Iranian Journal of Microbiology
Volume 15 Number 2 (April 2023) 278-289

ORIGINAL ARTICLE

Plant growth promoting and antagonistic traits of bacteria isolated from forest soil samples

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Received: December 2022**, Accepted:** February 2023

ABSTRACT

Background and Objectives: Sustainability in agricultural systems without compromising the environmental quality and conservation is one of the major concerns of today's world. The excessive use of agrochemicals is posing serious threats to the environment. Therefore identification of efficient plant growth promoting (PGP) bacteria as an alternative to chemically synthesized fertilizers is of great interest.
Materials and Methods: In the present investigation, forest soil samples collected were used for isolation of efficient plant growth promoting bacteria.
Results: Total of 14 bacteria were isolated, and tested for various PGP properties. Out of the 14 isolates, four isolates labelled as BKOU-1, BKOU-8, BKOU-13 and BKOU-14 showed significant plant growth promoting traits, hydrolytic enzyme production and effectively restricted the mycelial development of phyto-pathogenic fungi (*Fusarium oxysporum* and *Macrophomina phaseolina*). 16 S rRNA gene sequences of the bacterial isolates BKOU-1, BKOU-8, BKOU-13 and BKOU-14 were found to have maximum identity with *Bacillus aerius*, *Bacillus infantis*, *Alcaligenes faecalis* and *Klebsiella Oxytoca* respectively. All four bacterial isolates nucleotide sequences were submitted to GenBank and NCBI accession numbers were generated as follows: OL721916, OL721918, OL721919 and OL721926.
Conclusion: According to the findings of the study, these PGPR could be employed as biofertilizers/ biopesticides to boost crop yield of different crops in sustainable manner.

Keywords: Plant growth promoting bacteria (PGPR); *Bacillus aerius*; *Bacillus infantis*; *Alcaligenes faecalis*; Antagonistic activity; 16S rRNA sequencing; Biofertilizers

INTRODUCTION

In terms of both population and economics, India is one of the nations that is growing the fastest. By 2050, the Indian population is projected to reach 1.5 billion, and the country would need more than 300 million metric tonnes of food grains, about twice as much as it currently produces. In order for the food production to keep up with the rapid increase in the population, the yield plateau urgently needs to be raised. Since it is constrained by a number of physical, environmental and social variables, expanding the area under cultivation is not a practical solution. Farmers have been more reliant on chemical fertilisers and pesticides to increase food output during the previous few decades. According to several scholars,

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one of the greatest techniques for increasing global agricultural productivity is to use chemically manufactured fertilisers (1, 2). However excessive usage of chemically manufactured fertilisers has disrupted ecosystems all around the world. Such fertilisers are not only costly, but they can also harm the ecosystem by polluting soil and groundwater. They also have a detrimental effect on beneficial soil microbes, reduce soil fertility, promote pest resistance, and can remain in food grains, affecting human health (2-4). Therefore, alternative techniques must be prioritized that improve soil fertility and enhance crop production without the use of chemical fertilizers.

Sustainable agricultural practices like employing plant growth-promoting rhizobacteria (PGPR) or microbial inoculants are coming to the limelight in intensive agriculture globally. PGPR are a more sustainable choice which may improve plant fitness by increasing the plant's ability to use available nutrients (5). Rhizosphere is considered as narrow region of soil peculiarly influenced by existing plant root system (2, 6). This region is found to have ample nutrients in comparison with bulk soil. This is due to lodgement of extensive range of exudates from plants which include lipids, organic acids, amino acids, sugars and other secondary metabolites, creates a unique and dynamic environment for the microorganisms which affect plants and other associative microorganisms. Many microorganisms are highly dependent for their survival on preformed substrates exuded by plant roots (7-9). In turn, the soil microflora inhabiting the rhizosphere can cause dramatic changes in plant growth and development by producing plant growth regulators (PGRs) or biologically active substances, or by altering endogenous levels of PGRs, and/or by facilitating the supply and uptake of nutrients and providing other benefits (10). Plant growth-promoting rhizobacteria (PGPR) positively impacts plant growth both directly and indirectly (5, 11). Direct effects of PGPB on plant growth include the production and/or synthesis of phytohormones such as auxins, gibberellins, ethylene, cytokinins, and abscisic acid (ABA) (12, 13) potassium, zinc and phosphorus solubilization and the creation of iron chelating compounds (siderophore) (2, 14-16). Plant growth can also be aided indirectly by PGPB through disease control by suppression of pathogens. It has long been known that PGPB can impede the growth of disease-causing plant pathogens by releasing hydrolytic enzymes, antibiotics, and volatile chemicals including hydrocyanic acid and ammonia (17, 18). A diverse array of bacteria, including species of *Burkholderia, Rhizobium, Pseudomonas, Bradyrhizobium, Azospirillum, Bacillus, Azotobacter, Arthobacter, Alcaligens, Klebsiella, Enterobacter, Serratia* and many others, have been shown to facilitate plant growth by various mechanisms (19-21). The current study was focused on the isolation of efficient/novel plant growth promoting bacteria from the rhizosphere of forest samples that can be employed as bio-fertilizers or bio-pesticides as an alternative to chemically generated fertilisers.

MATERIALS AND METHODS

Sampling process and bacterial isolation. The samples of soil were collected from forest sites of Chintoor, East Godhavari district Andhra pradesh at 17.7434° N, 81.3977° E co-ordinates and Sujatha nagar, Bhadradri Kothagudem, Telangana, India at 17.4848° N, 80.5728° E co-ordinates to isolate PGPR strains. After digging 5 to 10 cm deep, soil samples were collected in sterile polythene bags. The soil samples collected were then safely transported to the botany laboratory of the Osmania University, Hyderabad, India, and were stored at 4°C for further investigation. 10 g of rhizosphere soil was mixed with 90 ml of sterile distilled water in a flask and was shaken on a rotary shaker for 1 h. Following this, 1 ml suspension from the flask was added to 9 ml vial and successive dilutions were made up to 10-7 dilution. 0.1 ml of this solution was put to nutritional agar (NA) medium plates and the plates were incubated at 30°C. Morphologically different colonies were picked further for purification on fresh NA medium by employing spread plate technique. The purified cultures were stored in nutrient agar slants at 4°C in refrigerator.

PGP traits of the isolated bacteria under *in vitro* conditions. Under *in vitro* conditions, the isolated rhizobacteria were tested for PGP properties such as ACC deaminase activity, hydrocyanic acid (HCN), ammonia, indole acetic acid (IAA), phosphate solubilization and siderophore synthesis. Qualitative estimation of IAA production was carried out as per the methodology of Mir et al. (2). In brief the bacterial isolates were spot inoculated on nutritional agar medium containing 5 mM L-tryptophan. After 48 hours of incubation, the inoculated site was covered with

a 10 mm-diameter nitrocellulose membrane (NCM) disc pre-soaked with a few drops of the Salkowski reagent. After a little while, a pink colour appeared which indicates IAA production. Siderophore production of the isolates was assessed using the Louden et al. (22) method by spot-inoculating test organism on chrome azurol- S (CAS) agar plates and incubating at 30°C for 2-3 days in the dark. A yellow to orange halo that formed around bacterial colonies was supposed to indicate the production of siderophores. HCN production by the rhizosphere bacterial isolates was determined by employing the methodology of Ahmad et al. (23). In brief actively grown isolates were streaked on nutrient agar medium amended with 0.44% glycine and overlaid with Whatman No.1 filter paper (pre saturated with 0.5% picric acid and 2% sodium carbonate solution w/v) was placed inside the lid of Petri dish and plates were incubated at 30 ± 2°C for 3-5 day. Colour change of the filter paper from yellow to brown indicates HCN production. Production of ammonia by bacterial isolates was determined by adopting the methodology of Di Benedetto et al. (24). Actively grown bacterial cultures were inoculated in 10 ml of peptone water broth and incubated at 30°C and 120 rpm for 4 days. After incubation, 0.5 ml of Nessler's reagent was added in each tube and change in colour from deep yellow to brown indicates ammonia generation. Solubilization of phosphate by isolated bacteria was carried out as per the methodology of Mehta & Nautiyal (25). In brief actively grown bacterial isolates were spot inoculated on NBRIP medium and plates were incubated at 30 ± 2°C for 5-78 days. Development of halo zones surrounding the colonies was a sign of successful phosphate solubilization. ACC deaminase was qualitatively estimated using the Penrose and Glick, (26) recommended approach. In brief actively grown bacterial cultures were inoculated on DF salt minimal medium supplemented with 3 mM ACC and the plates were incubated at 30°C for 3-4 days. DF medium without ACC served as the negative control, while DF medium containing $(NH_4)_2SO_4$ served as the positive control. The ability of the isolates to grow on ACC plates indicated that they exhibited ACC deaminase activity.

Screening of rhizobacterial isolates for multiple hydrolytic extracellular enzymes. Rhizobacterial isolates were screened for various hydrolytic enzymes under *in vitro* conditions such as protease, amylase, cellulase and lipase. Test for protease production by the rhizobacterial isolates was carried out as per the methodology of Bhattacharya et al. (27). In brief bacterial isolates were spot inoculated on skim milk agar plates. Production of halo zones surrounding the colonies indicates protease activity. Rhizobacterial isolates were inoculating on starch agar medium, incubated for 48 hrs at 30°C. After the incubation period, the plates were saturated with iodine solution, maintained for a minute and solution was drained out, formation of colourless zones around the bacterial colonies indicated amylase production (28). Actively growing bacterial cultures were inoculated onto carboxy methyl cellulose congo red medium, incubated at 30°C for 2-3 days. Halo zones presence surrounding bacterial colonies indicated cellulase enzyme production (2). Activity for lipase was tested by inoculating the actively growing culture on Tween 80 agar medium and incubating the plates at 30°C for 3-4 days. The appearance of halo zones around the bacterial colonies shows that the lipase enzyme is being produced (27).

Antagonistic activity *in vitro* against *Fusarium oxysporum* and *Macrophomina phaseolina*. The antifungal activity of the isolated bacteria was assessed using dual culture technique. *Fusarium oxysporum* and *Macrophomina phaseolina*, the two phytopathogenic fungus, were grown on PDA medium for this investigation. A clump of mycelium 5 mm in diameter was removed from an active fungal culture and put in the centre of a Petri plate containing potato dextrose agar. Actively growing individual rhizobacterial cultures were streaked in straight line 2.5 cm away from the fungal plug placed onto potato dextrose agar plate and inoculated with same fungus without bacterial culture served as control followed by incubation at 30°C for seven days. Fungal growth inhibition was evaluated and the percentage inhibition compared to the control was obtained by using the formula: I% = [(C-T)/C] ×100 (2), Where I = inhibition % of mycelial growth, C = radial growth of the pathogen without antagonists, T = radial growth of the pathogen with antagonists.

Identification of bacterial isolates at molecular level. In order to identify each isolate at the molecular level, a colony of each strain was inoculated in nutritious broth till the log phase. The genomic DNA was extracted using the Sambrook et al. (1989) method. The universal primers 27 F and 1492 R were em-

ployed for amplifying the 16S rRNA regions, as per Pandey et al. (29). PCR reaction setup and thermal profiling conditions were performed according to methods described by Zakhia et al. (30). Amplified PCR products were confirmed by electrophoresis in 1.5% agarose gels containing ethidium bromide and visualized using UV-transilluminator. Macrogen Inc. in Seoul, Korea purified and sequenced all of the PCR-generated products. Clustal X software was used to align the acquired sequences, phylogenetic trees were built using MEGA X software. BLAST method was utilised to match the obtained sequences with that of the NCBI and Ez-Taxon databases (31). Neighbour joining method was utilised to generate the dendrogram. The nucleotide sequences of isolated bacteria were submitted to NCBI GenBank and accession numbers were received.

RESULTS

Isolation of rhizobacteria. A total of 14 bacteria were obtained from forest soil samples collected from Chintoor, East Godhavari district, Andhra Pradesh, and Sujatha nagar, Bhadradri Kothagudem, Telangana, India. The bacterial isolates are named as BKOU followed by a numeric digit. All the 14 bacterial isolates were labelled as BKOU-1, BKOU-2, BKOU-3, BKOU-4, BKOU-5, BKOU-6, BKOU-7, BKOU-8, BKOU-9, BKOU-10, BKOU-11, BKOU-12, BKOU-13 and BKOU-14 (Table 1).

Plant growth promoting traits of the isolated bacteria *in vitro*. As indicated in Table 2 and Fig. 1, all the bacterial isolates were examined for plant growth boosting features like IAA, ammonia, phosphate solubilization, ACC deaminase, siderophore and HCN synthesis. In the present study, seven of the 14 isolates produced IAA and among these isolates, BKOU-1, BKOU-8, BKOU-13, and BKOU-14 exhibited the highest intensity (+++) of pink colour, indicates higher production capacity for IAA a PGP hormone. Isolate BKOU-6 has shown moderate intensity (++) of pink colour and BKOU-2 and BKOU-10 has shown very low intensity (+) with pink colour indicating production of very low quantity of production of IAA (Fig. 1A). The development of orange halos surrounding the bacterial colonies verified and identified siderophore production. The study has shown that 6 isolates (BKOU-1. BKOU-3, BKOU-5, BKOU-8, BKOU-13 and BKOU-14) showed production of orange halos indicating the siderophore production (Fig. 1BA). All the 14 isolates have shown the capability of producing ammonia. Out of the 14 isolates, BKOU-1 and BKOU-14 have shown strong activity (+++) for ammonia production, while BKOU-5, BKOU-8, BKOU-10 and BKOU-13 have shown moderate activity (++) for ammonia production and isolates BKOU-2, BKOU-3, BKOU-4, BKOU-6, BKOU-7, BKOU-9, BKOU-11 and BKOU-12 have demonstrated mild activity (+) for ammonia synthesis (Fig. 1D). By altering the colour of the filter paper from orange to brown, seven of the fourteen isolates were able to show the ability to produce hydrocyanic acid (HCN). Four isolates, BKOU-1, BKOU-8, BKOU-13, and BKOU-14, showed strong activity (+++) for HCN generation, while three others, BKOU-3, BKOU-7, and BKOU-10, showed mild activity (+) (Fig. 1E). Phosphorus is one of the most limiting nutrient for plant development next only to nitrogen. Amongst the 14 isolates, 7 have shown the ability to solubilize tricalcium phosphate on NBRIP medium. Even amongst the 7 isolates showing phosphate solubilization capability, isolates BKOU-1, BKOU-8, BKOU-13 and BKOU-14 have shown highest solubilization zone (+++) and isolates BKOU-2, BKOU-6 and BKOU-10 have shown mild (+) solubilization zone (Fig. 1C). Six isolates namely BKOU-1, BKOU-2, BKOU-8, BKOU-11, BKOU-13, and BKOU-14, grew on DF minimum medium supplemented with ACC, demonstrating that the bacteria have the ability to synthesize ACC deaminase.

Hydrolyzing extracellular enzymes and antagonistic activity screening. All the 14 bacterial isolates

Table 1. Source of sample collection details

Labels of isolated bacteria	Source	Place of collection
BKOU-1, BKOU-2, BKOU-3, BKOU-4, BKOU-5, BKOU-6, BKOU-7, BKOU-8	Soil	Chintoor, East Godhavari , Andhra pradesh, India
BKOU-9, BKOU-10, BKOU-11, BKOU-12, BKOU-13 and BKOU-14	Soil	Sujatha nagar, Bhadradri Kothagudem, Telangana India

Table 2. *In vitro* screening of isolates for PGPR characteristics.

Isolate name	IAA	Siderophore	P- solubilisation	HCN	Ammonia	ACC deaminase
BKOU-1	+++	+	+++	+++	+++	+
BKOU-2	+	-	+	-	+	+
BKOU-3	-	+	-	+	+	-
BKOU-4	-	-	-	-	+	-
BKOU-5	-	+	-	-	++	-
BKOU-6	++	-	+	-	+	-
BKOU-7	-	-	-	+	+	-
BKOU-8	+++	+	+++	+++	++	+
BKOU-9	-	-	-	-	+	-
BKOU-10	+	-	+	+	++	-
BKOU-11	-	-	-	-	+	+
BKOU-12	-	-	-	-	+	-
BKOU-13	+++	+	+++	+++	++	+
BKOU-14	+++	+	+++	+++	+++	+

+++: Strong activity, ++: moderate activity, +: mild activity, -: no activity

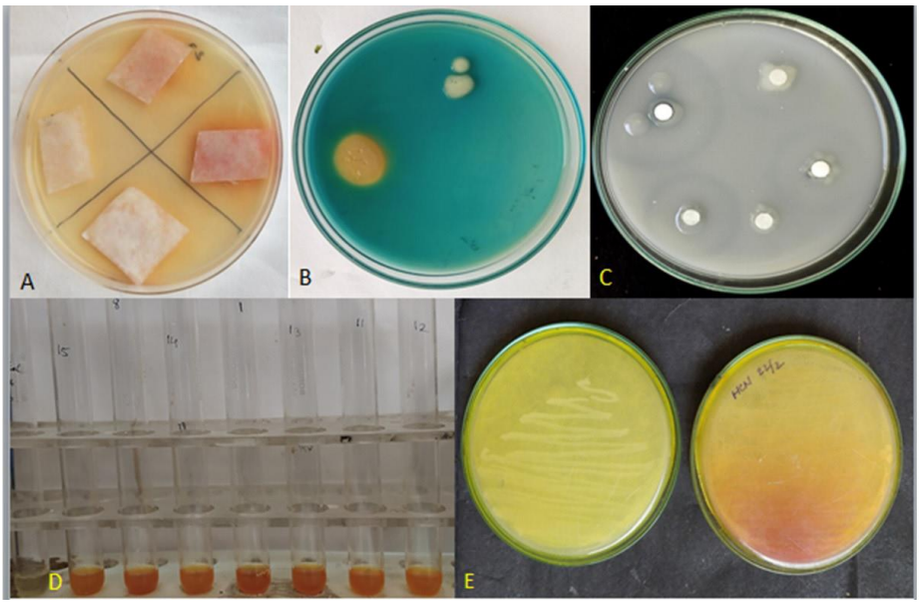

Fig. 1. (A) Indole acetic acid (IAA) production by the bacterial isolates, (B) Production of siderophore, (C) Phosphate solubilization by bacterial isolates, (D) Ammonia production by bacterial isolates and (E) Hydrocyanic acid production by bacterial isolates.

were analysed qualitatively for the synthesis of hydrolytic enzymes like protease, lipase, cellulase and amylase using skim milk agar, Tween 80 agar, carboxy methyl cellulose Congo red media and starch agar medium. 8 of the 14 isolates had a definite zone of clearance surrounding the colonies on skim milk agar medium, indicating the synthesis of protease enzyme. Amongst the 8 isolates, BKOU-1 and BKOU-14 isolates have shown strong (+++) protease production and BKOU-8 and BKOU-13 have shown moderate (++) production (Fig. 2A). Eight of the fourteen bacterial isolates produced lipase (Fig. 2B and Table 3) while nine produced cellulase (Fig. 2C). Seven of the 14 bacterial isolates were found to be amylase posi-

tive, as demonstrated by colourless zones around the colonies on starch agar medium. Even amongst the 7 isolates, BKOU-8 and BKOU-13 isolates showed strong production (+++) of amylase and BKOU-1 and BKOU-14 have shown moderate (++) production. Using the dual culture approach, it was determined whether all 14 bacterial isolates were hostile to the soil-borne phytopathogens *Fusarium oxysporum* and *Macrophomina phaseolina*. *Fusarium oxysporum* mycelium development was observed to be inhibited by 5 isolates, with isolate BHKOU-1 showing the greatest suppression (79%) followed by BKOU-8 (76%), BKOU-13 (54%), BKOU-14 (50%) and BKOU-2 (26%). Four isolates suppressed the mycelium growth of *Macrophomina phaseolina*, with highest level of inhibition displayed by BKOU-8 at 64%, followed by BKOU-1 (62%), BKOU-13 (50%) and BKOU-14 (44%). The results are depicted in Table 3.

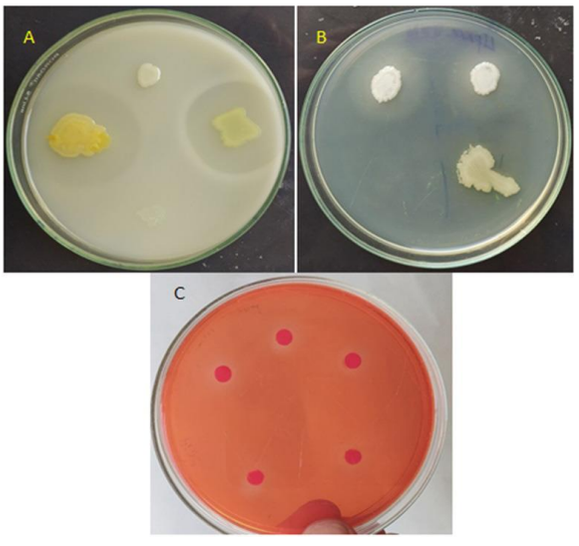

Fig. 2. A) Protease production, B) Lipase production, C) Cellulase production

Identification of the bacterial isolates. The PGPR characteristics of 14 bacteria isolated from forest soil samples were qualitatively evaluated. Out of the 14 isolates, four isolates labelled as BKOU-1, BKOU-8, BKOU-13 and BKOU-14 have shown significant PGP properties such as IAA, ammonia, HCN, siderophore, ACC deaminase and hydrolytic enzyme activity and phosphate solubilization. The same four isolates have shown significant antagonistic action towards phytopathogenic fungi (*Fusarium oxysporum* and *Macrophomina phaseolina*). The four isolates BKOU-1, BKOU-8, BKOU-13 and BKOU-14 were chosen for further evaluation at molecular level. The 16S rDNA gene amplicon of about 1.5 kb was yielded after PCR amplification and was delivered to Macrogen Inc. in Seoul, Korea for sequencing. The Macrogen Inc. sequences (941 base pair for BKOU-1, 871 bp for BKOU-8, 992 bp for BKOU-13, and

Table 3. Hydrolytic enzyme and antagonistic activity of isolated bacterial strains

Isolate	Amylase	Cellulase	Lipase	Protease	% growth inhibition of plant pathogenic fungi	
					Fusarium oxysporum	*Macrophomina phaseolina*
BKOU-1	++	++	+++	+++	79 ± 0.15	62 ± 0.16
BKOU-2	-	+	-	+	26 ± 0.21	-
BKOU-3	-	+	-	+	-	-
BKOU-4	+	-	+	-	-	-
BKOU-5	+	-	-	-	-	-
BKOU-6	-	-	+	+	-	-
BKOU-7	-	+	-	-	-	-
BKOU-8	+++	++	+++	++	76 ± 0.08	64 ± 0.3
BKOU-9	+	-	-	-	-	-
BKOU-10	-	-	+	+	-	-
BKOU-11	-	+	-	-	-	-
BKOU-12	-	+	+	-	-	-
BKOU-13	+++	+++	+++	++	54 ± 0.5	50 ± 0.13
BKOU-14	++	+++	+++	+++	50 ± 0.34	44 ± 0.18

+++: Strong activity, ++: moderate activity, +: mild activity, -: no activity, Data expressed as mean ± Standard error (SE) of values from replication of three experiments

1034 bp for BKOU-14) were compared to comparable sequences in GenBank, aligned with the Clustal W software, and the dendrogram was derived using the Neighbor-joining method (Fig. 3). The bacterial isolates BKOU-1, BKOU-8, BKOU-13 and BKOU-14 with their 16 S rRNA gene sequences were found to have maximum identity with *Bacillus aerius, Bacillus infantis, Alcaligenes faecalis* and *Klebsiella Oxytoca* respectively. All four bacterial isolates' nucleotide sequences were submitted to GenBank, and the following NCBI accession numbers were obtained: BKOU-1: OL721916, BKOU-8: OL721918, BKOU-13:OL721919 and BKOU-14: OL721926 (Table 4).

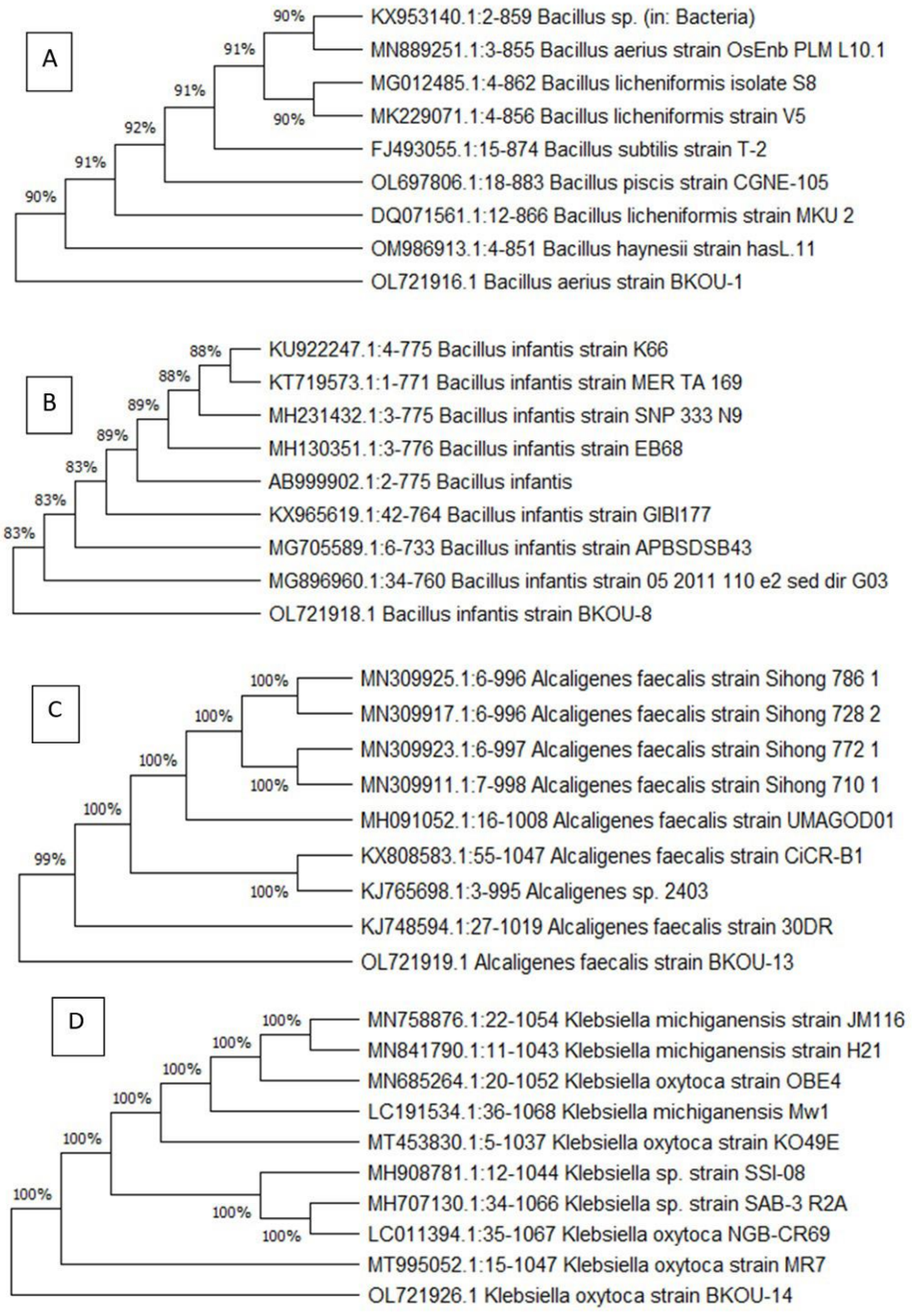

Fig. 3. Phylogenetic tree of the isolates A) BKOU-1, B) BKOU-8, C) BKOU-13 and D) BKOU-14

Table 4. Molecular identification of potential PGP bacterial isolates based on 16S rRNA sequence.

Isolate label	Source	16S rRNA Sequence Length	Hit strain	GenBank accession numbers
BKOU-1	Rhizosphere	941 bp	*Bacillus aerius*	OL721916
BKOU-8	Rhizosphere	871 bp	*Bacillus infantis*	OL721918
BKOU-13	Rhizosphere	992 bp	*Alcaligenes faecalis*	OL721919
BKOU-14	Rhizosphere	1034 bp	*Klebsiella oxytoca*	OL721926

DISCUSSION

Chemical fertilizers and their excessive use has led to perturbations in the ecosystem across the globe and usage of PGPR is an excellent substitute for chemical fertilizers and pesticides with the potential for plant growth promotion and bio control and this was demonstrated by many studies (20, 21). Microbial environment is more in soil than any other environment and this is the reason why rhizosphere soil was chosen for sample collection and the same has been the primary focus area for agriculture based research (32). The present study was aimed to isolate efficient focused on rhizobacteria from soil of the forest areas of Chintoor, East Godhavari district Andhra pradesh and Sujatha nagar, Bhadradri Kothagudem, Telangana, India, which can be employed as biofertilizers/ biopesticides to boost crop yield of different crops in sustainable manner. The isolates BKOU-1, BKOU-8, BKOU-13 and BKOU-14 with their 16 S rDNA gene sequences were found to have maximum identity with *Bacillus aerius, Bacillus infantis, Alcaligenes faecalis* and *Klebsiella oxytoca* respectively.

Currently, awareness on application of beneficial microbes as biofertilizers and biocontrol agents in agricultural practice is increasing, to maintain soil health and improve crop quality and quantity. Plant growth promoting bacteria are a class of heterogeneous soil bacteria found around and/or on surface of plant root system and involved in growth and development of plant through secretion of a broad list of chemical metabolites in vicinity of rhizosphere region. These bacteria boost plant growth and development directly by either increasing mobilization of nutrients such as nitrogen, phosphorus, zinc, potassium etc. or regulating phytohormone levels like auxins, gibberellins, cytokinin and ethylene and/or indirectly by suppressing the growth of disease causing phytopathogens through excretion of antibiotics, hydrolytic enzymes and other volatile compounds like HCN and NH_3 (33, 34).

IAA promotes plant cell elongation, division differentiation, enhance seed, tuber germination, initiates lateral and adventitious roots and biosynthesis of several metabolites etc., making it the most critical phytohormone for plant growth (2, 20, 34, 35). In this investigation, 7 of 14 bacterial isolates produced IAA (Table 2) and amongst the 7 isolates, *Bacillus aerius* strain BKOU-1, *Bacillus infantis* strain BKOU-8, *Alcaligenes faecalis* strain BKOU-13 and *Klebsiella oxytoca* strain BKOU-14 showed the highest intensity (+++) of IAA production. IAA is the main auxins for plant growth promotion and its production is more in rhizobacterial species than from the non-rhizosphere soil (36). The results of the present investigation are in line with the previous studies of many researchers who reported the production of IAA by various genera of bacteria belonging to *Bacillus, Pseudomonas, Alcaligenes faecalis, Serratia* and *Klebsiella* (35, 37-41). Similarly, Hyder et al. (42) reported the production of IAA by *Pseudomonas putida, P. aeruginosa, P. libanensis, Bacillus megaterium, Bacillus cereus* and *Bacillus subtilis* isolated from the rhizosphere of chilli. Phosphate solubilization has been of great interest to microbiologists, rhizobacteria's ability to solubilize insoluble phosphates can increase the supply of phosphorous and boost plant yield and growth of plants (43). According to many studies, rhizosphere soil contains a higher concentration of bacteria with phosphate-solubilizing ability than bulk soil (44). Using phosphate solubilizing PGPR can meet the phosphate needs of plants in a sustainable manner as a substitute biotechnological solution. A lot of bacterial genera together with *Bacillus, Pseudomonas, Enterobacter, Acinetobacter, Alcaligenes faecalis, Ochrobactrum, Burkholderia, Serratia, Klebsiella, Arthrobacter, Rhizobium* etc., are determined to be potential phosphate solubilising microorganism (19, 20, 33, 45, 46). In the current study 7 isolates have shown phosphate solubilization capability and even amongst the 7 isolates, *Bacillus aerius* strain BKOU-1, *Bacillus*

infantis strain BKOU-8, *Alcaligenes faecalis* strain BKOU-13 and *Klebsiella oxytoca* strain BKOU-14 isolates have shown highest phosphate solubilization. The isolated rhizosphere bacteria's ability to solubilize insoluble phosphates is consistent with a prior investigation of Baig et al. (47); Lee and Hong, (48); Bhardwaj et al. (37); Benaissa et al. (38); Kakar et al. (39); Miljaković et al. (40); Babar et al. (46) who reported phosphate solubilization by various genera of bacteria belonging to *Bacillus*, *Alcaligenes faecalis* and *Klebsiella*. Various organic acids are released by PGPR, which decreases the pH of the culture media results in phosphate solubilization (20). In the present investigation, six isolates showed production of siderophore. Because of the poor solubility of Fe3+ in soil, iron availability as a micronutrient is limited. Some rhizosphere bacteria have the capability to produce siderophore, a low molecular weight iron chelators, which can make iron available for plant growth and development and make it unavailable to phytopathogenic fungi hence also aid in defense against pathogens (2, 49). Rhizobacterial HCN synthesis is acknowledged as a phytopathogen's bio-control agent (50). In this study, 7 of the 14 isolates produced hydrocyanic acid (HCN) by changing the colour of the filter paper from orange to brown. Of the 7 isolates, three isolates *Bacillus aerius* strain BKOU-1, *Bacillus infantis* strain BKOU-8 and *Alcaligenes faecalis* strain BKOU-13 have shown strong activity (+++) for HCN production. The results of the present study are in line with the previous studies of various researchers who reported the production of HCN by *Bacillus subtilis, Bacillus licheniformis, Bacillus circulans, Alcaligenes faecalis, Bacillus mojavensis,* and *Bacillus cereus* (20, 38, 41). In the present investigation all the isolates were able to produce ammonia (Table 2). Plant growth is aided by the production of ammonia by PGPR, which supplies nitrogen to the host plant and thus promotes root and shoot elongation (51). In addition to plant growth, ammonia production by PGPR inhibits phytopathogenic spore germination and fungal growth suppression (52).

Hydrolytic enzymes produced by PGPR act as an important mechanism to prevent plant pathogenesis by inducing lysis of microbial cell walls. Hydrolytic enzymes act by breaking down the cell walls of fungal pathogens leading to death of the cells (35). In the current study, 7 of the 14 isolates tested have shown the ability to produce amylase, while 8 tested positive for protease production. Nine out of fourteen isolates were positive for cellulase synthesis and eight tested positive for lipase production. PGPR with the capacity to synthesize lytic enzymes restrict the growth of phytopathogens and thus enable bio-control against these pathogens in a more sustainable way vis-à-vis chemical fungicides. The findings of the present study are in line with the studies of Miljaković et al. (40) and Rasool et al. (20) who reported the production of various hydrolytic enzymes such as chitinase, proteases, β-1, 3-glucanase, cellulase etc., by various genera of *Bacillus* and *Alcaligenes faecalis.* Bio-control minimizes the need for chemical fungicides and thus reduces the problem of soil infertility caused due to chemical fungicides. The antagonistic action of PGPR against the fungus is a very important feature for plant growth and pest management. In this work, bacterial isolates *Bacillus aerius* strain BKOU-1, *Bacillus infantis* strain BKOU-8, *Alcaligenes faecalis* strain BKOU-13 and *Klebsiella oxytoca* strain BKOU-14, inhibited the development of phytopathogens like *Fusarium oxysporum* and *Macrophomina phaseolina* in dual culture assay. The results of the present study are in line with the previous studies of various researchers who reported antagonistic activity of *Alcaligenes faecalis, Bacillus* sp., against various phytopathogens such as *Rhizoctonia solani, Botrytis cinerea, Fusarium graminearum, Fusarium fujikuroi; Sclerotium rolfsii, Fusarium oxysporum, Macrophomina phaseolina* (20, 39, 53-57).

CONCLUSION

Present study concludes that four bacteria isolated from soil samples of forest lands (*Bacillus aerius* strain BKOU-1, *Bacillus infantis* strain BKOU-8, *Alcaligenes faecalis* strain BKOU-13 and *Klebsiella oxytoca* strain BKOU-14) had an excellent plant growth promoting properties as well as antagonistic activity against the phyto-pathogens. It can be concluded from the findings of this study that these strains can be used as bio-inoculants in a sustainable manner either individually or in consortium for plant growth promotion in different crops.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Botany at Osmania University Hyderabad, India, for pro-

viding laboratory facilities.

REFERENCES

1. Sharma K, Sharma S, Prasad SR. PGPR: renewable tool for sustainable agriculture. *Int J Curr Microbiol App Sci* 2019; 8: 525-530.
2. Mir MI, Hameeda B, Quadriya H, Kumar BK, Ilyas N, Kee Zuan AT, et al. Multifarious indigenous diazotrophic rhizobacteria of rice (*Oryza sativa* L.) rhizosphere and their effect on plant growth promotion. *Front Nutr* 2022; 8: 781764.
3. Alori ET, Babalola OO. Microbial inoculants for improving crop quality and human health in Africa. *Front Microbiol* 2018; 9: 2213.
4. Ahmad I, Zaib S (2020). Mighty microbes: plant growth promoting microbes in soil health and sustainable agriculture. *In Soil Health Springer, Cham.* pp 243-264.
5. Dutta S, Podile AR. Plant growth promoting rhizobacteria (PGPR): the bugs to debug the root zone. *Crit Rev Microbiol* 2010; 36: 232-244.
6. Bandyopadhyay P, Bhuyan SK, Yadava PK, Varma A, Tuteja N. Emergence of plant and rhizospheric microbiota as stable interactomes. *Protoplasma* 2017; 254: 617-626.
7. Ahkami AH, White III RA, Handakumbura PP, Jansson C. Rhizosphere engineering: Enhancing sustainable plant ecosystem productivity. *Rhizosphere* 2017; 3: 233-243.
8. Souza RD, Ambrosini A, Passaglia LM. Plant growth-promoting bacteria as inoculants in agricultural soils. *Genet Mol Biol* 2015; 38: 401-419.
9. Pascale A, Proietti S, Pantelides IS, Stringlis IA. Modulation of the root microbiome by plant molecules: the basis for targeted disease suppression and plant growth promotion. *Front Plant Sci* 2020; 10: 1741.
10. Singh VK, Singh AK, Kumar A. Disease management of tomato through PGPB: current trends and future perspective. *3 Biotech* 2017; 7: 255.
11. Glick BR (2020). Introduction to plant growth-promoting bacteria. In Beneficial plant-bacterial interactions. *Springer, Cham.* pp: 1-37.
12. Basu A, Prasad P, Das SN, Kalam S, Sayyed RZ, Reddy MS, et al. Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints, and prospects. *Sustainability* 2021; 13: 1140.
13. Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, et al. Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Front Plant Sci* 2018; 9: 1473.
14. Suleman M, Yasmin S, Rasul M, Yahya M, Atta BM, Mirza MS. Phosphate solubilizing bacteria with glucose dehydrogenase gene for phosphorus uptake and beneficial effects on wheat. *PLoS One* 2018; 13(9): e0204408.
15. Mishra J, Arora NK. Secondary metabolites of fluorescent pseudomonads in biocontrol of phytopathogens for sustainable agriculture. *Appl Soil Ecol* 2018; 125: 35-45.
16. Singh S, Kumar V, Sidhu GK, Datta S, Dhanjal DS, Koul B, et al. Plant growth promoting rhizobacteria from heavy metal contaminated soil promote growth attributes of *Pisum sativum* L. *Biocatal Agric Biotechnol* 2019; 17: 665-671.
17. Ahemad M, Kibret M. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *J King Saud Univ Sci* 2014; 26: 1-20.
18. Kumar A, Singh VK, Tripathi V, Singh PP, Singh AK. Plant growth-promoting rhizobacteria (PGPR): perspective in agriculture under biotic and abiotic stress. *In Crop Improvement Through Microbial Biotechnol* 2018; pp. 333-342.
19. Nevita T, Sharma GD, Pandey P. Composting of rice-residues using lignocellulolytic plant-probiotic *Stenotrophomonas maltophilia*, and its evaluation for growth enhancement of Oryza sativa L. *Environ Sustain* 2018; 1: 185-196.
20. Rasool A, Mir MI, Zulfajri M, Hanafiah MM, Unnisa SA, Mahboob M. Plant growth promoting and antifungal asset of indigenous rhizobacteria secluded from saffron (*Crocus sativus* L.) rhizosphere. *Microb Pathog* 2021; 150: 104734.
21. Hamid B, Zaman M, Farooq S, Fatima S, Sayyed RZ, Baba ZA, et al. Bacterial plant biostimulants: A sustainable way towards improving growth, productivity, and health of crops. *Sustainability* 2021; 13: 2856.
22. Louden BC, Haarmann D, Lynne AM. Use of blue agar CAS assay for siderophore detection. *J Microbiol Biol Educ* 2011; 12: 51-53.
23. Ahmad F, Ahmad I, Khan MS. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol Res* 2008; 163: 173-181.
24. Di Benedetto NA, Campaniello D, Bevilacqua A, Cataldi MP, Sinigaglia M, Flagella Z, et al. Isolation, screening, and characterization of plant-growth-promoting bacteria from durum wheat rhizosphere to improve N and P nutrient use efficiency. *Microorganisms* 2019; 7: 541.
25. Mehta S, Nautiyal CS. An efficient method for qualitative screening of phosphate-solubilizing bacteria. *Curr Microbiol* 2001; 43: 51-56.
26. Penrose DM, Glick BR. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol Plant* 2003;

118: 10-15.
27. Bhattacharya A, Chandra S, Barik S. Lipase and protease producing microbes from the environment of sugar beet field. *Indian J Agric Biochem* 2009; 22: 26-30.
28. Yassin SN, Jiru TM, Indracanti M. Screening and characterization of thermostable amylase-producing bacteria isolated from soil samples of afdera, Afar region, and molecular detection of amylase-coding gene. *Int J Microbiol* 2021; 2021: 5592885.
29. Pandey P, Kang SC, Maheswari DK. Isolation of endophytic plant growth promoting *Burkholderia* spp. MSSP from root nodules of *Mimosa pudica*. *Curr Sci* 2005; 89: 177-180.
30. Zakhia F, Jeder H, Domergue O, Willems A, Cleyet-Marel JC, Gillis M, et al. Characterisation of wild legume nodulating bacteria (LNB) in the infra-arid zone of Tunisia. *Syst Appl Microbiol* 2004; 27: 380-395.
31. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018; 35: 1547-1549.
32. Smalla K, Sessitsch A, Hartmann A. The Rhizosphere: soil compartment influenced by the root. *FEMS Microbiol Ecol* 2006; 56: 165.
33. Bhattacharyya PN, Jha DK. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World J Microbiol Biotechnol* 2012; 28: 1327-1350.
34. Shameer S, Prasad TNVKV. Plant growth promoting rhizobacteria for sustainable agricultural practices with special reference to biotic and abiotic stresses. *Plant Growth Regul* 2018; 84: 603-615.
35. Tsegaye Z, Gizaw B, Tefera G, Feleke A, Chaniyalew S, Alemu T, et al. Isolation and biochemical characterization of plant growth promoting (PGP) bacteria colonizing the rhizosphere of Tef crop during the seedling stage. *J plant Sci phytopathol* 2019; 3: 13-27.
36. Patten CL, Glick BR. Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. *Appl Environ Microbiol* 2002; 68: 3795-3801.
37. Bhardwaj G, Shah R, Joshi B, Patel P. *Klebsiella pneumoniae* VRE36 as a PGPR isolated from *Saccharum officinarum* cultivar Co99004. *J App Biol Biotech* 2017; 5: 47-52.
38. Benaissa A, Djebbar R, Abderrahmani A. Diversity of plant growth promoting Rhizobacteria of *Rhus tripartitus* in arid soil of Algeria (Ahaggar) and their physiological properties under abiotic stresses. *Adv Hortic Sci* 2018; 32: 525-534.
39. Kakar KU, Nawaz Z, Cui Z, Almoneafy AA, Ullah R, Shu Q-Y. Rhizosphere-associated *Alcaligenes* and *Bacillus* strains that induce resistance against blast and sheath blight diseases enhance plant growth and improve mineral content in rice. *J Appl Microbiol* 2018; 124: 779-796.
40. Miljaković D, Marinković J, Balešević-Tubić S. The significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegetable crops. *Microorganisms* 2020; 8: 1037.
41. Manasa M, Ravinder P, Gopalakrishnan S, Srinivas V, Sayyed RZ, El Enshasy HA, et al. Co-inoculation of *Bacillus* spp. for growth promotion and iron fortification in sorghum. *Sustainability* 2021; 13: 12091.
42. Hyder S, Gondal AS, Rizvi ZF, Ahmad R, Alam MM, Hannan A, et al. Characterization of native plant growth promoting rhizobacteria and their anti-oomycete potential against *Phytophthora capsici* affecting chilli pepper (*Capsicum annum* L.). *Sci Rep* 2020; 10: 13859.
43. Liu M, Liu X, Cheng B-S, Ma X-L, Lyu X-T, Zhao X-F, et al. Fang. Selection and evaluation of phosphate-solubilizing bacteria from grapevine rhizospheres for use as biofertilizers. *Span J Agric Res* 2016; 14(4): e1106.
44. Dasgupta D, Sengupta C, Paul G. Screening and identification of best three phosphate solubilizing and IAA producing PGPR inhabiting the rhizosphere of *Sesbania bispinosa*. *Int J Innov Res Sci Eng Technol* 2015; 4: 3968-3979.
45. Wan W, Qin Y, Wu H, Zuo W, He H, Tan J, et al. Isolation and characterization of phosphorus solubilizing bacteria with multiple phosphorus sources utilizing capability and their potential for lead immobilization in soil. *Front Microbiol* 2020; 11: 752.
46. Babar M, Saif-Ur-Rehman, Rasul S, Aslam K, Abbas R, Athar HU, et al. Mining of halo-tolerant plant growth promoting rhizobacteria and their impact on wheat (*Triticum aestivum* L.) under saline conditions. *J King Saud Univ Sci* 2021; 33: 101372.
47. Baig KS, Arshad M, Shaharoona B, Khalid A, Ahmed I. Comparative effectiveness of *Bacillus* spp. possessing either dual or single growth-promoting traits for improving phosphorus uptake, growth and yield of wheat (*Triticum aestivum* L.). *Ann Microbiol* 2012; 62: 1109-1119.
48. Lee EY, Hong SH. Plant growth-promoting ability by the newly isolated bacterium *Bacillus aerius* MH1RS1 from indigenous plant in sand dune. *J Korean Soc Environ Eng* 2013; 35: 687-693.
49. Abo-Zaid GA, Soliman NAM, Abdullah AS, El-Sharouny EE, Matar SM, Sabry SAF. Maximization of siderophores production from biocontrol agents, *Pseudomonas aeruginosa* f2 and *Pseudomonas fluorescens* JY3 using batch and exponential fed-batch fermentation. *Processes* 2020; 8: 455.
50. Choudhary DK, Johri BN. Interactions of *Bacillus* spp. and plants - with special reference to induced systemic resistance (ISR). *Microbiol Res* 2009; 164: 493-513.
51. Marques APGC, Pires C, Moreira H, Rangel AOSS,

Castro PML. Assessment of the plant growth promotion abilities of six bacterial isolates using *Zea mays* as indicator plant. *Soil Biol Biochem* 2010; 42: 1229-1235.

52. Swamy MK, Akhtar MS, Sinniah UR. Response of PGPR and AM fungi toward growth and secondary metabolite production in medicinal and aromatic plants. *In Plant Soil Microbes, Springer: Cham, Switzerland* 2016; pp. 145-168.
53. Guleria S, Walia A, Chauhan A, Shirkot CK. Molecular characterization of alkaline protease of *Bacillus amyloliquefaciens* SP1 involved in biocontrol of *Fusarium oxysporum*. *Int J Food Microbiol* 2016; 232: 134-143.
54. Khan MS, Gao J, Zhang M, Chen X, Moe TS, Du Y, et al. Isolation and characterization of plant growth-promoting endophytic bacteria *Bacillus stratosphericus* LW-03 from Lilium wardii. *3 Biotech* 2020; 10: 305.
55. Castaldi S, Masi M, Sautua F, Cimmino A, Isticato R, Carmona M, et al. *Pseudomonas fluorescens* showing antifungal activity against *Macrophomina phaseolina*, a severe pathogenic fungus of soybean, produces phenazine as the main active metabolite. *Biomolecules* 2021; 11: 1728.
56. Kumari P, Bishnoi SK, Chandra S. Assessment of antibiosis potential of *Bacillus* sp. against the soil-borne fungal pathogen *Sclerotium rolfsii Sacc.* (*Athelia rolfsii* (Curzi) Tu & Kimbrough). *Egypt J Biol Pest Control* 2021; 31: 1-11.
57. Sharf W, Javaid A, Shoaib A, Khan IH. Induction of resistance in chili against *Sclerotium rolfsii* by plant-growth-promoting rhizobacteria and *Anagallis arvensis*. *Egypt J Biol Pest Control* 2021; 31: 1-11.