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Rhizosphere: An Ideal Site for PGPR Screening

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ABSTRACT

Rhizosphere is the thin layer of soil surrounding plant roots and play important role in plant-bacterial interactions. This rhizospheric region around plant roots is an area rich in plant growth promoting bacteria. These plant-associated bacteria usually promote plant growth through various direct or indirect mechanisms including phosphorous solubilization, phytohormone production, nitrogen fixation, iron sequestration via siderophores and production of extracellular polymeric substances etc. PGPR modify root system of plants by the production of various phytohormones which facilitate the uptake of nutrients from soil more efficiently resulting in enhanced plant growth. **Objective:** To study the growth conditions of bacterial isolates. **Methods:** In the current study, rhizobacterial isolates have been isolated from indigenous environment and characterized macroscopically, microscopically, and biochemically. These isolates have been biochemically identified using Bergey's Manual of systematic bacteriology and using ABIS 7 online software and evaluated for their various growth promoting attributes. **Results:** AS2 was identified as Bacillus sp., while AS3 and AS4 were identified as Pseudomonas sp. All three strains exhibited auxin production, nitrogen fixation, and HCN production capabilities. However, AS4 lacked ammonification and zinc solubilization potential, and AS3 lacked ACC deaminase activity. **Conclusions:** It is concluded that these bacterial isolates have ability to promote plant growth. These bacterial isolates can be further used for plant stimulating agents for sustainable agriculture practices.

INTRODUCTION

Rhizosphere is the area of soil around plant roots. It is biologically active area enriched in diverse microbes. Bacterial count in the rhizosphere is high because of the exudates released by roots that contain products mainly sugars, amino acids, vitamins, proteins, carbohydrates etc. which are source of nutrients for bacteria [1, 2]. These rhizospheric bacteria can promote plant growth and health through various mechanisms and are known as plant growth promoting rhizobacteria (PGPR). PGPR include various genera of soil bacteria that promote growth of the associated plants [3]. Growth promoting bacteria may be free living (rhizospheric) or are present in association with plant structures such as root nodules. Some can also reside as phyllo spheric bacteria on the surface of leaves or may inhabit certain plant tissues as endophytes within root or stem [4]. These rhizospheric bacteria possess wide variety of mechanisms that stimulate plant growth. These mechanisms include phosphate solubilization, phytohormone and siderophore production, nitrogen fixation and inhibit growth of various pathogenic bacteria present around plant roots. Hence, PGPR bacteria can act as biofertilizers, biocontrol agents, biopesticides and phytostimulants. These beneficial bacteria besides increasing plant growth and health, play important role in maintaining soil fertility and sustainability. Eighty percent of the rhizobacteria can produce indole acetic acid (auxin) which stimulate the formation of lateral roots and root hair that enhance root surface area and facilitate increased uptake of essential nutrients and efficient use of water [5]. Moreover, bacterially produced auxins also stimulate cell division and elongation of plants cell. Various studies have been indicated that auxins enhance the formation of adventitious roots to absorb nutrients and water from the rhizosphere, thus, improving plant growth [6]. Various

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PGPR strains have potential to reduce the toxic effects of heavy metals by converting them to lesser toxic forms which eventually reduce bioavailability of these metals within soil (bioremediation). Hence, PGPR can stimulate plant growth by reducing metal accumulation within plant tissues [7]. In the current study, indigenous rhizobacterial strains have been isolated from the rhizosphere and their growth conditions have been observed. For the identification, these isolates have been characterized macroscopically, microscopically and biochemically. Moreover, minimum inhibitory concentrations against various heavy metals have also been evaluated.

METHODS

Bacteria strains have been isolated from rhizospheric soil samples collected from Bahawalpur, Pakistan (29.3544° N, 71.6911° E) by serial dilution method following by Ahmed *et al.*, [8]. All the bacterial isolates were grown on L-agar medium. For the optimization of growth conditions, bacterial isolates were grown using temperature range from 5°C to 55°C (5, 15, 25, 37, 45, 55), salt concentrations (2, 4, 6, 8 and 10%), pH's (5, 7, 9, 11), incubation period (24, 48, 72, 96, 120, 144 hours) and their optical density was recorded at 600 nm using spectrophotometer. Bacterial isolates were morphologically characterized by observing bacterial colony and cell morphology following Cappuccino and Sherman [9]. Various characteristics of bacterial colonies i.e., color, margins, elevation, shape, opacity and size were observed. Similarly, various traits of bacterial cell i.e., cell shape, arrangement, gram staining and spore staining were examined using oil emulsion lens. Bacterial isolates were also evaluated for their potential to resist various concentrations of heavy metals (Zn, Fe, Hg, Cu, Cr, Pb and Ag). Bacterial isolates were biochemically characterized following Hussain *et al.*, [10]. A number of biochemical tests were performed and bacterial isolates were identified using Bergey's Manual of Systematic Bacteriology and also by using ABIS 7 online software. The principal tests performed were Lactose fermentation (LAC), Methyl Red test (MR), Citrate Utilization test (CIT), Voges-Proskauer test (VP), Urease test (URE), Oxidase activity (OX), H_2S production (H_2S), Gelatin hydrolysis (GEL), Mannitol fermentation (MAN), Starch hydrolysis (STA), Indole test (IND), Oxidative fermentation test (OXF) and Blood hemolytic test (BH). Plant growth promoting traits of isolated bacterial strains were evaluated including auxin production potential [11] using tryptophan precursor, 1-Amino cyclopropane-1-carboxylate (ACC) deaminase activity was analyzed using colorimetric method following Li *et al.*, [12], ammonification potential was determined using Nessler's reagent [13], hydrogen cyanide (HCN) production potential was determined following Marques *et al.*, [14], nitrification ability using nitrogen-free bromothymol blue media following Goswami *et al.*, [15], and zinc solubilization potential was determined using tris-minimal media containing zinc oxide as insoluble zinc source following by Khanghahi *et al.*, [16].

RESULTS

Fifteen bacterial strains were isolated from collected rhizospheric soil sample out of which three auxin producing bacterial strains i.e., AS2, AS3 and AS4 were selected for further studies. All the bacterial isolates have shown maximum growth after 24 hours at 37 ℃ in the presence of 2 % NaCl and at pH 7-8. However, bacterial strain AS4 has shown maximum growth at pH 9 (Figure 1).

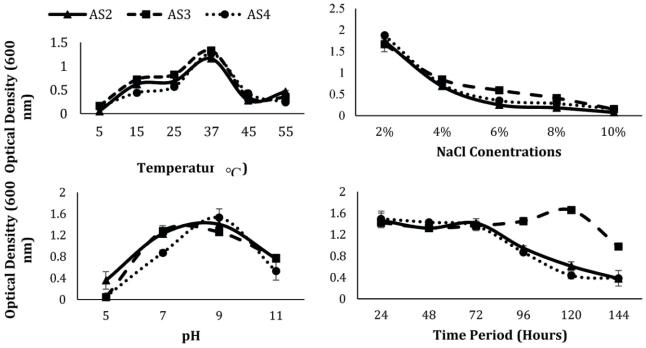

Figure 1: Impact of various temperatures (5, 15, 25, 37, 45, 55℃), salt concentrations (2, 4, 6, 8 and 10%), pH's (5, 7, 9 and 11), incubation period (24, 48, 72, 96, 120, 144 hours)

Colonies formed by bacterial isolates i.e., AS2, AS3, AS4 were creamy in color, dentate margins, round, opaque and have flat elevation except AS4 that exhibit concave elevation. All colonies vary in size. Size of bacterial colonies exhibited by strains AS2, AS3, AS4 were recorded as 5, 30 and 22 mm respectively. All three bacterial isolates were Gram-positive and spore formers. Cell shape of bacterial strain AS2 was observed as rod whereas cells of AS3 and AS4 were round in shape. All the bacterial cells were present in chains. Various biochemical tests have been performed and results were recorded. Using Bergey's Manual and ABIS 7 online software bacterial strains AS2, AS3 and AS4 have been identified as *Bacillus* sp., *Pseudomonas* sp. and *Pseudomonas* sp. (Table 1).

Table 1: Characterization of bacterial isolates

Bacterial isolates	Macroscopic characterization						Microscopic characterization			
	Color	Margin	Elevation	Shape	Opacity	Size (mm)	Gram Staining	Gpore Staining	Cell shape	Cell Arrangement
AS2	creamy	dentate	Flat	round	opaque	5	Gram-positive	Spore forming	Rod	Chains
AS3	creamy	Slightly dentate	Flat	round	opaque	5	Gram-positive	Spore forming	Rod	Chains
AS4	creamy	Slightly dentate	Concave	round	opaque	5	Gram-positive	Spore forming	Rod	Chains

Bacterial isolates	Biochemical characterization												
	LAC	MR	CIT	VP	URE	OX	H_2S	GEL	MAN	STA	IND	OXF	BH
AS2	-	+	-	+	-	+	+	-	-	-	-	Facultative	β-hemolysis
AS3	-	+	-	+	-	+	+	-	-	-	-	Strictly fermentative	β-hemolysis
AS4	-	+	-	+	-	+	-	-	-	-	-	Strictly fermentative	β-hemolysis

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[LAC- lactose fermentation; MR- methyl red test; CIT- citrate utilization test; VP- voges-proskauer test; URE- urease test; OX- oxidase activity; H_2S- H_2S production; GEL- gelatin hydrolysis; MAN- mannitol fermentation; STA- starch hydrolysis; IND- indole test, OXF- oxidative fermentation test; BH- blood hemolytic test

Minimal inhibitory concentration of bacterial isolates against various heavy metals have been recorded in Figure 2.

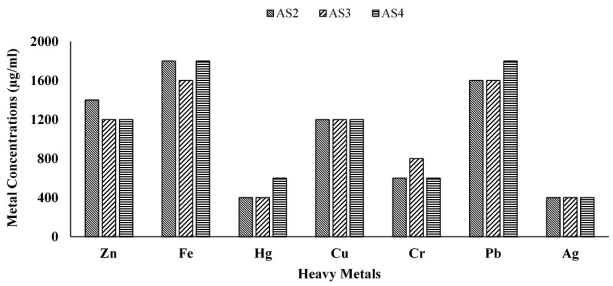

Figure 2: Minimal inhibitory concentration of bacterial isolates against various heavy metals (Zn, Fe, Hg, Cu, Cr, Pb, Ag)

All bacterial strains showed auxin, nitrogen fixing and hydrogen cyanide production potential. Bacterial strain AS4 lack ammonification and zinc solubilization potential and bacterial strain AS3 lack ACC deaminase activity (Table 2).

Table 2: *In vitro* analysis of PGP attributes

Sr. No.	Strain codes	Auxin production potential	Ammoni-fication	Nitrogen fixation	Zinc solubilization potential	HCN production	ACC Deaminase activity
1	AS2	+	+	+	-	+	+
2	AS3	+	+	+	+	+	-
3	AS4	+	-	+	-	+	+

DISCUSSION

Plant growth promoting bacteria (PGPB) are the microbes that have phytostimulatory activities. They can colonize the microhabitats in rhizosphere such as rhizoplane and root endosphere and act as root microbiome [17]. They possess various direct and indirect potentials having mechanisms that act synergistically to stimulate plant growth [18]. The focus of current study is the screening of rhizobacteria from rhizosphere. Rhizobacteria play an important role in enhancing plant growth by regulating various plant metabolic pathways and improving soil nutrients. The role of PGPR in agriculture have been extensively studied by different scientists mainly due to their growth stimulatory activity in environment friendly manner. Mercado-Blanco [19] reported various bacterial genera as a potential plant growth stimulator such as *Bacillus*, *Azotobacter*, *Burkholderia*, *Pseudomonas*, *Azorhizobium Bradyrhizobium* etc. In the current study, three bacterial isolates have been screened from rhizospheric soil sample. Growth conditions of these isolates have been optimized and it was observed that all bacterial isolates have shown maximum growth after 24 hours, at 37 ℃ in the presence of 2 % NaCl and at pH 7-8. These isolates were further characterized microscopically and macroscopically to examine specific attributes of bacterial isolates. Bacterial isolates were single streaked to observe their macroscopic features. Gram staining results have shown that all the bacterial isolates were Gram-positive rods and cocci. Gram-positive bacteria can retain the color of primary dye due to their thick peptidoglycan cell wall whereas Gram-negative bacteria were unable to retain color of primary dye to their lesser peptidoglycan content within cell walls. Instead, they appear pink due to safranin [20]. PGPR have developed several mechanisms that act in synergistic manner to regulate plant growth. These mechanisms either directly or indirectly influence plant health. The direct mechanism involves the nutrient acquisition facility to plants by improving soil nutrient status via biosynthesis of phytohormones, mineral solubilization and atmospheric nitrogen fixation whereas indirect methods involve plant defense responses that induce systemic acquired resistance against various biotic and abiotic environmental conditions and protecting plants from intruders [21, 22]. In the current study, the isolated bacterial strains have been identified biochemically as *Bacillus* sp. and *Pseudomonas* sp. Moreover, various plant growth promoting attributes i.e., auxin production ability, mineral solubilization potential, ACC deaminase activity, ammonification potential, HCN production potential, biological nitrogen fixing ability, biofilm and EPS production potential of the selected bacterial isolates were evaluated. Various rhizospheric bacteria are capable of producing auxins as a part of their metabolic activity. Rhizobacterial indole-3-acetic acid (IAA) production is one of the most prominent PGP attributes adopted by more than 80% of rhizobacteria [23]. The bacterially produced IAA is structurally and functionally similar to plant IAA and is important signaling molecule in maintaining and establishing plant-microbe interactions [24]. These rhizobacterial species have been reported as potential plant growth stimulating agents. Moreover, *Bacillus* sp. have also been reported to produce auxins. Auxin is most common type of phytohormone produced by most of PGPR. This exogenous bacterial auxin greatly affects plant's endogenous hormonal status and control various growth and developmental processes. Auxin primarily affects root morphology and architecture by promoting the growth of adventitious and lateral roots and hence stimulate plant growth by enhancing nutrient uptake and water absorption. Literature has showed that rhizobacteria have positive effect on root health and regulate other physiological mechanisms by triggering the hormonal signals from root

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to shoot [25]. Moreover, beside auxin production, these bacterial strains also have ability to solubilize phosphate and have ACC deaminase activity [26]. PGPR have also reported to promote plant growth in metal contaminated soils [27]. This bioremediation activity of *Bacillus* sp. and *Pseudomonas* sp. have also been studied by Pandey *et al.*, and Wani *et al.*, [28, 29]. Hence, plants inoculated with these bacterial species may enhance plant growth and can be used as biofertilizers.

CONCLUSIONS

In the current study indigenous bacterial isolates have been screened from rhizospheric soil sample. It is assumed that these bacterial isolates have ability to promote plant growth and hence, can be used for sustainable agricultural practices.

Authors Contribution

Conceptualization: AA, AT
Methodology: AA, AT
Formal Analysis: AA, AT
Writing-review and editing: AA, AT

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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