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Evaluation of Plant Growth Promoting Properties of Endophytic Bacteria Isolated from *Asparagus racemosus* Willd

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Abstract

Endophytic microorganisms residing in various parts of the plants enhance the growth of the host plant by producing several bioactive compounds and are usually with no associated negative effects. Endophytic bacteria were isolated from the root tubers of the medicinal plant *Asparagus racemosus* for assessing their plant growth promoting properties. The strains were morphologically and biochemically characterized and screened for Indole 3 acetic acid, ACC deaminase and siderophore production and phosphate solubilisation. Among the 26 strains isolated, four strains such as AR6, AR10, AR11 and AR12 were found to have multiple plant growth promoting properties and were identified as *Pseudomonas* sp., *Priestia* sp., *Brucella* sp. and *Enterobacter* sp. respectively by morphological, biochemical and 16S rRNA partial gene sequence analysis. All these strains are capable of producing Indole acetic acid, ACC deaminase and siderophore while *Priestia* sp. and *Brucella* sp. have the ability to dissolve insoluble phosphate. The highest IAA concentration (310.5µg/mL) was produced by *Enterobacter* sp. followed by *Priestia* sp. and *Brucella* sp. and the production was also confirmed by HPLC analysis. *Priestia* sp. was found to produce the highest concentration of Siderophore (76.44 units) and have higher capacity to solubilise phosphate than the other strains. The highest ACC deaminase production was shown by *Enterobacter* sp. The present study indicates that the isolated strains may enhance the growth of the host plant as they possess multiple plant growth promoting properties.

***Key words*:** Endophytes, Indole acetic acid, Siderophore, Phosphate solubilisation, ACC deaminase

Microbial community ubiquitously inhabiting most of the plant species has been receiving more attention nowadays as they form an untapped and underexploited group of organisms capable of producing several bioactive compounds. Endophytes are bacterial or fungal microorganisms that colonize healthy plant tissues intercellularly and/or intracellularly without causing any apparent symptoms of disease [1]. They have been commonly isolated from roots, leaves and stems and a few from flowers, fruits and seeds [2]. Even though endophytes share an ecological niche similar to that of pathogens, their existence is regarded beneficial to the host plant [3]. They produce secondary active metabolites that protect plants from phytopathogens. More over the exo-enzyme production, reported from these microbes has been suggested to have a profound role in aiding host plant colonization. Endophytes may enhance plant growth by phytohormone production and support plant growth under adverse biotic and abiotic stress [4]. Recently, bacterial endophytes have been used in different biotechnological sectors, such as bio-fertilizers to improve crop production thereby significantly reducing the chemical input into the environment [5]. In nanotechnology, these are used in the fabrication of various nanoparticles to be utilized in various fields [6].

The bacterial endophytes enhance the growth of host plants by releasing phytohormones such as indole-3-acetic acid (IAA), gibberellins, cytokinins and by phosphate solubilization, N_2 fixation, antibiotic production and siderophore production [7]. IAA production has been reported in numerous endophytic bacterial strains. IAA produced by the bacteria may increase root growth, root length and number of lateral roots and thereby increases root surface area which helps the plants to absorb maximum nutrients from the soil [8]. IAA also regulates several aspects of plant development such as stem elongation, apical dominance, tropism, and lateral root initiation, as well as fruit, meristem and root hair development by cell division and elongation [9]. Recent studies revealed that IAA has an ability to protect the plants from external stress conditions [10]. Plant auxin pool can be altered by the presence of bacterial auxins and this may affect the processes regulated by IAA.

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The siderophore production by endophytes helps the plants in iron sequestration and increases the concentration of bio available iron to inner tissues [11. They hinder the growth of soil pathogens by limiting their iron availability [12]. There are mainly three types of siderophores are produced by bacteria namely hydroxamates, catecholates and carboxylates. Phosphorus is the second limiting nutrient required for plant growth and development. It is involved in several important metabolic pathways like nutrient uptake, biological oxidation, and energy metabolism [13]. Plants can assimilate a very small proportion of phosphate directly from the soil. Microbes can dissolve insoluble organic and inorganic phosphorous containing compounds by producing low molecular weight organic acids and extracellular enzymes. Higher concentration of ethylene in plants may cause growth inhibition or even death. ACC, which is the precursor of plant ethylene, can be cleaved into ammonia and α-ketobutyrate by the action of 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme [14]. Thus, ACC deaminase producing endophytic bacteria can decrease the amount of ethylene produced in the host plants [15].

In the present study *A. racemosus*, a tuberous plant having enormous medicinal properties was selected for studying the interaction between host plant and the endophytes. The tubers of this plant are believed to be useful against several ailments including stomach disorders, muscular spasm, diarrhea, cancer, inflammation, night blindness, kidney problems and throat complaints [16]. The present study aimed to isolate, characterize and identify the endophytic bacterial strain from the root tubers of *A. racemosus* and evaluation of that isolates for their growth promoting properties.

MATERIALS AND METHODS

Isolation of endophytic bacteria

Root tubers of *A. racemosus* were collected from various parts of Kerala and used for endophytic bacterial isolation. The healthy tubers were thoroughly washed with running tap water to remove soil particles and were cut into small pieces of 1 to 2 cm length with a sterile knife. For removing the rhizospheric bacteria the pieces were surface sterilized by sequential immersion in 70% ethanol for 1 minute, followed by 5% sodium hypochlorite for 10 minutes and again in 70% ethanol for 30 seconds. Finally, samples were washed with sterile distilled water for 5 times and the final wash was used for spreading on the control plate with nutrient agar medium (g/L; Peptone - 5, Yeast Extract - 3, Beef Extract - 2, NaCl - 5, Agar - 18). The surface sterilized samples were aseptically dried using sterile tissue paper and then trimmed and made into smaller pieces for inoculating into previously prepared nutrient agar plate. Some pieces were macerated using 10mL phosphate buffer saline (g/L- sodium chloride 8, potassium chloride 0.2, disodium hydrogen phosphate 1.44 and potassium dihydrogen phosphate 0.24, and pH 7.4) and used for inoculation. Uncrushed pieces of samples were also used for inoculation. The plates were incubated at 28 °C for 5 days. The absence of colonies in the control plate indicates the successful disinfection of samples. Individual colonies having morphological differences were selected and purified by continuous subculture. The strains were temporarily stored at 4 °C for further studies.

Biochemical characterization and molecular identification

The micro-morphological and physio-biochemical characters of the strains were analyzed using standard protocol [17]. For molecular identification of bacterial strains 16S rRNA partial gene sequence-based homology analysis was used. Genomic DNA was isolated using NucleoSpin® Tissue Kit and the quality of the DNA isolated was checked using agarose gel electrophoresis. The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using primers such as 16S-RS-F (5'-CAGGCCTAACACATGCAAGTC -3') and 16S-RS-R (5'-GGGCGGWGTGTACAAGGC – 3'). PCR was carried out in a 50 µL reaction volume containing 5µl 2X Phire Master Mix, 4µl D/W, 0.25 µl of forward and reverse primers each and 1µl DNA. It was carried out for 35 cycles with the initial denaturation at 95 °C for 5 min, cyclic denaturation at 95 °C for 30 s, annealing at 60 °C for 40 s and extension at 72 °C for 60s with a final extension of 7 min at 7 °C. The PCR product was checked by agarose gel electrophoresis purified and was further subjected to sequencing. The sequence data was checked by BLAST analysis [18].

Detection of plant growth promoting properties

Screening for Indole Acetic Acid production

The bacterial isolates were inoculated into 20 mL of nutrient broth supplemented with 0.2% (v/v) of L-tryptophan and incubated for 10 days at 28 °C. After incubation, the culture was centrifuged at 3,000 rpm for 20 min and the supernatant was used for analyzing indole- 3 acetic acid production [19]. 1ml of culture supernatant was mixed with 2 mL of Salkowski reagent (0.5 M FeCl3, 35% v/v HClO4) and placed in dark for 30 minutes. Formation of red colour indicates the presence of IAA and the optical density was recorded at 530 nm for determining the amount of IAA produced by each isolate. Uninoculated nutrient broth with tryptophan was used as control. The positive isolates were inoculated into 200mL of nutrient broth containing 0.2% (V/V) tryptophan and incubated for 10 days. Then the culture was centrifuged at 3000rpm for 20 minutes for removing bacterial cells and extracted twice using ethyl acetate. The extract was vacuum dried in a rotary evaporator at 40 °C. Then it was dissolved in methanol and subjected to reverse phase HPLC analysis for the confirmation of IAA production.

Siderophore production

Qualitative assay

The strains were screened for siderophore production on plates containing blue agar CAS medium made of chrome azurol S (CAS) and hexadecyltrimethylammonium bromide (HDTMA) as indicators [20]. The blue agar CAS medium was prepared by adding 850 mL of autoclaved MM9 salt medium [added with 32.24 g piperazine-N, N0 -bis 2- ethanesulfonic acid (PIPES) at pH 6], 100 mL of blue dye, 30mL of filter sterilized 10% Casaminoacid solution, 10 mL of 20% glucose solution and agar. The bacterial isolate was inoculated into the CAS agar plates and incubated at 28 °C. Production of siderophore was indicated by the development of yellowish orange halo around the colonies after 48 hours.

Quantitative estimation

The sideropohores produced were quantitatively determined by colorimetric assay using culture supernatants of strains growing in MM9 medium (buffer with 100mM PIPES). 0.5mL of culture supernatant was mixed with 0.5mL CAS assay solution. To this 10µL shuttle solution (0.2M 5- Sulfosalicylic acid) was added. Siderophores if produced can reduce the blue colour of the solution by removing iron from the dye complex. Loss of colour of the solution can be measured by taking the absorbance at 630nm. The minimal medium was used as blank

and minimal medium containing CAS assay solution and shuttle solution as reference.

Percent (%) siderophore units produced by bacteria can be measured by the following formula:

$$[(A_r - A_s)/A_r) \times 100]$$

Phosphate solubilization

Qualitative study

Phosphate solubilization ability of the isolated strains were evaluated using Pikovskaya medium (g/L-glucose 10, tri-calcium phosphate 5, ammonium sulphate 0.5, sodium chloride 0.2, magnesium sulphate heptahydrate 0.1, potassium chloride 0.2, ferrous sulfate heptahydrate 0.002, yeast extract 0.5, manganese (II) sulfate dehydrate 0.002, agar 20, pH 7.0) containing 2.4 mg/mL bromophenol blue [21]. The bacterial strains were aseptically inoculated in the Pikovskaya medium agar plates and incubated at 30 °C for 2–3 days. Formation of clear zone around the colonies indicates the ability of isolates to solubilize tricalcium phosphate.

Quantitative assay

The strains found to have the ability of phosphate solubilization were quantitatively analyzed using NBRIP medium (Glucose 10g/L, $MgCl_2.6H_2O$ 5g/L, $Ca_3(PO_4)_2$ 5g/L, $MgSO_4.7H_2O$ 0.25g/L, KCl 0.2g/L, $(NH_4)_2SO_4$ 0.1g/L). Strains were incubated in this medium for 9 days with a shaking speed of 100 rpm. Samples taken after every 24 hours were centrifuged at 10000 rpm for 10 minutes and filtered using Whatman No. 1 filer paper followed by 0.22 µm Milipore membrane. Available phosphate was determined by Murphey and Riley Method. In this method assay reagent was prepared by mixing 125 mL of 5N H_2SO_4 and 37.5mL of Ammonium molybdate (20g in 500mL) thoroughly followed by addition of 75ml of 0.1M Ascorbic acid and 12.5 mL of Pottassium Antimonyl tartarate (1mg/ml). 8 mL of the above reagent was added to 40 mL of the sample solution and the optical density was measured at 880nm.

1-Aminocyclopropane-1- carboxylate (ACC) deaminase production

Qualitative study

ACC deaminase production was screened using DF salts minimal medium [Potassium dihydrogen phosphate 4g/L, disodium hydrogen phosphate 6g/L, magnesium sulphate heptahydrate 0.2g/L, ferrous sulphate heptahydrate 0.1g/L, boric acid 10 µg/L, manganese(II) sulfate 10 µg/L, zinc sulphate 70 µg/L, copper(II) sulfate 50 µg/L, molybdenum (VI) oxide 10 µg/L, glucose 2 g/L, gluconic acid 2 g/L, citric acid 2 g/L, agar 12 g/L) amended with 0.2% ammonium sulphate (w/v), Dworkin and Foster [22]. Isolated strains were aseptically inoculated in to the medium and the presence of bacterial colonies after 2 days of incubation indicates the ability to produce ACC deaminase.

Quantitative assay

A minimal medium with ACC as the sole nitrogen source induces ACC deaminase production. 15mL of rich medium was taken in two culture tubes and each tube was inoculated with 5µL of bacterial culture. The culture was incubated overnight in a shaking water bath with a shaking speed of 200rpm. Then it was centrifuged at 8000 rpm for 10 minutes at 4 °C. The pellet was washed with DF salt minimal medium and it was centrifuged again at 8000rpm for 10 minutes at 4 °C. Then it was transferred to fresh culture tube and suspended in 7.5 ml of DF salt minimal medium. Bacterial culture having 3mM ACC concentration was prepared by mixing 40µL of thawed 0.5M ACC solution to the culture tube. The culture was placed in shaking water bath at 200 rpm for 24 hours at 30 °C. Then the culture was centrifuged at 8000 rpm for 10 minutes at 4 °C and the pellet was washed with 5mL 0.1 M Tris- HCl (pH 7.6). The washing was repeated twice to remove the growth medium completely. The pellet was transferred to 1.5mL microcentrifuge tube and centrifuged at 16000 rpm for 5 minutes. The supernatant was removed and the pellet was suspended in 600µL of 0.1M Tris-HCl having pH 8.5. 30 µL of toluene was added to the tube and vortexed well for 30S. 200µL of the toluenized cells are transferred to fresh 1.5mL centrifuge tube and 20µL of 0.5M ACC was added, vortexed and incubated at 30 °C for 15 minutes. I mL of 0.56M HCl was added and the mixture was vortexed and centrifuged at 16000rpm for 5 minutes. 1mL of supernatant was vortexed with 800µL of 0.56M HCl. 300µL of 0.2% 2,4-dinitrophenylhydrazine in 2M HCl was added to the glass tube, vortexed and incubated at 30 °C for 30 minutes. Then 2 mL of 2N NaOH was added to this solution and the absorbance was measured at 540 nm. The absorbance of the assay reagents in the presence of ACC was taken as reference. The absorbance of bacterial extract and assay reagents in the absence of ACC was subtracted from the absorbance of mixture containing bacterial extract, assay reagents and ACC. Activity was determined using a standard curve of α ketoglutarate.

RESULTS AND DISCUSSION

Isolation of endophytic bacteria

In the present study endophytic bacterial strains were isolated from tubers of *Asparagus racemosus* after removing epiphytic microorganisms by surface sterilization. 26 morphologically distinct microbial colonies observed after repeated isolation in the nutrient agar plates after 48 hours of incubation were confirmed as endophytes because of the absence of colonies in the control plates which was inoculated with the final wash of sample tissues. These strains were subcultured continuously to get pure colonies and were stored at 4 °C for further studies. 4 strains (AR6, AR10, AR11 and AR12) with multiple plant growth promoting activities were selected for detailed study.

Biochemical characterization and molecular identification

Strains were tested for various substrates utilization, enzyme production and for the ability to produce acids from different carbohydrates (Table 1). AR6, AR10, AR11 and AR12 were identified as *Pseudomonas* sp., *Priestia* sp., *Brucella* sp. and *Enterobacter* sp. respectively by BLAST analysis of 16S rRNA gene sequence (Fig 1-4).

During unfavourable environmental conditions plants can recruit beneficial microorganisms from the soil [23]. There are several reports regarding the isolation of endophytic *Enterobacter sp.* from *Musa* spp. [24], Moso Bamboo [25], maize [26] etc. Isolation of *Pseudomonas* sp. from different hosts has been reported and demonstrated as growth promoting agents of several crop plants [27]. Reports indicating *Priestia* sp. as endophyte inhabiting halophyte *Bolboschoenus planiculmis* [28], *Cicer arietinum* [29] *Triticum aestivum* [30] etc. are also available. Previous studies also reported the isolation of *Brucella* sp. from halophyte *Reaumuria soongorica* [31] and *Cicer arietinum* [29]. These microorganisms may enhance the growth of plants by producing bioactive compounds.

Table 1 Biochemical characterization of endophytic bacterial strains

S. No.	Biochemical tests	AR6	AR10	AR11	AR12	Acid production from carbohydrates	AR6	AR10	AR11	AR12
1.	Growth on macconkey agar medium	+	+	+	+	Fructose	-	-	+	+
2.	Indole test	-	-	-	-	Salicin	-	-	+	+
3.	Methyl red test	-	-	+	+	Rhamnose	-	-	-	-
4.	Voges Proskauer test	+	+	+	+	Mannitol	-	-	-	+
5.	Citrate test	-	+	+	+	Xylose	-	-	-	+
6.	Casein test	-	-	+	-	Lactose	-	-	-	-
7.	Starch hydrolysis	-	-	+	-	Galactose	-	-	+	+
8.	Gelatin hydrolysis	+	+	+	+	Inositol	-	-	-	-
9.	Nitrate reduction	-	-	+	+	Sucrose	-	-	+	-
10.	Catalase	+	+	+	+					
11.	Oxidase	-	+	+	+					
12.	Esculin hydrolysis	-	-	-	-					
13.	H_2S gas production	+	-	-	-					
14.	Urease	-	-	-	-					

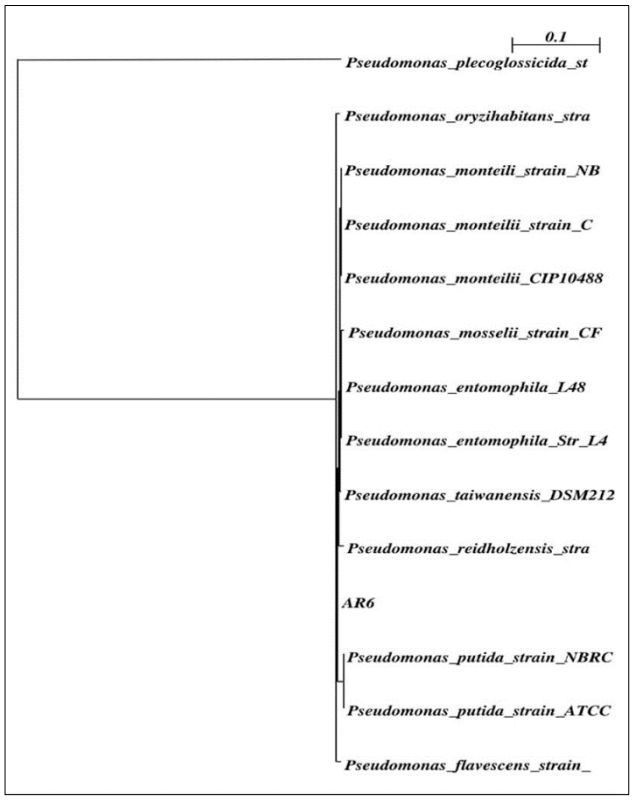

Fig 1 Phylogenetic relationship of isolate AR6 by maximum likelihood method based on 16S rRNA sequence

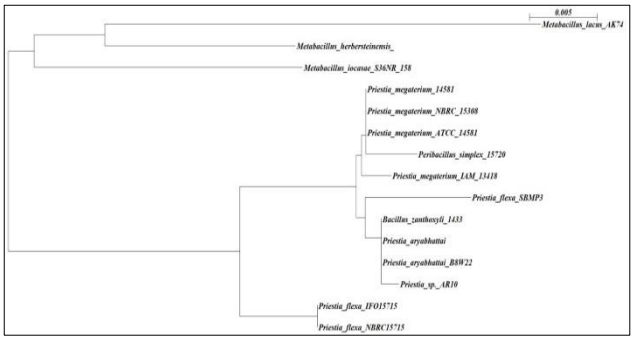

Fig 2 Phylogenetic relationship of isolate AR10 by maximum likelihood method based on 16S rRNA sequence

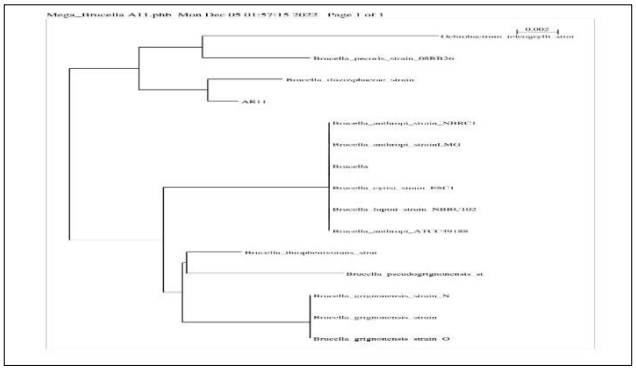

Fig 3 Phylogenetic relationship of isolate AR11 by maximum likelihood method based on 16S rRNA sequence

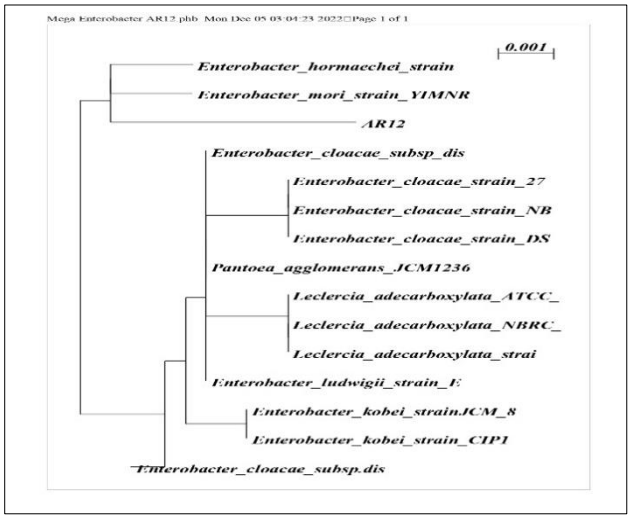

Fig 4 Phylogenetic relationship of isolate AR12 by maximum likelihood method based on 16S rRNA sequence

Detection of plant growth promoting properties

The strains were screened for the ability to produce Indole acetic acid, Siderophore and ACC deaminase enzyme and for phosphate solubilization capacity. All the four isolates were Indole 3-acetic acid (IAA), Siderophore and ACC deaminase producers and *Priestia* sp. and *Brucella* sp. were found to have phosphate solubilization capacity (Table 2).

Indole 3-acetic acid production

Isolated strains inoculated into nutrient broth containing tryptophan and incubated for 10 days to screen for indole acetic acid production. The formation of red colour in the culture supernatant mixed with Salkowski reagent after 30 minutes of incubation in dark indicates that all the four strains are capable of indole acetic acid production. This was confirmed using pure Indole acetic acid as positive control and uninoculated culture medium as negative control. Indole 3-acetic acid (IAA) production was confirmed by the comparison of peaks of standard Indole 3-acetic acid (IAA) with that of the bacterial

extracts formed in HPLC Analysis. The retention time of peaks showed by AR6, AR10, AR11 and AR12 were 1.002, 0.965, 1.038 and 1.098 respectively which are comparable to that of Standard IAA (Fig 5).

Table 2 Plant growth promoting properties of bacterial isolates

Name of the isolate	Identification by 16S rRNA analysis	Plant growth promoting properties			
		IAA production	Siderophore production	Phosphate solubilization	ACC deaminase production
AR6	*Pseudomonas* sp.	+	+	-	+
AR10	*Priestia* sp.	+	+	+	+
AR11	*Brucella* sp.	+	+	+	+
AR12	*Enterobacter* sp.	+	+	-	+

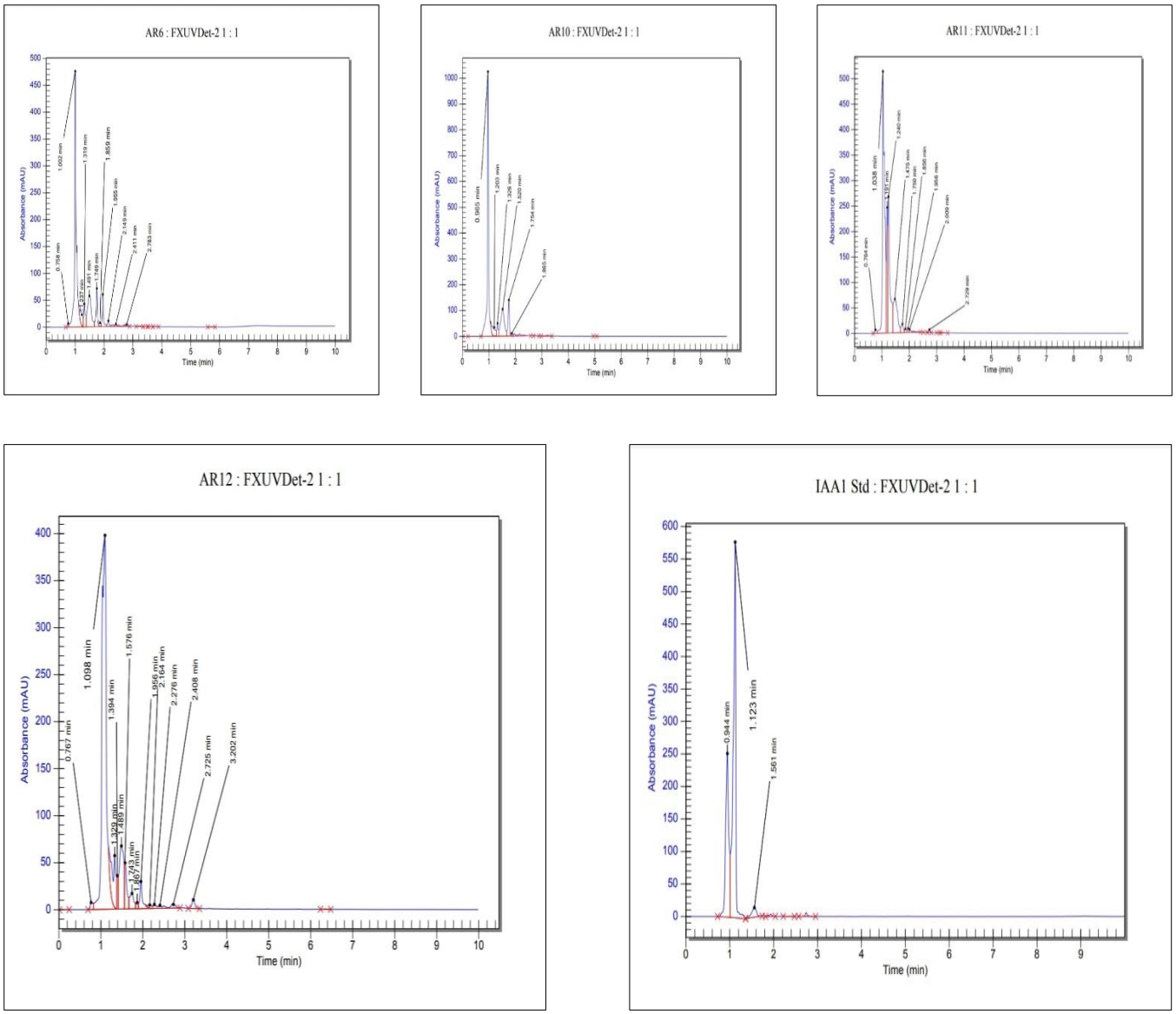

Fig 5 HPLC Chromatogram of AR6, AR10, AR11, AR12 and IAA standard

The absorbance of red colour formed due to the reaction of IAA with Salkowski reagent was measured at 530 nm. The concentration of IAA was estimated using standard IAA curve. The highest concentration was produced by *Enterobacter* sp. (310.5µg/mL) after 6 days of incubation followed by *Preistia* sp. (273µg/mL) after 8 days and *Brucella* sp. (244.7µg/mL) after 8 days of incubation. The lowest IAA concentration was observed in the culture of *Pseudomonas* sp. (51.5µg/mL) (Fig 6).

Tryptophan is the precursor of IAA and several Trp-dependent and Trp-independent pathways are present in plants and bacteria for its synthesis [32]. Endophytic *Enteobacter* sp. isolated from *Ocimum sanctum* has been reported to produce 17.715µg/mL IAA when cultured in a tryptophan containing nutrient broth [33]. IAA production by *Priestia* sp. isolated from rhizospheric soil has been reported by Chakraborty *et al*. [34]. Lin *et al.* [] also reported a production of 24.3 ± 1.37 µg/mL IAA by a soil isolated *Priestia* sp. The ability of IAA production by different *Pseudomonas* sp. has been reported in several studies [35]. Umang *et al.* [36] reported the production of 591.8 µg/mL IAA by *Pseudomonas putida* UB1 strain isolated from rhizospheric soil.

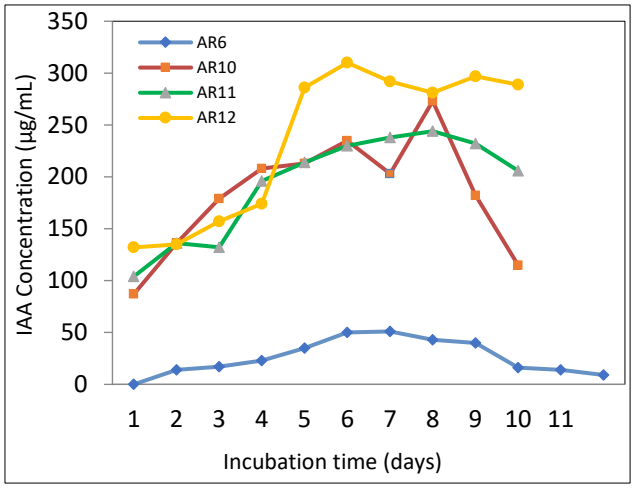

Fig 6 Indole acetic acid production by the bacterial isolates

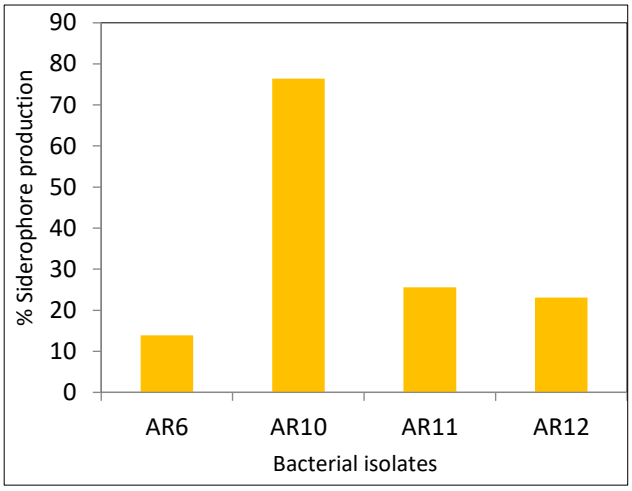

Fig 7 Siderophore production by the bacterial isolates

Siderophore production

Siderophore producing bacterial strains can be identified by the formation of yellow or orange halo around the colonies cultured on the blue agar Chromo azural S medium. All the strains, were found to be siderophore producing. These strains were quantitatively analyzed for percent siderophore production using CAS liquid medium. *Priestia* sp. showed highest production that is 76.44 units and *Pseudomonas* sp. was found to have the lowest production capacity (13.92 units). *Brucella* sp. and *Enterobacter sp*. were observed to have 25.59 and 23.09 units respectively (Fig 7).

Siderophore production is an important characteristic of plant growth-promoting bacteria which enhances plant growth by improving iron chelation properties and plays a significant role in controlling phytopathogens [37]. Various studies demonstrated the ability of endophytic bacteria to secrete siderophores which facilitates iron absorption by host plants. All the strains of *Enterobacter* sp. isolated from *Musa acuminata* are siderophore producers [38]. Previous studies have shown that siderophors are secreted by plant growth promoting bacterial strain *Paenibacillus* sp. [39]. Siderophore producing ability (0.23 ± 0.04) by *Priestia* sp. isolated from soil has been reported by Lin *et al* [40].

Phosphate solubilization

Presence of clear zones around the colonies of AR10 and AR11 grown in the Pikovskaya medium after 2-3 days of incubation indicates that they are able to solubilize tricalcium phosphate. NBRIP medium was used for assessing the phosphate solubilization capacity of the bacterial strains. The culture samples were assayed for 10 days and the available phosphate was determined using phosphate standard curve. *Priestia sp*. released a maximum of 95.5 µg/mL phosphate after 7 days of incubation and *Brucella* sp. produced a maximum of 88.5 µg/mL phosphate after 6 days (Fig 8).

Several rhizospheric and endophytic microorganisms can mineralize organic phosphates and solubilize inorganic phosphates through hydrolysis or by using phosphatases enzymes. Oteino *et al.* [41] reported that *Pseudomonas* sp. isolated from *Miscanthus giganteus* has the capacity to solubilize insoluble phosphate compounds. According to Lin *et al.* [40] *Priestia* sp. isolated from soil showed 87.5 ± 0.21 $mg \cdot L^{-1}$ phosphate solubilization activity.

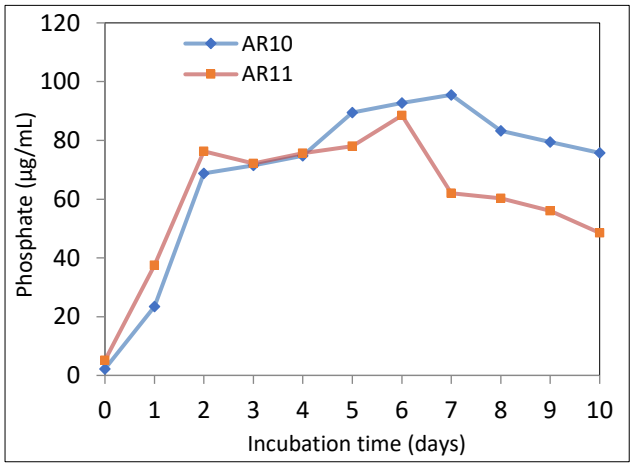

Fig 8 Phosphate solubilization by bacterial isolates

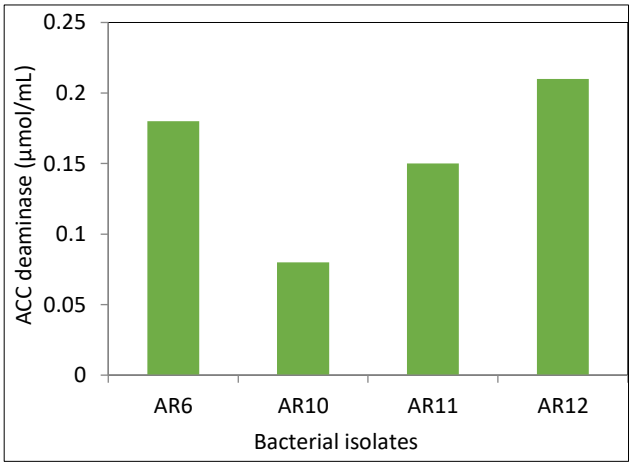

Fig 9 ACC deaminase production by bacterial isolates

ACC deaminase production

Growth of bacterial colonies in DF salt minimal medium is the positive result of 1-aminocyclopropane-1-carboxylate (ACC) deaminase production. All the strains gave positive results and were analyzed for the amount of enzyme produced. *Enterobacter* sp. showed higher production (0.21µg/mL) compared to other three strains and the lowest production was observed in *Priestia* sp. (0.08 µg/mL) (Fig 9).

1-aminocyclopropane-1-carboxylate (ACC) deaminase production from both rhizospheric and endophytic bacterial strains has been reported by several authors [42]. This enzyme lowers the ethylene levels in host plants and thereby promotes growth during stress conditions. Previous reports of

Rhizobacterium *Enterobacter cloacae* ZNP-4 exhibiting an activity of 188.90 ± 7.30 nmol of α-ketobutyrate / mg protein/ h is available [43]. Glick [15] reported that *Pseudomonas fluorescens* promotes the growth of groundnut seedlings and exhibited an activity of 342 nm α ketobutyrate / mg / h in liquid assay. *Pseudomonas putida* isolated from the rhizosphere of tomato plant showed an activity of 23.7+4.3 µmol α ketobutyrate / mg protein / h in quantitative assay [44].

CONCLUSION

Root tubers of Asparagus harbor a vast diversity of microorganisms and tubers collected from different locations show great variations in microbial inhabitants. Even though isolation of *Enterobacter* sp. and *Pseudomonas* sp. from different plant species has been reported in several studies, the reports indicating the presence of all the major growth promoting properties in same isolates are rare. The availability of limited information about the strains *Priestia* sp. and *Brucella* sp. as endophytes indicates their high host specificity. Most of the isolated strains were found to have multiple growth promoting properties which was usually rare. *Enterobacter* sp. produced comparatively higher concentration of Indole acetic acid and ACC deaminase in liquid culture medium. The highest production of siderophore and the phosphate solubilizing ability were found in *Priestia* sp. From the present study it was clear that these strains may enhance the growth of host plant and provide protection against stress and pathogens. These endophytic bacterial strains can be used as biofertilizers for improving the soil quality which forms a major solution to the environmental problems created by the over use of chemical fertilizers.

LITERATURE CITED

1. Wilson D. 1995. Endophyte: the evolution of a term, and clarification of its use and definition. *Oikos* 73(2): 274-276.
2. Lodewyckx C, Vangronsveld J, Porteous F, Moore ERB, Taghavi S, Mezgeay M, van der Lelie D. 2002. Endophytic bacteria and their potential applications. *Crit. Rev. Plant Sciences* 21: 583606
3. Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW. 1997. Bacterial endophytes in agricultural crops. *Can. Jr. Microbiology* 43(10): 895-914.
4. Eid AM, Salim SS, Hassan SE-D, Ismail MA, Fouda A. 2019. Role of endophytes in plant health and abiotic stress management. *In*: Microbiome in Plant Health and Disease: Challenges and Opportunities. (Eds) Kumar, V., Prasad, R., Kumar, M., Choudhary, D.K. Springer: Singapore. pp 119-144.
5. ALKahtani MD, Fouda A, Attia KA, Al-Otaibi F, Eid AM, Ewais EE-D, Hijri M, St-Arnaud M, Hassan, SE-D, Khan N. 2020. Isolation and characterization of plant growth promoting endophytic bacteria from desert plants and their application as bioinoculants for sustainable agriculture. *Agronomy* 10: 1325.
6. Hassan SED, Fouda A, Radwan AA, Salem SS, Barghoth MG, Awad MA, Abdo AM, El-Gamal MS. 2019. Endophytic actinomycetes *Streptomyces spp* mediated biosynthesis of copper oxide nanoparticles as a promising tool for biotechnological applications. *Jr. Biol. Inorg. Chem*. 24: 377-393.
7. Kumar A, Singh R, Yadav A, Giri DD, Singh PK, Pandey KD. 2016. Isolation and characterization of bacterial endophytes of *Curcuma longa* L. *3 Biotech* 6: 60.
8. Boiero L, Perrig D, Masciarelli O. 2007. Phytohormone production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. *Appl. Microbiol. Biotechnology* 74: 874880.
9. Taiz L, Zeiger E. 2010. Chapter 19 Auxin: The first discovered plant growth hormone, in: Plant Physiology. (Eds) L. Taiz, E. zeiger 5th Ed. Sunderland, Massachusetts U.S.A. Sinauer Associates Inc. pp 545-582.
10. Bianco C, Defez R. 2009. *Medicago truncatula* improves salt tolerance when nodulated by an indole 3 acetic acid - overproducing *Sinorhizobium meliloti* strain. *Jr. Exp. Botany* 60(11): 30973107.
11. Dimkpa C. 2016. Microbial siderophores: production, detection and application in agriculture and environment. *Endocytobiosis Cell Res*. 27: 7-16.
12. Ahmed E, Holmstrom SJM. 2015. Siderophore production by microorganisms isolated from a Podzol soil profile. *Geomicrobiol Journal* 32: 397-411.
13. Nesme T, Metson GS, Bennett EM. 2018. Global P flows through agricultural trade. *Glob. Environ. Change* 50: 133-141.
14. Honma M, Shimomura T. 1978. Metabolism of 1-aminocyclopropane-1-carboxylic acid. *Agric. Biol. Chem*. 43: 1825-1831.
15. Glick BR, Cheng Z, Czarny J, Duan J. 2007. Promotion of plant growth by ACC deaminase-containing soil bacteria. *Eur. Jr. Plant Pathology* 119: 329-339.
16. Garde GK, Sarth Vagbhat. 1970. Marathia Translation of Vagbhat‟s Astangahridya. *Uttarstana, Aryabhushana Mudranalaya* 40-48.
17. Bergey DH, Hendricks D, Holt JG, Sneath PH. 1984. *Bergey's Manual of Systematic Bacteriology*. Williams & Wilkin. Vol. 2.
18. Zhang Z, Schwartz S, Wagner L, Miller W. 2000. A greedy algorithm for aligning DNA sequences. *Jr. Comput. Biol*. 7: 203-214.
19. Rahman A, Sitepu IR, Tang S-Y, Hashidoko Y. 2010. Salkowski's reagent test as a primary screening index for functionalities of Rhizobacteria isolated from wild dipterocarp saplings growing naturally on medium-strongly acidic tropical peat soil. *Biosci. Biotechnol. Biochem.* 74: 2202-2208.
20. Schwyn B, Neilands JB. 1987. Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.* 160(1): 47-56.
21. Jasim B, Joseph AA, John CJ, Mathew J, Radhakrishnan EK. 2014. Isolation and characterization of plant growth promoting endophytic bacteria from the rhizome of Zingiber officinale. *3 Biotech* 4(2): 197-204.
22. Dworkin M, Foster JW. 1958. Experiments with some microorganisms which utilize ethane and hydrogen. *Jr. Bacteriology* 75(5): 592-603.
23. Liu H, Brettell LE, Qiu Z, and Singh BK. 2020 Microbiome-mediated stress resistance in plants. *Trends Plant Sciences* 25: 733-743.

24. Gloria M, Benjamin V, Fernanda M, Katia R, Lydia FY, Masuo JK, Blondy B, Monica C, Paolo Di Mascio, James FW, Miguel JB. 2019. *Enterobacter cloacae*, an endophyte that establishes a nutrient-transfer symbiosis with banana plants and protects against the Black Sigatoka pathogen. *Front. Microbiol., Sec. Plant Pathogen Interaction*. Vol. 10: Art. 804.
25. Yuan ZS, Fang Liu F, Zhang GF. 2017. Isolation of culturable endophytic bacteria from moso bamboo (*Phyllostachys edulis*) and 16s rDNA diversity analysis. *Archives of Microbiology* 199(4): 1259-1266.
26. Naveed M, Mitter B, Yousaf S. 2014. The endophyte *Enterobacter sp*. FD17: a maize growth enhancer selected based on rigorous testing of plant beneficial traits and colonization characteristics. *Biol. Fertil. Soils* 50: 249-262.
27. Kumar A, Munder A, Aravind R, Eapen SJ, Tummler B, Raaijmakers JM. 2013. Friend or Foe: Genetic and functional characterization of plant endophytic *Pseudomonas aeruginosa*. *Environmental Microbiology* 15: 764-779.
28. Hwang HH, Chien PR, Huang FC, Yeh PH, Hung SW, Deng WL, Huang CC. 2022. A plant endophytic bacterium *Priestia megaterium* strain BP-R2 isolated from the halophyte *Bolboschoenus planiculmis* enhances plant growth under salt and drought stresses. *Microorganisms* 10(10): 2047.
29. Khanna A, Raj K, Kumar P. 2022. Antagonistic and growth-promoting potential of multifarious bacterial endophytes against *Fusarium* wilt of chickpea. *Egypt Jr. Biol. Pest Control* 32: 17.
30. Shahid M, Zeyad MT, Syed A, Singh UB, Mohamed A, Bahkali AH, Elgorban AM, Pichtel J. 2002. Stress-tolerant endophytic isolate *Priestia aryabhattai* BPR-9 modulates physio-biochemical mechanisms in wheat (*Triticum aestivum* L.) for enhanced salt tolerance. *Int. Jr. Environ. Res. Public Health* 19(17): 10883.
31. Li L, Gao L, Liu Y. 2021. Diversity of cultivable endophytic bacteria associated with halophytes in Xinjiang of China and their plant beneficial traits. *Jr. Arid Land* 13: 790-800.
32. Pattern CL, Glick BR. 2002. Role of *Pseudomanas putida* indo lactic acid in development of the host plant root system. *App. Eniron. Microbeal*. 68: 3795-3801.
33. Panigrahi S. Mohanty CC, Rath. 2020. Characterization of endophytic bacteria *Enterobacter cloacae* MG0014 isolated from *Ocimum sanctum* with Indole acetic acid (IAA) production and plant growth promoting capabilities against selected crops. *South African Journal of Botany* 134: 17-26.
34. Chakraborty D, Nagarajan S, Aggarwal P, Gupta VK, Tomar RK, Garg RN, Sahoo RN, Sarkar A, Chopra UK, Sundara Sarma KS, Kalra N. 2008. Effect of mulching on soil and plant water status, and the growth and yield of wheat (*Triticum aestivum* L.) in a semi-arid environment. *Agricultural Water Management* 95(12): 1323-1334.
35. Karnwal A. 2009. Production of indole acetic acid by fluorescent Pseudomonas in the presence of L-tryptophan and rice root exudates. *Journal of Plant Pathology* 91(1): 61-63.
36. Umang B, Patel K, Trivedi UB. 2013. Optimization of indole acetic acid production by *Pseudomonas putida* UB1 and its effect as plant growth-promoting rhizobacteria on mustard (*Brassica nigra*). *Agric. Res.* 2: 215-221.
37. Kumar P, Thakur S, Dhingra GK, Singh A, Pal MK, Harshvardhan K, Dubey RC, Maheshwari DK. 2018. Inoculation of siderophore producing rhizobacteria and their consortium for growth enhancement of wheat plant. *Biocatalysis and Agricultural Biotechnology* 15: 264-269.
38. Singh S, Choure K, Rai PK, Gour SS, Agnihotri VK. 2022. Evaluation of plant growth-promoting activities of endophytic bacteria of *Musa acuminata* and their characterization. *Journal of Applied Biology and Biotechnology* 10(5): 94-101.
39. Liu Q, Yang K, Ge. 2017. Promotion of iron nutrition and growth on peanut by *Paenibacillus illinoisensis* and *Bacillus sp*. strains in calcareous soil. *Brazilian Journal of Microbiology* 48(4): 656-670.
40. Lin, Xiao-Rui, Han-Bing Chen, Yi-Xi Li, Zhi-Hua Zhou, Jia-Bing Li, Yao-Qiang Wang, Hong Zhang, Yong Zhang, Yong-He Han, Wang SS. 2022. *Priestia* sp. LWS1 is a selenium-resistant plant growth-promoting bacterium that can enhance plant growth and selenium accumulation in *Oryza sativa* L. *Agronomy* 12(6): 1301.
41. Oteino N, Lally RD, Kiwanuka S, Lloyd A, Ryan D, Germaine KJ, Dowling DN. 2015. Plant growth promotion induced by phosphate solubilizing endophytic Pseudomonas isolates. *Front Microbiol*. 6: 745.
42. Hardoim PR, Van Overbeek LS, Elsas JD. 2008. Properties of bacterial endophytes and their proposed role in plant growth. *Trends Microbiology* 16: 463-471.
43. Singh RP, Pandey DM, Jha PN, Ma Y.2022. ACC deaminase producing rhizobacterium Enterobacter cloacae ZNP-4 enhance abiotic stress tolerance in wheat plant. *PLoS One* 17(5): e0267127.
44. Gravel V, Hani Antoun, Russell J. Tweddell. 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: Possible role of indole acetic acid (IAA). *Soil Biology and Biochemistry* 39: 1968-1977.