International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

IJFBS International Journal of Fauna And Biological Studies

ISSN 2347-2677
IJFBS 2018; 5(6): 105-113
Received: 17-09-2018
Accepted: 21-10-2018

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Study on isolation and characterization of rhizospheric *Bacillus* spp. for plant growth promoting activities

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Abstract
Plant growth promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and can enhance plant growth by a wide variety of mechanisms.
Bacteria are the most abundant among different rhizospheric microbes. Among Plant growth promoting bacteria, *Bacillus* is one of the most potential genera due to their spore forming ability, thereby increasing the adaptation of *Bacillus* strain to commercial formulation and field application
In the present study, an attempt was made to isolate, screen and select different isolates of *Bacillus* and subjected to different test to identify their morpho-physiological, biochemical and *In vitro* characterization to study their plant growth promoting activities. The Rhizospheric soil samples were collected from different plants i.e. *Triticumaestivum* (wheat), *Cynodondactylon* (doobghas), *Cicerarietinum* (chana), *Trifoliumamoenum* (bersin) and from different locations of Dehradun and processed to isolate different isolates of *Bacillus* on nutrient agar medium.
A total of 50 *Bacillus* isolates were isolated from the 22 soil samples. Interestingly, in the present study 17 isolates out of 50 were formed endospores, 5 isolates recorded as capsulated, 35 isolates were reduced the nitrate and all were recorded positive for IAA production. 6 isolates were found to be positive for siderophore production Biochemical characterization revealed that 12 isolates belonged to genus *Bacillus* belonging to five different species. Isolates NAB-1 seems to be *B. alvei.* Isolates NAB-8, NAB-11 and NAB-22 seem to be *B. laterosporus*. Isolates NAB-18, NAB-48, seems to be *B. subtillis*. Isolates, NAB-20, NAB-21 and NAB-25 seem to be *B. larvae*. Isolates NAB-23, NAB-43, NAB-44 seem to be *B. alcalophilus.*

Keywords: Morpho-physiological characterisation, PGPR, *Bacillus*, Rhizosphere, Dehradun

1. Introduction
Plant growth in agricultural soils is influenced by many abiotic and biotic factors. There is a thin layer of soil immediately surrounding plant roots that is an extremely important and active area for root activity and metabolism which is known as rhizosphere. A large number of microorganisms such as bacteria, fungi, protozoa and algae coexist in the rhizosphere and influence physical, chemical and biochemical activities.
Microbial communities are abundantly present in rhizosphere or areas under the influence of the root and its close vicinity. The rhizosphere gives support to many active microbial populations capable of exerting beneficial, neutral or detrimental effects on plant growth (Wahyudi *et al*., 2011) [1].
Bacteria are the most abundant among different rhizospheric microbes. Plants select those bacteria contributing most to their fitness by releasing organic compounds through exudates creating a very selective environment where diversity is low. Since bacteria are the most abundant microorganisms in the rhizosphere, it is highly probable that they influence the plants physiology to a greater extent, especially considering their competitiveness in root colonization (Barriuso *et al*., 2008) [2]. Rhizobacteria inhabit plant roots and exert a positive effect ranging from direct influence mechanisms to an indirect effect. So, the bacteria inhabiting the rhizosphere and beneficial to plants are termed Plant growth promoting bacteria. In the last few years, the number of PGPR that have been identified has seen a great increase, mainly because the role of the rhizosphere as an ecosystem has gained importance in the functioning of the biosphere. Various species of bacteria like *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia*have been reported to enhance the plant growth. These are naturally occurring soil

bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion (Saharan and Nehra, 2011) [3]. PGPR inoculation has been reported as an environmental friendly approach that play important role in crop protection, growth promotion or biological control of diseases (Dilantha *et al*., 2006) [4].

PGPR have been subjected to numerous investigations focused on biotechnological applications in agriculture, horticulture, forestry and environmental protection. Early studies in the 1950's began with a focus on nitrogen fixing bacteria. Since then, a large number of PGPR belonging to different bacterial classes and genera with multifunctional traits have been described (Rodríguez *et al*., 2008) [5].

The main aim of biotechnological development based on PGPR has been to develop soil inoculants that can contribute to sustainable agriculture, thereby diminishing the need for use of chemical fertilizers and pesticides (Viveros *et al*., 2010) [6].

Inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass production through direct effects on root and shoots growth. Inoculation of ornamentals, forest trees, vegetables, and agricultural crops with PGPR may result in multiple effects on early-season plant growth, as seen in the enhancement of seedling germination, stand health, plant vigor, plant height, shoot weight, nutrient content of shoot tissues, early bloom, chlorophyll content, and increased nodulation in legumes. PGPR are reported to influence the growth, yield, and nutrient uptake by an array of mechanisms. They help in increasing nitrogen fixation in legumes, help in promoting free-living nitrogen-fixing bacteria, increase supply of other nutrients, such as phosphorus, sulphur, iron and copper, produce plant hormones, enhance other beneficial bacteria or fungi, control fungal and bacterial diseases and help in controlling insect pests. There has been much research interest in PGPR and there is now an increasing number of PGPR being commercialized for various crops (Saharan and Nehra, 2011) [3].

Among PGPR cluster, *Bacillus* is one of the most potential genera due to their spore forming ability, thereby increasing the adaptation of *Bacillus* strain to commercial formulation and field application (Liu and Sinclair, 1993) [7].*Bacillus* is frequently isolated from rhizosphere, these species are also common plant endophyte. The gram positive bacterium *Bacillus subtilis* is known to positively influence plant growth, vitality, and the ability of the plant to cope with pathogens often resulting in higher yield. *B.mucilaginous* has been observed for its capability in solubilising potassium (Wu *et al*., 2005) [8] and phosphate (Idriss *et al*., 2002) [9]. It has also been reported that wheat yield increased up to 30% with Azotobacter inoculation and up to 43% with *Bacillus* inoculation, due to some exerted growth hormones such as indole acetic acid (IAA) (Kloepper *et al*., 1991) [10]. Other studies also revealed that inoculation of *Bacillus* with *Bradyrhizobiumjaponicum* enhanced the growth of soybean plant conducted to the increasing level of nodulation (Bai *et al*., 2003) [11]. Furthermore, (Woitke *et al*., 2004) [12] had proven the ability of *B. subtilis* to help hydroponically grown tomato plants to withstand salinity stress.

2. Objectives of the Study

Therefore the present study is aimed with the following objective:-

- Isolation of Bacillus isolates from different hosts plants.
- Biochemical characterization for identification.
- *In vitro* assessment for plant growth promoting activities

3. Materials and Methods

3.1 Collection of soil samples

The soil samples were collected from rhizosphere of healthy plants from different locations of Dehradun (Balawala, Gularghati, Ranjhawala, Doiwala, Raipur).

3.2 Isolation and maintenance of cultures

20 gm soil was taken in 100 ml of sterile distilled water and shaken vigorously. Dilutions (upto 10^{-8}) were prepared in sterile diluents and 0.1 ml of appropriate diluted suspension was pipetted on Nutrient Agar Medium (see appendix) plates aseptically and spreaded on the medium. The plates were incubated at 27 °C in incubator for 24-48 hrs. *Bacillus* colonies were isolated on the basis of rhizoid and mucoid whitish appearance. Isolated bacilli were checked for purity by restreaking on NAM plates. Again a single colony was picked and streaked and maintained on nutrient agar slants.

3.3 Morpho-physiological characterization

All the bacterial isolates were further subjected to different test to identify their morpho-physiological characteristics. The bacterial isolates were streaked on the NAM plates and incubated at 27 °C for 24-48 hrs. After incubation the colonies of the all streaked bacteria were studied for their colony characteristics like colony margin, shape, colour etc. Afterwards all the isolates were subjected to different test like Gram-staining, endospore staining and capsule staining.

3.3.1 Gram staining

Gram staining of all the isolates was done following the standard procedure (Gram, 1884). Briefly, a fixed bacterial smear was subjected to four different reagents i.e. Crystal violet (primary stain), Iodine solution (mordant), Alcohol (decolourising agent), Safarin (counter stain) and then the smear was observed under oil immersion (100x) objective.

3.3.2 Endospore staining

Endospore staining of all the isolates was done following the standard procedure as described by Ferdinand cohn (1872). Briefly, a fixed bacterial smear was steamed and flooded with malachite green and then the smear was observed under oil immersion (100x) objective.

3.3.3 Capsule staining

Capsule staining of all the isolates was done following the procedure of Anthony (1931). Briefly, a bacterial smear was flooded with crystal violet and 20% copper sulphate solution and then the smear was observed under oil immersion (100x) objective.

3.4 Biochemical characterization

All the isolates were evaluated for different biochemical tests as per Bergey's Manual of Determinative bacteriology (1986). Details are as below:-

3.4.1 Acid from carbohydrates

Different carbohydrate sources were used to evaluate their utilization and production of acid by the organism. All the isolates were inoculated on the slants of basal medium (see

appendix) for acid production from carbohydrates and incubated at 35^{O}C. The carbohydrates which were used are:-
a) D-Glucose, b) L-Arabinose, c) D-Xylose, d) D-Mannitol
After 24-48 hrs, slants were observed for the production of acid by observing by yellow colour on the basal medium.

3.4.2 Production of indole
Test was performed using indole production medium (see appendix) and all the bacterial isolates were inoculated in indole medium at 35 °C for 2-3 days. When the growth was observed then 2 ml of test solution (see appendix) was added and shaken vigorously. The positive tests were observed for a pink to red colour in the alcohol layer which separated on standing indicated the presence of indole.

3.4.3 Voges-proskauer reaction (Acetyl methyl carbinol production)
The tubes of voges-proskauer broth (see appendix) were inoculated and observed for acetyl methyl carbinol production after incubation for 3 days by mixing 3 ml of 40% (w/v) sodium hydroxide with the culture and then 0.5-1 mg of creatin was added. Then inoculated tubes were observed for the production of a red colour after 30-60 min at room temperature.

3.4.4 Utilization of citrate
Test was performed using citrate utilization medium (see appendix) and all the bacterial isolates were inoculated on the slants of citrate agar and incubated for 35 °C. After 24-48 hrs positive isolates appeared blue on the citrate agar.

3.4.5 Reduction of nitrate to nitrite
Test was performed using nitrate broth (see appendix) and all the bacterial isolates were inoculated in nitrate broth at 35 °C temp for 2-3 days. A strip of potassium iodide was moistened with a few drops of 1N hydrochloric acid and then a loopful of culture was touch on the strip. The positive reactions were observed for the production of purple colour indicated the presence of nitrite.

3.4.6 Hydrolysis of starch
Test was performed using starch amended NAM (see appendix) and all the bacterial isolates were inoculated on the plates of starch agar and incubated for 28 °C temperature. After 24-48 hrs, as the growth were observed, all the plates of starch agar were flooded by iodine solution. The positive isolates which hydrolyzed the starch formed the clear zone around the growth.

3.4.7 Hydrolysis of casein
Test was performed using the milk agar (see appendix) and all the bacterial isolates were inoculated on the plates of milk agar with one streak and incubated at 30 °C for 3 days. The positive isolates produced a clear zone around the growth on the milk agar indicated the decomposition of casein.

3.4.8 Hydrolysis of gelatin
Test was performed using the gelatin agar (see appendix) and all the bacterial isolates were inoculated in the tubes of gelatine agar and incubated at 28 °C for 3-4 days. After incubation, these slants were re incubated at 20 °C for 4 hrs. These slants were observed for liquefaction of agar i.e. the positive reaction.

3.4.9 Production of catalase
Test was performed for the production of catalase in which all the bacterial isolates were placed on the different slides with the help of streaking loop and then 10% of hydrogen peroxide was added on the culture. The positive reaction was recorded with formation of effervescences.

3.5 *In vitro* characterization of plant growth promoting activities
All the isolates were evaluated for the following plant growth promoting activities:-

3.5.1 Siderophore production
Siderophore production ability of the isolate was assessed on chrome azurol 'S' agar medium (appendix). The respective ability was evaluated in two steps. In first step, petriplates with chrome azurol 'S' agar medium were prepared. Four to five *Bacillus* isolates (on one medium plate) were spot inoculated and incubated at 27 °C for 72 hrs. After incubation plates were observed for a yellow halo around the bacterial growth. Isolates found positive for siderophore production reconfirmed in the second step. In this step only single isolate per plate was round streaked at three places on the chrome 'S' agar plates and incubated as as above. After incubation the plates were observed for siderophore production.

Siderophore production efficiency:-Siderophore production efficiency was estimated by the following formula:-

$$\text{Siderophore production efficiency} = \frac{\text{Diameter of zone of siderophore production}}{\text{Diameter of bacrteial growth}} \times 100$$

3.5.2 IAA production
All *Bacillus* isolates were screened for IAA production. The test culture was inoculated in 10.0 ml of nutrient agar broth for both, with tryptophan and without tryptophan and incubated at 27 °C for 24-48 hrs. After incubation, contents were centrifuged at 10,000 rpm for 10 mins. 2.0 ml of supernatant from the above tube was taken in a separate test tube and 0.1 ml of Salkowaski solution (0.5 M $FeCl_3$ in 35% hypochlorous acid) was added to it. Development of pink colour (within 10 minutes) indicates the production of IAA in filtration by the test isolates.

3.5.3 Ammonia production
Test was performed for the production of ammonia in which all the bacterial isolates were tested. A loopful of freshly grown cultures were placed on different slides and then a drop of Nessler's reagent was added on the culture of each slides. Development of yellow colour was a positive test for the ammonia production (Cappuccino and Sherman, 1992) [13].

4. Results & Discussion
Isolation of rhizospheric *Bacillus* isolates
On the Nutrient agar medium plates, different bacterial colonies were observed after 24-48 hrs of incubation at 27 ^{0}C. A total of 50 rhizospheric isolates were isolated and selected for further studies (Table-2, Fig.1). All the isolates were isolated from the 22 rhizospheric soil samples of different plants from different locations and were processed to isolate and identify different isolates of *Bacillus* on nutrient agar medium (Table-3). Maximum isolates i.e. 39 were isolated from DoobGhas (*Cynodondactylon)* plant where minimum isolates i.e. 2 were isolated from Chana (*Cicerarietinum)*

plant.

Table 4.1: Host plants and respective number of isolates collected from them

S. No.	Host plant	Location	Total No. of Isolates
1.	Wheat (*Triticumaestivum*)	Doiwala, Balawala,	**6**
2.	Doobghas (*Cynodondactylon*)	Gularghati,Balawala,Raipur, Ranjhawala	**39**
3.	Chana *(Cicerarietinum)*	Balawala,	**2**
4.	Bersin *(Trifoliumamoenum)*	Doiwala	**3**
Total			**50**

Table 4.2: Isolation of different *Bacillus spp.* isolates from different hosts plants.

Sample No.	Location	Plant	Isolates
1	Field (hostel front), Balawala	DoobGhas (*Cynodondactylon*)	NAB-1
2	Field (Balawala)	DoobGhas (*Cynodondactylon)*	NAB-2
3	Host, Balawala	Wheat (*Triticumaestivum)*	NAB-3
4	Hostel garden , Balawala	DoobGhas (*Cynodondactylon)*	NAB-4
5	Hostel ground, Balawala	DoobGhas (*Cynodondactylon)*	NAB-5
6	College road(field), Balawala	DoobGhas (*Cynodondactylon)*	NAB-6
7	Host (Balawala field)	Wheat (*Triticumaestivum)*	NAB-7
8	Host (Balawala field)	Wheat (*Triticumaestivum*	NAB-8
9	Gularghati road	DoobGhas (*Cynodondactylon)*	NAB-9, NAB-10
10	Gularghati	DoobGhas (*Cynodondactylon)*	NAB-11 to NAB-15
11	Host , Balawala	Wheat (*Triticumaestivum)*	NAB-16
12	Host , Balawala	Wheat (*Triticumaestivum)*	NAB-17
13	Balawala	Chana (*Cicerarietinum*)	NAB-18
14	Balawala	Chana (*Cicerarietinum)*	NAB-19
15	Near college (field), Balawala	DoobGhas (*Cynodondactylon)*	NAB-20 to NAB-25
16	Doiwala	Bersin (*Trifoliumamoenum*)	NAB-26 to NAB-28
17	College front, Balawala	Wheat (*Triticumaestivum)*	NAB-29
18	Host (Balawala)	DoobGhas (*Cynodondactylon)*	NAB-30 to NAB-34
19	Raipur road (Floriculture)	DoobGhas (*Cynodondactylon)*	NAB-35 to NAB-38
20	Host, Balawala	DoobGhas (*Cynodondactylon)*	NAB-39
21	Balawala (Home garden)	DoobGhasm (*Cynodondactylon)*	NAB-40 to NAB-45
22	Bhagwandas chowk, Raipur	DoobGhas (*Cynodondactylon)*	NAB-46 to NAB-50

Morpho-physiological characterisation

All the isolates seem to be *Bacillus* were further streaked on the fresh NAM plates to study morpho-physiological characters.

Grams staining revealed that all the collected isolates were rod shaped, single and some in chains and blue in colour. Therefore all the isolates were Gram-positive bacilli. Endospores staining showed that from all the collected isolates 17 bacterial isolates were formed endospores as they appeared as red coloured vegetative bacilli with intracellular green coloured spores. Capsule staining revealed that from all the collected isolates, 5 isolates were recorded positive as they showed capsules in contrast to deep purple colour of the cell.

Table 4.3: Morpho-physiological characterization of different isolates.

Isolate No.	Colour	Form	Density	Surface	Cell arrangement	Gram's reaction	Endospore	capsule
NAB-1	White	Circular	Opaque	Rough	Chains	+	+	-
NAB-2	White	Irregular	Opaque	Glistening, Mucoid	Diplobacilli	+	-	+
NAB-3	White	Irregular	Opaque	Glistening, Mucoid	Single	+	-	-
NAB-4	White	Irregular	Translucent	Rough	Chains	+	+	-
NAB-5	White	Irregular	Translucent	Rough	Single	+	-	-
NAB-6	Cream	Irregular	Translucent	Rough	Chains	+	+	-
NAB-7	Cream	Circular	Transparent	Glistening	Diplobacilli	+	-	-
NAB-8	White	Irregular	Transparent	Rough	Diplobacilli	+	-	-
NAB-9	Dullwhite	Irregular, Feathry	Translucent	Rough	Diplobacilli	+	+	-
NAB-10	Dull white	Irregular	Translucent	Rough	Single	+	+	-
NAB-11	Dull white	Irregular	Opaque	Wrinkled	Small chains	+	-	-
NAB-12	Dull white	Circular	Opaque	Glistening	Single	+	-	-
NAB-13	White	Irregular, Rhizoid	Translucent	Rough	Chains	+	+	-
NAB-14	Cream	Irregular	Translucent	Glistening	Diplobacilli	+	-	-

NAB-15	White	Irregular	Opaque	Wrinkled	Diplobacilli	+	-	-
NAB-16	White	Irregular, Rhizoid	Transparent	Rough	Small chains	+	+	-
NAB-17	Cream	Irregular	Translucent	Glistening	Single	+	-	+
NAB-18	White	Irregular	Translucent	Glistening	Single rods	+	-	-
NAB-19	White	Irregular	Translucent	Wrinkled	Diplobacilli	+	-	-
NAB-20	White	Irregular	Translucent	Glistening	Single	+	-	+
NAB-21	White	Irregular	Translucent	Rough	Diplobacilli	+	+	-
NAB-22	White	Circular	Translucent	Wrinkled	Small chain	+	-	-
NAB-23	Cream	Irregular	Translucent	Glistening, Mucoid	Diplobacilli	+	-	+
NAB-24	Cream	Irregular	Translucent	Glistening, Mucoid	Diplobacilli	+	-	+
NAB-25	Cream	Irregular	Translucent	Glistening	Diplobacilli	+	-	-
NAB-26	Cream	Irregular	Translucent	Rough	Small chains	+	+	-
NAB-27.	White	Irregular	Translucent	Wrinkled	Small chains	+	+	-
NAB-28.	White	Irregular	Translucent	Rough	Diplobacilli	+	+	-
NAB-29.	Cream	Irregular	Translucent	Glistening	Single rods	+	-	-
NAB-30	White	Irregular	Translucent	Glistening	Diplobacilli	+	-	-
NAB-31	White	Irregular	Translucent	Glistening	Diplobacilli	+	-	-
NAB-32	White	Irregular	Translucent	Glistening	Single rods	+	-	-
NAB-33	White	Irregular	Translucent	Glistening	Diplobacilli	+	-	-
NAB-34	Dull White	Circular	Opaque	Smooth	Small chains	+	-	-
NAB-35	White	Irregular	Translucent	Rough	Chains	+	+	-
NAB-36	White	Irregular	Translucent	Glistening	Single rods	+	-	-
NAB-37	Cream	Irregular	Translucent	Wrinkled	Diplobacilli	+	+	-
NAB-38	Cream	Irregular	Translucent	Wrinkled	Diplobacilli	+	+	-
NAB-39	Cream	Circular	Translucent	Wrinkled	Diplobacilli	+	-	-
NAB-40	Cream	Irregular	Translucent	Rough	Small chains	+	+	-
NAB-41	Cream	Irregular	Translucent	Glistening	Single rods	+	-	-
NAB-42	White	Irregular,Feathery	Opaque	Rough	Chains	+	+	-
NAB-43	White	Irregular	Translucent	Glistening	Single rods	+	-	-
NAB-44	White	Irregular	Opaque	Smooth	Chains	+	-	-
NAB-45	Cream	Irregular	Transparent	Glistening	Diplobacilli	+	-	-
NAB-46	Cream	Irregular	Translucent	Glistening	Diplobacilli	+	-	-
NAB-47	Dull White	Irregular	Translucent	Smooth	Small chains	+	+	-
NAB-48	Dull White	Irregular	Translucent	Smooth	Small chains	+	-	-
NAB-49	White	Irregular	Translucent	Glistening	Single rods	+	-	-
NAB-50	White	Irregular	Translucent	Glistening	Diplobacilli	+	-	-

Table 4.4: Different biochemical tests performed to characterize bacterial isolates.

Isolate No.	Indoleproduction	Acid production from different sugars				Vogues proskauer	Citrate utilization	Starch hydrolysis	Gelatine Hydrolysis	Casein Hydrolysis	Nitrate reduction
		D-Glucose	L-Arabinose	D-Xylose	D-Mannitol						
NAB-1	-	+	-	-	-	+	-	+	+	+	-
NAB-2	-	+	-	-	+	+	-	+	+	-	-
NAB-3	-	+	+	-	+	+	-	-	+	-	-
NAB-4	-	+	-	+	+	-	-	-	+	-	-
NAB-5	-	+	+	-	+	-	-	-	+	-	-
NAB-6	-	+	+	+	+	+	-	+	+	-	+
NAB-7	-	+	-	+	+	-	-	-	+	-	+
NAB-8	-	+	-	+	+	-	-	-	+	+	+
NAB-9	-	+	-	+	-	-	-	+	+	+	+
NAB-10	-	+	+	-	+	+	-	+	+	-	-
NAB-11	-	+	-	-	+	-	-	+	+	+	+
NAB-12	-	+	+	-	+	+	-	+	+	-	-
NAB-13	-	+	+	+	+	-	-	-	+	+	+
NAB-14	-	+	+	-	+	-	-	-	+	+	+
NAB-15	-	+	-	+	-	+	-	+	+	+	+
NAB-16	-	+	-	+	-	-	+	+	+	-	+
NAB-17	-	+	-	+	+	-	-	+	+	-	+

NAB-18	-	+	+	+	+	+	+	+	+	+	+
NAB-19	-	+	-	+	-	-	+	-	+	-	-
NAB-20	-	+	+	-	+	-	-	-	+	-	+
NAB-21	-	+	+	+	+	-	-	+	+	+	-
NAB-22	-	+	+	-	+	-	-	-	+	+	+
NAB-23	-	+	+	+	+	-	-	-	+	+	-
NAB-24	-	+	-	+	+	-	-	-	+	-	-
NAB-25	-	+	-	-	+	-	-	-	+	+	+
NAB-26	-	+	-	-	-	-	-	-	+	+	+
NAB-27	-	+	-	-	+	-	-	-	+	-	+
NAB-28	-	+	+	+	+	-	-	-	+	-	-
NAB-29	-	+	-	+	-	+	-	-	+	+	-
NAB-30	-	+	-	+	-	-	-	-	+	-	+
NAB-31	-	+	+	+	-	-	-	-	+	-	+
NAB-32	-	+	-	+	-	-	-	-	+	+	+
NAB-33	-	+	-	+	-	-	-	-	+	+	+
NAB-34	-	+	-	-	-	+	-	+	+	-	+
NAB-35	-	+	-	+	-	-	+	-	+	-	+
NAB-36	-	+	-	+	-	-	+	-	+	-	+
NAB-37	-	+	-	+	-	+	-	+	+	-	+
NAB-38	-	+	-	-	-	+	+	-	+	-	+
NAB-39	-	+	-	+	+	+	-	+	+	+	+
NAB-40	-	+	-	+	+	+	-	-	+	-	+
NAB-41	-	+	-	+	+	+	+	-	+	+	-
NAB-42	-	+	+	+	+	+	-	-	+	+	+
NAB-43	-	+	+	+	+	-	-	+	+	+	-
NAB-44	-	+	-	+	+	-	-	+	+	+	+
NAB-45	-	+	-	+	-	-	-	-	+	-	+
NAB-46	-	+	-	+	+	-	-	+	+	+	+
NAB-47	-	+	+	+	+	-	-	+	+	-	+
NAB-48	-	+	-	+	+	+	-	+	+	+	+
NAB-49	-	+	-	+	-	+	-	-	+	-	+
NAB-50	-	+	-	-	-	-	-	-	+	-	+

5. Biochemical tests

Carbohydrate fermentation test

Results of carbohydrate fermentation test showed that all the isolates fermented the Glucose. However only 17 isolates were positive for Arabinose, 35 isolates were recorded positive for Xylose and 30 *Bacillus* isolates showed the positive result for fermentation of Mannitol as they all produced the yellow colour on the basal medium.

Among 50 isolates all were recorded negative for the production of indole, 18 isolates were positive for Voges-proskauer test, 7 isolates were recorded as positive for utilization of citrate, 35 were positive in which nitrate reduced to nitrite,20 isolates showed zone of hydrolysis on starch, 23 were positive for hydrolysis of Casein. All the isolates were recorded positive for hydrolysis of gelatin when inoculated in the tubes of nutrient gelatine and 48 were recorded positive for production of catalase.

Characterization for plant growth promoting activities

All the bacterial isolates were evaluated for siderophore production. The test was performed on CAS agar medium. Results showed that out of 5*0 Bacillus* isolates, 6 were found positive for the trait. Isolate NAB-10 showed maximum efficiency (66.3%) for siderophore production followed by NAB-18 (55%), NAB-38(50%). Least efficiency was shown by NAB-50 i.e. 20%.

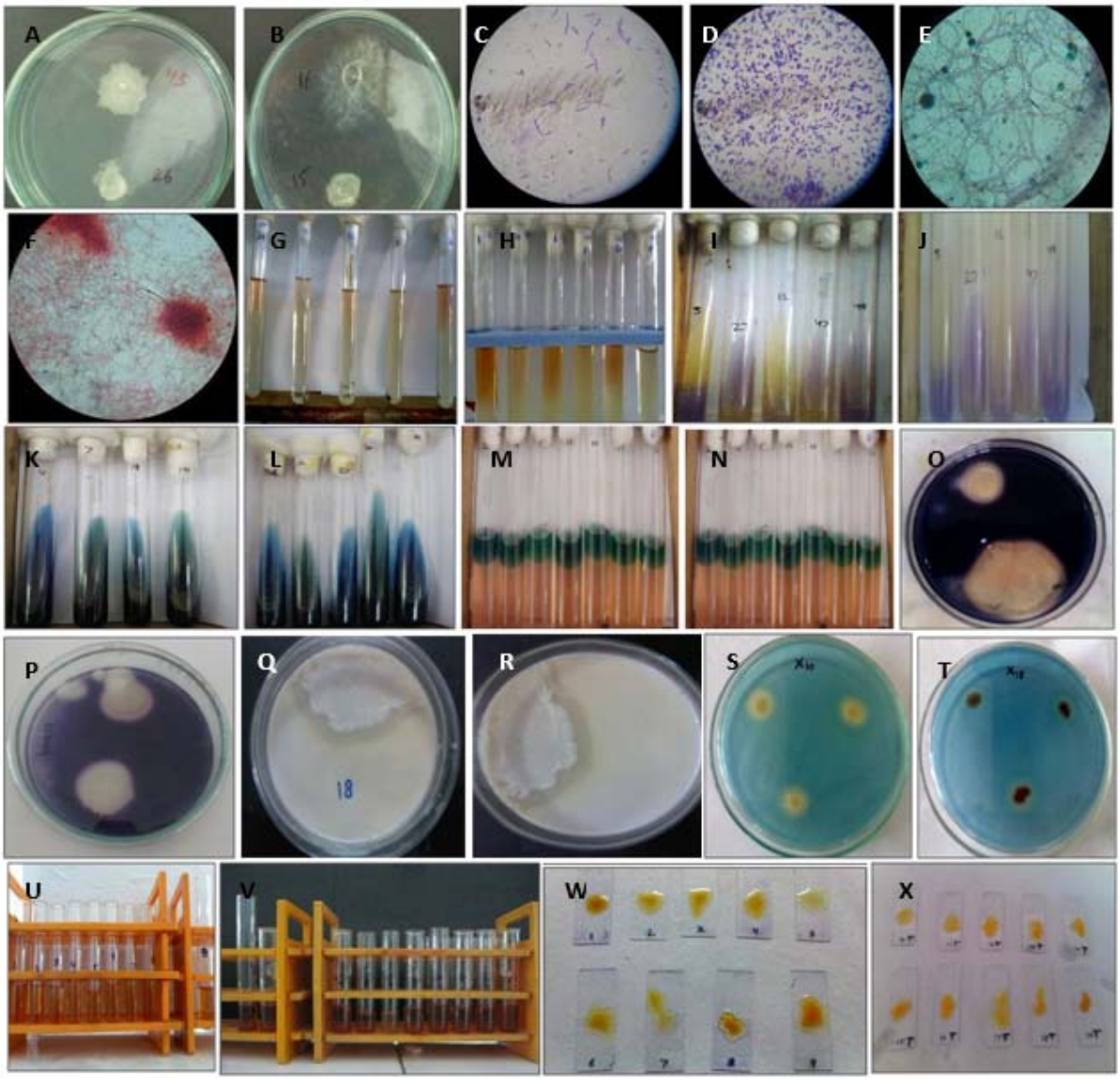
The evaluation of IAA was done with and without the amendment tryptophan. All the *Bacillus* sp isolates were able to synthesize IAA at various concentrations. Several *Bacillus* sp isolates obtained from this study were also able to produce IAA constitutively, even in the culture without tryptophan supplementation.

All the bacterial isolates were evaluated for the production of ammonia. Results showed that out of 50, 18 were showed the increased production of ammonia as they produced yellow colour after adding a drop of Nesseler's reagent and the remaining other bacterial isolates showed the small amount of ammonia production.

Table 4.5: Characterization of plant growth promoting activities.

Isolates	Ammonia production	Siderophore Production	IAA		Catalase
			With Tryptophan	Without Tryptophan	
NAB-1	++	-	+	+	+
NAB-2	+	-	+	+	+
NAB-3	+	-	+	+	+
NAB-4	++	-	+	+	+
NAB-5	+	-	+	+	+
NAB-6	+	-	+	+	+

NAB-7	+	-	+	+	+
NAB-8	++	-	+	+	+
NAB-9	++	-	+	+	+
NAB-10	+	18.3mm, (66.3%)	+	+	+
NAB-11	+	-	+	+	+
NAB-12	++	-	+	+	+
NAB-13	++	-	+	+	-
NAB-14	+	-	+	+	+
NAB-15	++	-	+	+	+
NAB-16	++	-	+	+	+
NAB-17	+	-	+	+	+
NAB-18	++	17.3 mm, (55%)	+	+	+
NAB-19	++	-	+	+	+
NAB-20	+	-	+	+	+
NAB-21	+	11.6 mm, (42%)	+	+	+
NAB-22	+	-	+	+	+
NAB-23	+	-	+	+	+
NAB-24	+	-	+	+	+
NAB-25	++	-	+	+	+
NAB-26	++	-	+	+	+
NAB-27	++	-	+	+	+
NAB-28	++	-	+	+	+
NAB-29	++	-	+	+	+
NAB-30	++	-	+	+	+
NAB-31	++	-	+	+	+
NAB-32	+	-	+	+	+
NAB-33	+	-	+	+	+
NAB-34	+	-	+	+	+
NAB-35	+	-	+	+	+
NAB-36	+	-	+	+	+
NAB-37	++	-	+	+	-
NAB-38	+	17.3mm, (50%)	+	+	+
NAB-39	+	-	+	+	+
NAB-40	+	-	+	+	+
NAB-41	+	-	+	+	+
NAB-42	+	-	+	+	+
NAB-43	+	-	+	+	+
NAB-44	+	-	+	+	+
NAB-45	+	-	+	+	+
NAB-46	+	-	+	+	+
NAB-47	+	-	+	+	+
NAB-48	+	13.6mm, (26%)	+	+	+
NAB-49	+	-	+	+	+
NAB-50	+	8.3mm, (20%)	+	+	+



A & B - Different morphology of *Bacillus*, C & D - Gram's reaction show Gram's positive rods of Bacilli, E & F - Different isolates show endospores, G&H- Voges-Proskauer reaction, I& J- Carbohydrate fermentation, K & L - Citrateutilization, M& N- Indole test, O&P – Starch hydrolysis, Q&R – Casein hydrolysis, S&T – Siderophore production, U&V - IAA Production, W & X - Ammonia production

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