Original Article

Isolation of Plant Growth Promoting Bacteria from the Rhizosphere of Different Plants and Assessment of Their Plant Growth Promotion Potential

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Abstract:

Plant are benefitted in different aspects by symbiotic bacteria. Environmental conditions, Plant conditions and type of pathogens determine these important services for plants **Objective:** The research was conducted to assess the plant growth enhancing effects of wheat and cabbage rhizobacteria on the growth of wheat plant **Methods:** For this purpose, total 49 bacteria were isolated and characterized from the rhizosphere of wheat and cabbage plants. The isolates were assessed for plant growth promoting properties such as: indole acetic acid production, phosphate solubilization, antibacterial activity and heavy metal resistance. Indole acetic acid was found to be produced by 7 isolates and phosphate solubilization was shown by 20 isolates. Antibacterial activity was determined against four clinical isolates like *Staphylococcus aureus, Klebsiella sp., Escherichia coli* and *Pseudomonas aeruginosa* **Results:** Antibacterial activity against *Staphylococcus aureus* was shown by 38 isolates, 12 isolates showed antibacterial activity against *Escherichia coli* and *Klebsiella sp*., whereas no isolate was found to be positive against *Pseudomonas aeruginosa.* Another plant growth enhancing trait (heavy metal resistance) was shown by 28 rhizobacteria. In order to evaluate the capability of isolates to enhance the plant growth, bio-inoculation assay was performed using wheat seeds **Conclusions:** Rhizobacterial inoculation increased the number of roots, shoots, leaves and roots and shoot length of wheat plantlets as compared to un-inoculated control.

Introduction:

The term rhizosphere was first introduced by the German microbiologist Hiltner in 1904. The area influenced by root exudates with dynamic microbial community is called rhizosphere. Rhizosphere consists of three components including rhizospheric soil, root itself and rhizoplane. There is a group of bacteria which reside in rhizosphere and colonize under the influence of root, known as rhizobacteria. In 1 g of rhizospheric soil approximately 10^7-10^9 CFU culturable rhizospheric bacteria are present [1]. While the number of bacterial species in 1 g of non rhizospheric soil varies between 2000 and 8.3 million [2, 3]. Mostly rhizobacteria are Gram negative rods with less Gram positive cocci, pleomorphic or rods [4]. Some rhizobacteria are capable of exerting positive effects on plant growth, named as plant growth promoting rhizobacteria (PGPR). PGPR have a critical role in promotion of plant growth and belongs to the following genera such as: *Enterobacter, Bacillus, Rhizobium, Arthrobacter, Pseudomonas, Azospirillium, Beijerinckia, Azotobacter, Burkholderia, Alcaligenes, Erwinia, Flavobacterium, Acinetobacter* and *Serratia* [5]. PGPR can be distinguished by the following intrinsic uniqueness's: PGPR should be capable of colonizing the root surface.To promote the plant growth, colonization of root is very crucial or first step of rhizobacteria that can lead to

exhibit beneficial effects [6, 7]. Root colonization by bacteria is a vital process in which bacteria exists on seeds or other plant parts by attaching and growing on root surface [8]. Colonization of bacteria is facilitated by dynamic bacterial characteristics such as: quorum sensing, production of fimbriae, production of specific cell surface components, motility and chemotaxis in response to root exudates [9-11]. Rhizobacteria should be able to survive, grow and establish their superiority over other soil microbes at least for a time which is needed to exhibit their plant growth promoting characteristics. They should stimulate the plant growth [8]. PGPRs enhance the plant growth by direct or indirect mechanisms of plant growth promotion. Direct enhancement effect of PGPR denotes to the phyto-hormones production such as gibberellic acid, ethylene, indole acetic acid (IAA) and cytokinins [12], providing fixed atmospheric nitrogen or by phosphorus compounds solubilization [13]. The indirect enhancement effect of PGPR denotes to the production of antifungal, metabolites and siderophore production [14]. This study was designed to isolate and characterize bacteria from wheat and cabbage rhizosphere and then to access the bio-inoculation effects of the isolates on plant growth promotion.

Methods:

Plant root adhering soils of wheat and cabbage plants were collected in sterile plastic bags from different fields of University of Agriculture Faisalabad. Collected Soil samples (1g/sample) were thoroughly mixed in 99 ml of autoclaved distilled water to make suspensions. Soil samples were further serially diluted (10^{-3}–10^{-6}). 100µl from each dilution was spread on Nutrient-agar plates and incubated at 37°C for 24 hours. After incubation different bacterial isolates were selected on the basis of colony morphology. The selected bacterial isolates were then purified on Nutrient-agar [15]. All the isolates were identified morphologically and biochemically[15]. After purification and characterization plant growth promoting properties of isolates were accessed. Phosphate solubilization was detected by line streak inoculation on pikovskaya agar. After 48 hours of incubation at 37°C, clear zone around growth was considered as positive result for phosphate solubilization [15].

For indole acetic acid quantification, 25 ml of L-broth was added in 100-ml Erlenmeyer flasks, autoclaved and cooled. In the autoclaved L-broth medium 2.5 ml of filter sterilized (0·2 µm membrane filter) tryptophan solution (1%) was added to achieve a final concentration of 1mg/ml. The flask contents were inoculated by adding 1.0 ml of 24 hours old bacterial broth cultures adjusted to an optical density of 0.5 measured at 550 nm by spectrophotometer. The flasks were incubated at 37ºC for 72 hours at 120 rev min^{-1} shaking. After completion of incubation, the contents were centrifuged. 2ml of Salkowski's reagent was added in 1ml of each bacterial supernatant and incubated at room temperature for 30 minutes in dark. After 30 minutes auxin compounds expressed as (IAA-equivalents) were determined by taking optical density at 535nm by spectrophotometer. The optical densities were then used to determine the concentration of IAA produced by bacteria using standard curve of IAA [15]. Heavy metal resistance of bacterial isolates was determined against Cr^{+3} heavy metals which is generally found in polluted environment. 5% stock of heavy metal was prepared in autoclaved distilled water. Varying concentrations (100, 300, 500, 700 and 1000µg/ml) of stocks was added in autoclaved Nutrient agar before pouring. Heavy metal supplemented medium was streaked with bacterial isolates and incubated at 37ºC for 24 hours. After completion of incubation bacteria which grew on heavy metal supplemented agar medium were considered as resistant and those which were unable to grow were considered as sensitive.

Antibacterial activity of bacterial isolates was evaluated against four different clinical isolates: *Staphylococcus aureus, Escherichia coli, Klebsiella sp.* and *Pseudomonas aeruginosa* by

well diffusion method. Bacteria were grown in L-broth for 48 hours. After 48 hours the bacterial cultures were centrifuged and supernatant were saved. Mueller Hinton media was prepared in distilled water and autoclaved. Plates containing Mueller Hinton agar were swab streaked with four different clinical isolates and wells were created with a sterile Pasteur pipette of even thickness. 50 µl bacterial supernatants were poured in each well marked with respective bacterium. The supernatants were allowed to diffuse in the agar for thirty minutes. Plates were then incubated at 37ºC for 24 hours. After completion of incubation bacterial wells with clear zones around them, were considered as resistant to the clinical isolate and those bacterial wells with no clear zones were considered as sensitive. To investigate the role of rhizosphere bacteria in stimulating the growth of plant germination experiments were carried out in laboratory. Mono cultures and consortium cultures of bacteria were used to inoculate the seeds of *Triticum aestivum.* The seeds were grown in plastic pots (5 seeds per pot) containing 80 g sterile garden soil. The seeds were inoculated with the bacterial suspensions by dipping seeds in bacterial suspensions for half an hour. The pots were moistened daily to maintain 60% of the whole water holding capacity (WHC). The plants were grown for 14 days. After 14 days plants were harvested. After growth, seedlings were harvested and different growth parameters were recorded. These parameters included: Percentage germination, Shoot lengths (cm), Root lengths (cm), Seedling lengths (cm), Number of roots, Number of shoots and Number of leave [16].

Results:

Total 49 isolates, 30 from wheat rhizosphere and 19 from cabbage rhizosphere were obtained. Isolates were identified morphologically and biochemically. Among all the bacterial isolates *Bacillus* and *Pseudomonas* genera were found to be the most dominant. Other isolates were from *Lactobacillus, Escherichia, Stapylococcus, Streptococcus, Micrococcus* and *Klebsiella* genera.

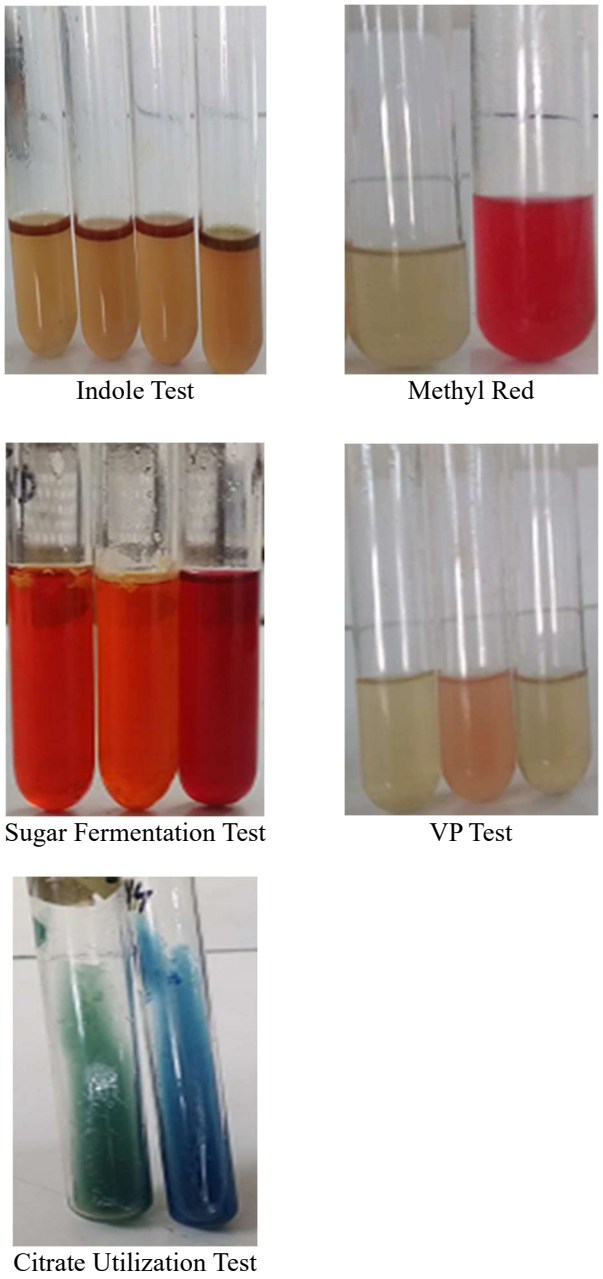

Figure 1: Biochemical Tests

Seventeen strains were able to solublize phosphate as seen in Fig. 2. Most of phosphate solublizing bacteria were found to belong to the genera of *Pseudomonas, Klebsiella, Staphylococcus* and *Bacillus.* The IAA production ability of isolated rhizobacteria was determined. IAA quantitation results showed that seven strains were good IAA producers, producing IAA upto 200, 175, 210, 225, 152, 203 and 255µg/ml respectively.

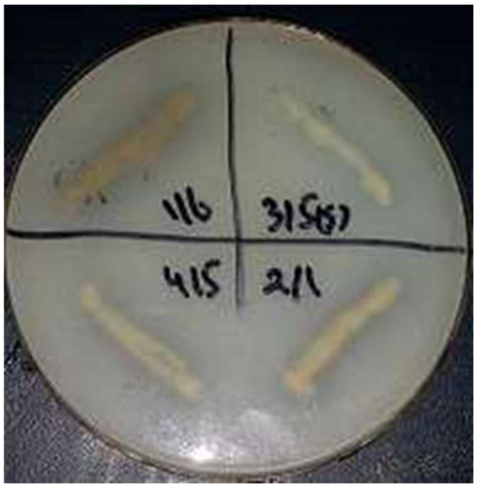

Figure 2: Phosphate Solubilization

Because of well reported importance of plant associated bacterial communities in relieving heavy metal toxicity of plants, the bacterial isolates were evaluated for their ability to tolerate heavy metal (Cr^{+3}). All bacterial isolates showed resistance to different concentrations of heavy metal except WS3/5a- *Staphylococcus spp*. which was sensitive to all concentrations of heavy metal. Whereas 22 isolates were found resistant to Cr^{+3} up to the concentration of 1000 µg ml^{-1}. To determine the antibacterial activity of isolated bacteria four different clinical isolates *Staphylococcus aureus, Klebsiella sp., Escherichia coli* and *Pseudomonas aeruginosa* were used. *Staphylococcus aureus* was sensitive to the antimicrobial compounds produced by majority of the isolates. Clinical isolates were found resistant to most of the isolated rhizobacteria. On the whole, 4 strains showed antibacterial activity towards all clinical isolates as shown in Fig. 3. The rest of the isolates gave variable results resisting one or two clinical isolates.

Figure 3: Antibacterial Activity

As plant growth promoting bacteria can enhance plant growth, health, condition and yield, the seeds of commercially important crops can be treated with these bacteria for getting better yields. Before any seed treatment the effect of inoculating strain should be properly assessed through pot and field experiments. In present study, to assess the effects of inoculated bacteria on plant growth, plant microbe interaction of isolated bacteria was carried out. The trend of percentage germination, no of roots and leaves remained consistent between control and majority of bacterized seeds. Most of bacterized seeds gave results of root and shoot length similar to the untreated seeds of control but six isolates (CS1/7a- *Bacillus megaterium*,WS4/5b-*Bacillus megaterium*, WS4/1-*Escherichia coli*, CS1/5- *Lactobacillus sp.*, WS1/6- *Micrococcus sp.*, WS1/5- *Klebsiella sp.*, and consortium of WS4/1, WS4/5b, CS1/5, consortium of WS1/5, CS1/5, CS1/7a, WS1/6, consortium of WS4/1, WS4/5b, CS1/7a, consortium of WS4/1, WS1/6, WS1/5, WS4/5b, CS1/5, CS1/7a) were able to affect root and shoot length, up to an observable level. The root length was enhanced up to 4 cm and shoot length was increased up to 0.5 cm in case of CS1/7a treated seeds. In case of WS4/5b a treated seed root length was increased by 7 cm and decrease in shoot length by 1 cm was observed. The increase of root length up to 6.25 cm and increase in shoot length by 4 cm was observed in case of WS4/1 treated seeds. Seed treatment with consortium of WS4/1, WS4/5b and CS1/5 resulted in a root length enhancement of 3 cm and increase in shoot length by 2.5 cm. Seed treatment with consortium of WS1/5, CS1/5, CS1/7a and WS1/6 resulted in a root length enhancement of 1.9 cm and increase in shoot length by 3.75 cm. In case of consortium containing WS4/1, WS4/5b and CS1/7a treated seeds root length was enhanced up to 2.9 cm whereas shoot length was increased up to 3 cm. The root length was increased up to 11.1 cm and shoot length was enhanced up to 4.1 cm in case of consortium containing six strains WS4/1,

WS1/6, WS1/5, WS4/5b, CS1/5 and CS1/7a. Most of root length enhancing bacteria was found to be those which also exhibited good IAA production potential.

Discussion:

Rhizosphere colonization by PGPR produces beneficial effects on plant growth, yield and development. There is a need to explore the soil and plant interactions with microbial communities of rhizosphere in order to control the microbial densities in rhizosphere for the benefit of plant. The absolute mechanism of plant growth enhancement is not clearly understood but some suppositions of the plant growth enhancing traits such as phosphate solubilization, indole acetic acid production, antibacterial activity and heavy metal resistance are assumed to be involved [17]. In present study 49 isolates were identified and screened for plant growth enhancing characteristics. 17 isolates were positive for phosphate solubilization. Among the rhizosphere microbes, the important phosphate solubilizing bacterial genera include *Bacillus* and *Pseudomonas* [18]. 7 isolates produced IAA upto 200, 175, 210, 225, 152, 203 and 255µg/ml respectively.. There is a report of isolation of IAA producing bacteria from wheat and banana rhizosphere respectively [19]. IAA production depends on substrate availability, growth stage and culture condition and its amount varies among different isolates [20].

Worldwide industrialization has increased the chromium contamination. 22 isolates showed chromium tolerance up to concentration of 1000 µg ml^{-1}. [21] Ovese*t al.*, 2013 reported the isolation of *P. aeruginosa* from the water contaminated with heavy metal, used to irrigate the mustard crop rhizosphereic soil. This strain showed chromium resistance up to concentration of 1800 µg ml^{-1}. All four clinical isolates *Staphylococcus aureus, Klebsiella sp., Escherichia coli* and *Pseudomonas aeruginosa* were found sensitive to antimicrobial compounds produced by only 4 isolates [20]. Whereas clinical isolates showed resistant to most of the isolates. Sayyed *et al.*, 2016 reported that fluorescent *P. aeruginosa* showed antibacterial activity against *S. aureus, B. subtilis* and *P. vulgaris.* In present study, to assess the type of interaction and effects of inoculated bacteria on plant growth plant microbe interaction of isolated bacteria was carried out. The trend of percentage germination, no of roots and leaves remained consistent between control and majority of bacterized seeds.

Most of bacterized seeds gave results of root and shoot length similar to the untreated seeds of control but inoculation withCS1/7a- *Bacillus megaterium*,WS4/5b-*Bacillus megaterium*, WS4/1-*Escherichia coli*, CS1/5- *Lactobacillus sp.*, WS1/6- *Micrococcus sp.*, and WS1/5- *Klebsiella sp.* enhanced root length however suppression in shoot length was observed in seeds treated with CS1/5, WS1/6, WS4/5b, WS1/5, consortium of WS1/6, WS1/5, CS1/7 a, consortium of WS4/1, WS4/5b, WS1/6, CS1/5 and consortium of WS1/6, WS1/5, CS1/5. CS1/7a did not affect shoot length. Most of root length enhancing bacteria was found to be those which also exhibited good IAA production potential. As Davies in 1995 reported that root is one of the plant's organs that is most sensitive to fluctuations in IAA, and respond to increasing amounts of exogenous IAA by elongation of the primary root and formation of lateral and adventitious roots. This could be the probable reason of root length enhancement in case of seed treatment with IAA producing strains.

Findings of this study suggested that Inoculation of seeds with PGPR can lead to replacement of chemical fertilizers with bio-fertilizers at commercial level. As bio-fertilizers are eco-friendly and safe to use.

Conclusions:

The results of this study indicate well diverse bacterial communities in the rhizosphere of wheat and cabbage plants. Most of the wheat and cabbage rhizospheric bacterial isolates were capable of producing indole acetic acid, phosphate solubilization, heavy metal resistance

and antibacterial activity. A variety of bacterial isolates hold a good potential to be used as plant growth enhancing bacteria. Seed inoculation with individual bacteria and bacterial consortium, enhanced the plant growth. On the basis of consequences of this study it can be concluded that harnessing the importance of indigenous microbial communities and their commercial exploitation can lead to enhanced agricultural production. This study can be used as a model to find out effective plant growth enhancing rhizobacteria from rhizosphere of different plants.

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