



Research Article

Isolation and Identification of Rhizospheric Bacteria from the Juniper Forest of Ziarat, Balochistan

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Abstract | The Greek juniper, the predominant and evergreen species in Ziarat, Balochistan, has significant socioeconomic and environmental significance, but it faces extinction due to severe predatory exploitation over the past century. Restoring the nutrient content in forest soils is made possible by phosphate-solubilizing bacteria, which are crucial functional groups in controlling the elemental cycle and plant nutrition. Similar to other possible plant growth-promoting (PGP) rhizobacteria, these functional bacteria may possess advantageous characteristics or cross-functional capabilities that are necessary for plants and can enhance their growth. The purpose of this study was to isolate, identify and describe specific PGP characteristics of three functional groupings of bacteria from the soils of Ziarat juniper forests. Based on their colonial growth on the appropriate functional media, the bacteria were isolated. They were then identified using molecular and specific biochemical properties. Their functional quantitative activities and PGP properties were evaluated using the phosphate solubilization test and the production of indole-3-acetic acid (IAA). Based on their individual high functional activities, root and shoot lengths, and improvements in seedling vigor, the study results revealed the numerous PGP features of a three chosen bacterial isolates. Positive characteristics of all isolates indicated that they were potential plant growth promoting rhizobacteria (PGPR). It was possible to characterize the most effective isolates as belonging to *Pseudomonas sihuiensis*, *Enterobacter mori* and *Pantoea conspicua*. According to the study's findings, these useful bacterial strains might be used in biofertilizer formulations to help crops flourish in acidic soils. To the best of our knowledge, this is the first study to use these species as PGPR.

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Keywords | Bacterial isolation, DNA extraction, IAA production, Juniper forest, Phosphate-solubilization activities, Species identification, Ziarat



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Introduction

Greek juniper is native to Balochistan and can be found there between 30° 37'N and 68° 3'E.

This species lies at elevations of 1200–3000 meters above sea level in solitary, arid valleys. Situated in the dry temperate forest zone, the region is typified by its steep terrain and uneven, craggy ridges. The

Balochistan juniper forest is one of the world's largest, most famous, biologically significant and unique forests (Bibi *et al.*, 2015). These forests which are part of our biological heritage and are the second-biggest forest in the world with resistant to drought tree species are important for the general well-being of the local community and multiple ecosystems. Juniper forest woodlands cover roughly 141,000 hectares with approximately 86,100 hectares found in Loralai and Ziarat districts (Sarangzai *et al.*, 2010).

The rhizosphere of plants is home to plant growth promoting rhizobacteria (PGPR), which can grow in, on, or around plant tissues and have a positive impact on plant development (Akhtar *et al.*, 2012). They have the ability to directly or indirectly promote plant growth (Kloepper and Schroth, 1980). PGPR can impact plant growth by a variety of processes, including the solubilization of inorganic phosphate, the synthesis of phytohormones, siderophores, and organic acids, the reduction of plant ethylene levels, N₂ fixation, and the biocontrol of plant diseases. In efforts to attain sustainability, the use of beneficial bacteria as biofertilizers and bio-control agents has recently garnered more attention globally, especially in horticulture, forestry, and agriculture (Datta *et al.*, 2011).

In recent years, there has been a significant increase in the number of PGPR that have been found, mostly due to the rhizosphere's growing significance as an ecosystem in the biosphere's operation. Numerous bacterial species have been shown to promote plant growth, including *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus*, and *Serratia*. Many PGPR inoculants that are now commercialized to support growth in at least one way, such as by suppressing plant disease (known as Bio-protectants), improving nutrient uptake (known as Bio-fertilizers), or producing phytohormones (known as Bio-stimulants) (Saharan and Nehra, 2011).

Plant height, root length, and dry matter production of the shoot and root are all significantly increased by the use of PGPR, which presents an appealing alternative to chemical fertilizers, herbicides, and vitamins. Today's ecological and economic issues have reignited interest in the use of bio-control agents and bio-fertilizers to minimize the use of expensive and environmentally damaging agrochemicals (Rodríguez *et al.*, 2006).

Natural rhizosphere bacteria in agro-systems have been significantly impacted by agrochemicals, specifically fertilizers and pesticides (Matson *et al.*, 1997). Plant-beneficial microbial bio-resources have the potential to promote environmentally friendly crop production by supplementing or replacing many of these high-intensity, harmful techniques (Hart and Trevors, 2005). Specifically, plant growth promoting rhizobacteria (PGPR) are becoming more and more recognized globally for their advantages in agriculture and environmental activities (Vessey, 2003).

According to a number of reports, certain bacterial species, especially those that colonize rhizospheres, have the capacity to release organic phosphates or to dissolve insoluble inorganic phosphate compounds like rock phosphate, tri-calcium phosphate, di-calcium phosphate, and hydroxyapatite. According to Khan *et al.* (2010), these bacteria provide the plants with soluble phosphates in exchange for root-borne carbon molecules, mostly sugars and organic acids, which are essential for bacterial growth. Phosphate Solubilizing Microbes (PSM) inoculation of crops has the potential to cut phosphate fertilizer application rates by 50% without appreciably lowering crop production (Ahmad *et al.*, 2007). According to current research, Phosphate solubilizing bacteria (PSB) could be helpful for bioleaching rare earth elements for mined ores or for phyto-remediation of heavy metal-impacted soil (Monica *et al.*, 2017).

Unfortunately, there hasn't been any research done on PGPR in Ziarat's juniper forest. Greek juniper is the dominant species of this genus naturally occurring in Ziarat, Balochistan with a danger of distinction. Better knowledge of the bacteria linked to this tree that have the ability to promote growth must thus be a top priority. In addition to the loss of animal and plant diversity, the extinction of this valuable plant species would have a significant impact on the environment by reducing the number of subsurface microorganisms, many of which are still unknown and may be directly related to juniper. The juniper forest in Ziarat is important not only for the environment but also for society and the economy because all parts of the tree are valuable: The bark produces high-quality resin that has numerous industrial uses, the timber has good qualities for building, furniture, and paper production, and the seeds are highly nutritious for humans and many animals. Therefore, using PGPR as inoculants in nurseries and for forestation

operations may be essential to reforesting degraded areas, reviving and growing juniper forests.

This study's objectives were to: (1) isolate and identify phosphate-solubilizing bacteria from the soil of dry temperate juniper forests; and (2) characterize the isolated bacteria biochemically and molecularly for their individual functional activities, including the production of IAA, the promotion of early plant growth and certain phenotypic and biochemical traits. Accordingly, this study screened and discovered P-solubilizing rhizobacteria (*Pseudomonas sihuiensis*, *Enerobacter mori* and *Pantoea conspicua*) associated with *J. excelsa* plant roots that exhibited bio-fertilizer features and had prospective uses as native P-solubilizing bacterial bio-fertilizers.

Materials and Methods

Study area

The area for the present research is situated around Chasnak valley in district Ziarat Balochistan, which have an area of roughly 3500 hectares. The dominant tree species of the area is *J. excelsa*. The study area latitude and longitude are 30° 37'N and between 68° 3'E, as well as in some isolated dry valleys from 1200 m to 3000 m above sea level. The area includes irregular and rugged ridges with steep terrain. The juniper tract falls within the dry temperate forest region (Bibi *et al.*, 2015).

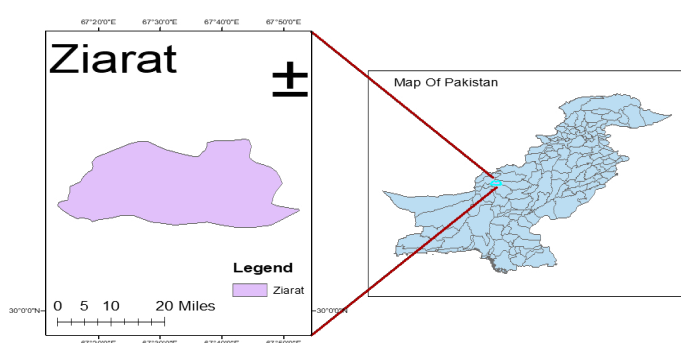


Figure 1: Map of Ziarat, Balochistan.

Survey of field area and samples collection

In order to gather rhizospheric soil, a through survey of the Chasnak valley have been conducted. Three samples (upper area soil, middle area soil and lower area soil) were collected randomly from Chasnak area. The depth of soil samples taken with a soil auger was 15-30 cm. Samples have been gathered in sterilized zipper bags, tagged and stored at 4°C for further analysis (Peck and Melsted, 2015).

Isolation of bacteria from rhizospheric soil (serial dilution plate technique method)

To isolate bacteria from rhizospheric soil, LB (Luria Bertani) media was used and soil microorganisms were isolated using the serial dilution technique. One gram of soil from the sample was suspended in 9 mL of distilled water and centrifuged thoroughly at 4000 rpm for 15 minutes in a sterilized falcon tube. The suspensions were serially diluted 10^{-1} to 10^{-9} after autoclaving for 30 minutes at 121°C. The spread plate method was used to isolate organisms from a diluted sample. After that 10 uL was pipetted onto nutrient agar plates and distributed with a glass L-shaped rod. The plates were incubated at 28 °C for 48 to 72 hours. The most prominent colonies based on colony morphology were separated and kept at 4 °C for further study (Jorquera *et al.*, 2008).

Streaking

The sterilized LB agar growth was poured in sterilized Petri plates under laminar flow hood air cabinet and left for 15 minutes to settle down. Three Petri dishes were randomly selected for spreading 10 µL from selected serial dilution was spread in Petri plates using sterilized L-shaped spreader, labeled the Petri plates and incubated at 28°C for 48 to 72 hours. After a period of 48 and 72 hours, single colony growth was checked and taken for re-streaking to get more pure single colonies using sterilized aluminum wire loop.

Preservation of isolated bacteria

Single pure colonies of bacteria isolated was taken under sterilized condition at laminar flow hood cabinet and was suspended in sterilized eppendorf tubes (1ml) containing 70% glycerol stock and preserved at -20 °C for further use.

Biochemical characterization of bacterial isolated

The following biochemical tests were carried out:

Cytochrome oxidase test

The purpose of the cytochrome oxidase test was to confirm the cytochrome oxidase enzyme presence in the bacterial colony. Under sterile condition, 24 hours old bacterial colony was applied (Khan and Bano, 2016) on to filter paper strip (DESTO Laboratories Karachi, Pakistan) impregnated with the chemical N, N, N', N'-Tetramethyl-p-phenylenediamine. The results were considered positive if color changes and finally becomes almost black (Steel, 1961).

Catalase test

To test for the presence of catalase in a 24-hours old bacterial colony, a drop of hydrogen peroxide (H_2O_2) was placed on a glass slide, and a 30 percent solution was added. If gas bubbles formed within five to ten seconds, it indicate the presence of the catalase enzyme (McFadden, 1980; Khan and Bano, 2016).

Indole acetic acid production test

The bacterial cultures had been grown for 48–72 hours in their specific medium, which contain: NaCl (10g/L), yeast extraction (5g/L), and L-tryptophane as a precursor (10g/L). To produce indole acetic acid, 50 milliliters (ml) of LB medium were inoculated with 1 ml of bacterial suspension and incubated at 28 °C. Once the cultures were fully grown, they were centrifuged at 3000 rpm for 30 minutes. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of Salkowski reagent (prepared by combining 50 ml of 35% perchloric acid with 1 ml of 0.5 MFeCl₃ solution). The production of indole acetic acid (IAA) was indicated by the development of a pink color after adding the Salkowski reagent. The optical density was measured at 530 nm using a spectrophotometer (Ahmad *et al.*, 2008).

P-solubilization index (SI) test

Under sterile condition the pure 24-hours old bacterial colonies with tricalcium phosphate were analyzed by spotting 10 micro litter on autoclaved Pikovskaya's media that contained: Ammonium sulphate (0.500g/L), calcium phosphate (5g/L), potassium chloride (0.200g/L), magnesium sulphate (0.100g/L), ferrous sulphate (0.0001g/L) manganese sulphate (0.0001g/L) and agar (15g/L) (Pikovskaya, 1948). The plates were incubated for seven days at 28°C. Phosphate solubilization was observed positive for colonies exhibiting transparent halos. Re-streaked the fresh PVK agar plates with the dominant colonies allowed for a subculture, which was then incubated at 28°C. The formula (Premono *et al.*, 1996) was used to calculate the Solubilization index (SI). According to Pradhan and Sukla (2006), the formula for calculating Solubilization Index is as follows: Colony diameter + Halo zone diameter/ Colony diameter.

Molecular characterization of bacterial isolated

Under sterile condition, 18 to 24 hours old isolated bacterial colony from glycerol stock preservation was grown on LB agar media and then pure single colony

was inoculated in LB broth and kept on incubator shaker (170 rpm) for 24 hours at 38 °C. Mini Qiagen Kit for DNA extraction was used. Following given steps were carried out for DNA extraction:

Procedure for bacterial Dna extraction through mini Qiagen kit

Taken a loop full colony and added 500µL of solution A. Added 2 µL of Solution B, 20 µL Solution C and vortex the suspension. Incubate at 65 °C for 1Hour. Added 500 µL of Solution D in it. Spin at 13000 rpm for 9 min. Taken upper aqueous layer in new tube and added 500 µL ice chilled Solution E in it. Incubated for 20 min at room temperature and then spin at 13000 rpm for 10 min. Discarded the supernatant and added 500 µL of Solution F in it Spin at 8000 rpm for 5 min. Discarded the supernatant and air dry the pellet. Added 40 µL of Solution G to the pellet and incubated at 65 °C for 30 min. The entire extracted, purified and isolated DNA was stored in -20 °C.

Polymerase chain reaction (PCR)

Primers were optimized for annealing temperature through gradient PCR method. The reaction mixture was used and conditions for PCR was: Master Mix in a volume of 5 µL, Forward primer in a volume of 1 µL, Reverse primer in a volume of 1 µL, PCR Water was in a volume of 6 µL and template was in a volume of 1 µL. The total volume for the reaction mixture was 14 µL.

Cycling conditions: amplification of 16-S rRNA

The 16-S rRNA gene was amplified in a PCR (Biometra, Germany) using 25 µ of Taq Master Mix. Thermo-cycler was taking 35 cycles to amplify the gene, temperature profile is as under: Holding stage was begins at 95 degrees Celsius for five minutes. Denaturation begins at 95 degrees Celsius for 35 seconds; Primer annealing at 58 degrees Celsius for 25 seconds; Primer extension at 72 degrees Celsius for 95 seconds and final extension at 72 degrees Celsius for 10 minutes, and hold at 4 degrees Celsius.

Primers used

Forward primer and reverse primer were used for 16-S rRNA gene amplification.

Gel electrophoresis

Purified PCR products were run on 1.5 % agarose gel. 30mL of 1.5 % gel is prepared by adding 0.45g of agarose in 30mL 1x TBE buffer. Solution was boiled

Table 1: Climate and geographic location of the research area.

S. No.	Total area (HA)	Forest type	Site	Altitude (m)	Latitude
1	3500	Dry temperate forest	Chasnak valley, district Ziarat	3000 m	30° 37'N and 68° 3'E

Table 2: Bacteria isolated from rhizospheric soil of *J. excelsa* species.

S. No.	Bacterial ID	Site	Forest tree	Location
1	SC	Lower of Chasnak (2200m)	<i>J. excelsa</i>	Chansak Ziarat
2	SB	Middle of Chasnak (2500m)	<i>J. excelsa</i>	Chasnak Ziarat
3	SA	Upper of Chasnak (2800m)	<i>J. excelsa</i>	Chasnak Ziarat

for 1.5 minutes in microwave and cooled down a bit before adding 5 µL Ethidium Bromide. 2 µL PCR samples were loaded on the gel along with control.

Statistical analysis

In the current study, different statistical tools have been used such as SPSS, standard deviation, variance, ANOVA and Microsoft Excel etc. Other parametric and statistical techniques and tools were also used based on the requirements of the following research.

Results and Discussion

Isolation of bacteria from Rhizospheric soil of *J. excelsa* species

The data was collected from Chasnak valley of district Ziarat, Balochistan having coordinate mentioned in Table 1. The bacteria isolated from *J. excelsa* were named as SC, SB and SA as given in Table 2 with location.

Biochemical characterization of bacterial isolated from Rhizospheric soil of *J. excelsa*

For biochemical characterization, bacteria isolated from rhizospheric soil of *J. excelsa* tree species having bacterial strains SA, SB and SC were tested, following four tests were carried out; showed in Table 3.

Table 3: Biochemical characterization of isolated bacteria from rhizospheric soil of *J. excelsa* tree species.

S. No.	Characterization	SA	SB	SC
1	Oxidase reaction	+	+	+
2	Catalase reaction	+	+	+
3	Indole acetic acid (IAA)	-	+	+
4	P-Solubilization	+	+	+

Cytochrome oxidase test

The purpose of the cytochrome oxidase test was to

confirm the cytochrome oxidase enzyme presence in the bacterial colony. Under sterile condition, 24 hours old bacterial colony was applied on to filter paper strip impregnated with the chemical N, N, N', N'-Tetramethyl-p-phenylenediamine. The results were considered positive for all three bacterial isolated stains (SA, SB, SC) as given in Table 3.

Catalase test

To test for the presence of catalase in a 24-hour old bacterial colony, a drop of hydrogen peroxide (H₂O₂) was placed on a glass slide, and a 30 percent solution was added. Gas bubbles formed within five to ten seconds, it indicated the presence of the catalase enzyme. All isolated bacterial strains showed catalase positive result as show in Table 3.

Indole acetic acid (IAA) production test

Indole acetic acid test were carried out for bacterial isolated. Isolated bacteria from middle (SB) and lower area (SC) rhizospheric soil showed positive test i.e., formation of red ring on the upper surface after addition of Salkowsky reagent. Isolated bacteria from upper area rhizospheric soil having strain SA showed negative test, given in Table 3. With the help of spectrophotometer, optical density was measured at 530, given in Table 4.

Table 4: Showing optical density of indole acetic acid for bacterial isolated strains at 530 nm.

S. No.	Bacteria isolated	Optical density at 530 nm
1	SA	0.917
2	SB	2.601
3	SC	2.550

Phosphate solubilization index (SI) test

Under sterile conditions, the pure 24 h old bacterial colonies, containing tricalcium phosphate was spotted

on autoclaved Pikovskaya's media (Pikovskaya, 1948) and was incubated for 7 days at 28°C. The Solubilization index (SI) was determined using the formula (Premono *et al.*, 1996). SI is derived from the following formula: Solubilization Index = Colony diameter + Halo zone diameter / Colony diameter (Pradhan and Sukla, 2006). All the bacterial isolated strains gave positive result for P-solubilization test, given in Table 3.

Molecular characterization of isolated bacteria

Quantification of DNA: The bacterial isolated strains SA, have 927 ng/μL, SB have 1387 ng/μL and SC have 1178 ng/μL DNA quantification at a wave length of 260/280, given in Table 5.

Table 5: Showing DNA quantification of bacterial isolated.

S. No.	Bacterial ID	ng/μL	260/280
1	SC	1178	1.87
2	SB	1387	1.93
3	SA	927	1.95

Identification of bacterial isolated through gene sequencing
Identified strains, SC, SB, and SA from rhizospheric soil of *J. excelsa* were carried out by 16-S rRNA sequence analysis. The comparison of sequence shows in Table 6, the bacteria having strain SC showed 99.55 % homology with *Pseudomonas sihuiensis*, bacterial isolated SB strain showed 99.76 % homology with *Enterobacter mori* species and the bacterial isolated strain SA showed 99.07 % homology with *Pantoea conspicua*.

Table 6: Alignment of 16-S rRNA and phylogenetic tree of bacterial isolated strains.

ID	Specie	Homology
SC	<i>Pseudomonas sihuiensis</i>	99.55 %
SB	<i>Enterobacter mori</i>	99.76 %
SA	<i>Pantoea conspicua</i>	99.07 %

Plant Growth Promoting Rhizobacteria (PGPR) are a group of microorganisms that live in the rhizospheric soil of various plant species. They promote plant growth through several processes, including the production of plant hormones (such as auxins and gibberellins), mineral solubilization, siderophore production, phytohormone synthesis, enhanced nutrient uptake, increased leaf foliage, higher protein content in leaves, and functioning as biofertilizers.

Additionally, PGPR protect plants from the adverse effects of oxidative stress by producing antioxidant enzymes that neutralize reactive oxygen species. These microorganisms and beneficial bacteria play an essential role in preserving soil ecology and fertility (Nagendran *et al.*, 2021). Plant Growth Promoting Rhizobacteria (PGPR) offers cross-protection by enhancing plant defence mechanisms, reducing pathogen resistance through induced systemic resistance, and mitigating abiotic stress by altering phytohormone metabolism (Khan *et al.*, 2020). These rhizobacteria play a vital role in protecting plants, improving soil health and promoting growth. Some well-known strains of beneficial bacteria include *Azospirillum*, *Pseudomonas*, *Serratia*, *Bacillus*, and *Rhizobium* species. These rhizospheric bacteria are renowned for their symbiotic relationships, promoting plant growth and soil fertility (Ijaz *et al.*, 2019).

The isolates in this study showed a number of PGPR-friendly traits as well as a variety of action mechanisms that point to their potential for promoting *J. excelsa* growth. The high quantities of IAA produced by bacteria, such as the isolates *Enterobacter mori* and *Pseudomonas sihuiensis* (SB, SC strains), for instance, surpass the IAA levels frequently reported (Husen, 2003; Yasmin *et al.*, 2007; Vasconcellos *et al.*, 2010), which is one of these advantageous characteristics. IAA produced by PGPR primarily impacts the root system, increasing the size and quantity of adventitious roots as well as their ramifications. This allows the roots to utilize a greater volume of soil, which supplies the plant with a significant amount of nutrients. The bacteria that produce a lot of root exudates therefore profit from this. However, depending on the amounts released into the root system, IAA's effects might vary. Depending on the type of plant, it may potentially inhibit plant growth.

Microbial activity is the primary source of phosphatases in soil, and their activity rises significantly in the rhizosphere (Rodriguez and Fraga, 1999). Therefore, looking for microbes in the rhizosphere that produce these enzymes becomes essential. Phosphatase production was a characteristic shared by all three isolates among the modes of action assessed in our investigation, and it might be a key characteristic for successful PGPR in the forest area. As a result, there is a broad range of applications for this PGPR. They can serve as inoculants in nurseries and could eventually become essential for producing tree seedlings for

degraded area reforestation projects. Applying these biofertilizers might lead to a significant reduction in the usage of chemical fertilizers, which would lower production costs and be a step toward the long-term growth of cleaner farming methods. Tests in nurseries and the field are still required for the isolates selected in this study. Producing inoculants and testing them in various vehicles would be the next step if their potential was verified. It is also necessary to investigate the isolates' survival in the rhizosphere and evaluate any potential alterations in the composition of the bacterial community after the strains' establishment (Ma *et al.*, 2021).

Today, the use of specific rhizobacteria as biofertilizers is considered crucial for ensuring high productivity and sustainability in the agricultural sector (Hakim *et al.*, 2021). Most rhizospheric bacteria interact favorably with plants, significantly boosting their growth and survival (Bizos *et al.*, 2020; Hakim *et al.*, 2021). Secondary metabolites are organic substances produced by various life forms, including fungi, plants, bacteria, and animals. Unlike primary metabolites essential for normal growth, development, and reproduction, secondary metabolites typically function as mediators in ecological interactions, providing organisms with selective advantages such as enhanced fertility or survivability. These compounds are often restricted to specific species within a phylogenetic group and frequently play a significant role in plant defense against interspecies attacks and herbivores. Plant secondary metabolites are categorized into several major molecular families based on their biosynthetic pathways, including flavonoids, terpenes, phenolics, steroids, and alkaloids (Kessler and Kalske, 2018).

Identification on the basis of phylogenetic analysis of 16-S *rRNA* gene showed that isolates belonged to *Pseudomonas*, *Enterobacter* and *Pantoea* genera (Swami *et al.*, 2016). Many rhizospheric bacteria from the genera, *Pantoea*, *Bacillus*, *Pseudomonas*, *Rhizobium*, *Agrobacterium*, *Alcaligenes*, *Acinetobacter*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Enterobacter*, *Erwinia*, *Flauobacterium*, *Klebsiella*, *Micrococcus*, *Mycobacterium*, *Burkholderia* have been identified as plant growth promoting bacteria (Nagargade *et al.*, 2018; Basu *et al.*, 2021).

In this study, the objective was to research eco-friendly and cultural bacteria to create a prospective

microbial consortium as bio-inoculants, to boost soil health, plant productivity and nutritional content. All the three isolated bacteria strains (*Pseudomonas sibuiensis*, *Enterobacter mori* and *Pantoea conspiciua*) consider as a novel species and have capability to boost plant growth (Sarkar *et al.*, 2021). Biochemical characterization for all three isolated strains showed significant improvement of positive indole acetic acid (IAA), P solubilization, positive catalase and oxidase result.

Novel bacteria were discovered firstly reported (Wu *et al.*, 2014) from a forest soil in Sihui City, South China. According to this study the rhizospheric soil from Ziarat juniper forests were collected from three different latitudes. The results showed the presence of phosphate solubilizing rhizobacteria at all three latitudes, which benefit the trees by solubilizing organic matter into a form that plants can readily absorb. Application of P-Solubilizing *Pantoea*, *Pseudomonas* and *Enterobacter* have resulted a plant growth promoting rhizobacteria on plant growth parameters of medicinal plant (Malleswari, 2014). In the present study, all three isolated strains are phosphate solubilizers. Moreover, genera such as: *Pantoea*, *Pseudomonas* and *Streptomyces* (Sturz and Nowak, 2000), *Klebsiella* and *Micrococcus* (Felici *et al.*, 2008; Swain and Ray, 2009), *Kitasatospora* (Shrivastava *et al.*, 2008) and *Staphylococcus* (Sati *et al.*, 2011) have previously been shown to exhibit PGP traits. Considering these above-mentioned facts, the PSB bestowed with multiple plant growth promoting traits have greater potential to be used in the future for enhancing the productivity and growth of plants (Sati *et al.*, 2011).

Conclusions and Recommendations

According to the study's findings, the natural rhizobacterial community in the soils of Greek juniper woods in Ziarat, Balochistan, contains a high effective and advantageous phosphate-solubilizing bacterial species. All of these useful bacterial strains have the potential to work in concert to enhance soil fertility and maybe stimulate plant growth through advantageous interactions. The PSB were identified across seven genera: *Pseudomonas*, *Klebsiella*, *Streptomyces*, *Pantoea*, *Kitasatospora*, *Micrococcus*, and *Staphylococcus*. Based on the study findings, two rhizobacterial isolates, SA (identified as *Pantoea conspiciua*) and SC (identified as *Pseudomonas sibuiensis*), showed potential as

bioinoculants for sustainable cultivation of agricultural crops. This study also represents the first reported analysis of cultural phosphate-solubilizing bacteria (PSBs) associated with the *J. excelsa* rhizosphere. Furthermore, additional studies targeting microbiome analysis of the rhizosphere are needed to uncover the actual diversity present.

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Novelty Statement

In this study, the objective was to research eco-friendly and culturable bacteria to create a prospective microbial consortium as bio-inoculants, to boost soil health, plant productivity and nutritional content. According to the research finding, all three bacterial isolated strains (*Pseudomonas sihuiensis*, *Enterobacter mori*, *Pantoea conspicua*) considered as a plant growth promoting rhizobacteria (PGPR) and have capability to boost plant growth through establishing a forest nursery.

Author's Contribution

Basir Ahmad: Wrote the original draft.

Lubna Ansari: Supervised the study

Shazia: Performed data analysis

Saqib Mehmood: Provided help in field for sample collection

Nasim Iqbal Butt: Proof read the draft.

Conflict of interest

The authors have declared no conflict of interest.

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