Nocardia* sp.
8	GK_NR_143	+	+	-	-	-	2.41±0.04	*Streptomyces* sp.

9	GK_NR_144	-	+	+++	-	-	0.66±0.34	*Xenophilus* sp.
10	GK_NR_145	+	+	-	+	-	0.12±0.12	*Streptomyces* sp.
11	GK_NR_146	+	+	-	-	-	0	*Streptomyces* sp.
12	GK_NR_149	+	+	+	-	-	**14.88±0.11**	*Brevibacterium* sp.
13	GK_NR_150	+	+	+	-	-	3.57±0.04	*Leifsonia* sp.
14	GK_NR_154	+	+	-	-	-	0.03±0.03	*Agromyces* sp.
15	GK_NR_156	+	++	-	-	-	0	*Staphylococcus* sp.
16	GK_NR_162	+	+	-	+	-	1.22±0.06	*Streptomyces* sp.
17	GK_NR_166	+	++	-	-	-	0	*Streptomyces* sp.
18	GK_NR_177	+	++	-	-	-	0	*Promicromonospora* sp.
19	GK_NR_179	+	+	-	-	-	0	*Streptomyces* sp.
20	GK_NR_180	+	+	-	-	-	0	*Streptomyces* sp.

21	GK_NR_182	+	+	-	++	-	0	*Streptomyces* sp.
22	GK_NR_186	+	+	-	+	-	0.03±0.04	*Streptomyces* sp.
23	GK_NR_188	-	+	-	-	-	0.35±0.36	*Janthinobacterium* sp.
24	GK_NR_194	-	+	+	-	+++	8.97±0.45	*Pseudomonas* sp.
25	GK_NR_195	-	++	-	-	-	0	*Janthinobacterium* sp.
26	GK_NR_196	-	+	-	-	-	0	*Pedobacter* sp.
27	GK_NR_197	-	+	-	-	-	1.88±0.06	*Janthinobacterium* sp.

'-' negative, '+' mild positive, '++' moderately positive, '+++' strongly positive, '++++' highly positive

Table 13. Bacterial characterization from the rhizosphere of snowbrush ceanothus from the greenhouse conditions based on gram stain, nitrogen fixation, phosphate solubilization, and catalase, siderophore, and IAA production

S. No.	Code	Gram stain	Catalase test	Siderophore production	Phosphate solubilization	Nitrogen fixation	IAA (ug/ml)	BLAST
1	GK_GR_41	-	++	+++	++	++	**14.08±0.58**	*Pseudomonas* sp.
2	GK_GR_42	-	++	+	+++	+++	6.05±0.27	*Pseudomonas* sp.
3	GK_GR_43	+	-	-	-	-	0.25±0.10	*Streptomyces* sp.
4	GK_GR_44	+	-	-	-	-	1.49±0.02	*Streptomyces* sp.
5	GK_GR_45	-	-	+	+++	+++	7.35±0.04	*Pseudomonas* sp.
6	GK_GR_51	-	+	++	-	-	1.69±0.05	*Variovorax* sp.
7	GK_GR_52	-	++	++++	+++	+++	11.33±1.23	*Pseudomonas* sp.
8	GK_GR_55	-	++	++++	++	+++	9.82±0.17	*Pseudomonas* sp.

9	GK_GR_58	+	+	-	-	-	1.08±0.04	*Streptomyces* sp.
10	GK_GR_59	+	+	-	-	-	2.19±0.06	*Priestia* sp.
11	GK_GR_60	-	+	++++	+++	++	12.27±0.04	*Pseudomonas* sp.
12	GK_GR_61	-	+	-	-	-	12.02±0.40	*Agrobacterium* sp.
13	GK_GR_64	-	+	+++	+	+++	10.60±0.17	*Pseudomonas* sp.
14	GK_GR_66	-	-	+++	-	++	3.82±0.03	*Pseudomonas* sp.
15	GK_GR_68	+	+	-	-	-	5.27±0.05	*Streptomyces* sp.
16	GK_GR_70	-	++	+	-	-	6.25±0.43	*Xenophilus* sp.
17	GK_GR_72	-	+	++	-	-	6.65±0.50	*Xenophilus* sp.
18	GK_GR_73	-	+	++	+	+	3.46±0.06	*Bacillus* sp.
19	GK_GR_74	+	++	+	-	-	3.09±0.17	*Priestia* sp.
20	GK_GR_75	+	-	-	-	-	8.96±0.33	*Priestia* sp.

21	GK_GR_79	-	-	+	-	-	7.00±0.57	*Acidovorax* sp.
22	GK_GR_81	-	-	+	-	-	2.01±0.09	*Pedobacter* sp.
23	GK_GR_88	+	+	-	-	-	3.18±0.10	*Priestia* sp.
24	GK_GR_90	-	+	++++	++	++	5.19±0.16	*Pseudomonas* sp.
25	GK_GR_94	-	+	+	+++	++	4.16±0.02	*Pseudomonas* sp.
26	GK_GR_97	+	++	++	-	-	0.03±0.05	*Peribacillus* ap.
27	GK_GR_98	-	++	++	+	+++	11.79±0.08	*Pseudomonas* sp.
28	GK_GR_99	-	++	+	-	-	2.39±0.07	*Brevundimonas* sp.
29	GK_GR_104	-	+	+++	+	+++	5.49±0.09	*Pseudomonas* sp.
30	GK_GR_106	-	+	+	+	+	5.82±0.23	*Ancylobacter* sp.
31	GK_GR_109	-	-	+++	++	++	0.49±0.19	*Pseudomonas* sp.
32	GK_GR_111	+	+	-	-	-	3.28±0.07	*Streptomyces* sp.

33	GK_GR_112	-	+	+++	-	+++	3.86±0.06	*Pseudomonas* sp.
34	GK_GR_115	-	+	++++	-	+++	4.68±0.06	*Pseudomonas* sp.
35	GK_GR_119	-	+	++	+++	+++	0.43±0.07	*Pseudomonas* sp.
36	GK_GR_122	+	+	-	-	-	6.29±0.09	*Streptomyces* sp.
37	GK_GR_124	+	++	-	-	-	0.05±0.03	*Streptomyces* sp.

'-' negative, '+' mild positive, '++' moderately positive, '+++' strongly positive, '++++' highly positive

Fig 13. Standard curve for the IAA test.