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ISOLATION AND CHARACTERIZATION OF PLANT GROWTH PROMOTING RHIZOSPHERIC SOIL BACTERIA

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Abstract: The rhizosphere is the zone of soil around the roots in which microbial biomass is directly impacted by the presence of roots. A beneficial rhizobacteria community is essential for growth and development of the plant host. In the present study, total 7 different samples were collected from different sites and total 19 bacterial isolates were obtained from different soil samples. All isolates were screened for different enzyme activities i.e. cellulase, amylase, protease, lipase, pectinase, chitinase. 16 isolates were positive for cellulase, 16 isolates were positive for amylase, 15 isolates were positive for protease, 14 isolates were positive for lipase, 18 isolates were positive for pectinase and 18 isolates were positive for chitinase. Isolates were also characterized for plant growth promoting activities like IAA, phosphate solubilization, nitrogen fixation, ammonia production, catalase, zinc solubilisation, seed germination and pot assay. 10 isolates were positive for IAA, 18 isolates were positive for phosphate solubilization, 17 isolates were positive for nitrogen fixation, 12 isolates were positive for ammonia production, many isolates gave promising result for seed germination and pot assay. Isolates were also checked for biocontrol activities such as HCN production and antimicrobial activity. 5 isolates were positive for HCN production, one isolate gave positive result for antibacterial activity and one isolate gave positive result for antifungal activity. Looking to overall activities together isolates B5 and B9 were found more efficient for all the activities under study.

Keywords – **Rhizobacteria, Extracellular enzyme activities, Plant growth promoting activities, Biocontrol activities**

1. INTRODUCTION

The phyllosphere and rhizosphere are two areas connected to the plants. Phyllosphere and rhizosphere both are beneficial to plants. Hiltner first coined the term “rhizosphere” as the area of microbial activity around roots (Hassan *et al*., 2019). The area of soil immediately around a plant’s roots is known as rhizosphere; this area is influenced by root exudates and the associated microorganisms. There are three separate, but interacting, components recognized in the rhizosphere. These are the rhizosphere (soil), the rhizoplane, and the root itself. The rhizosphere is the zone of soil influenced by roots through the release of substrates that affect microbial activity. The rhizoplane is the root surface, including the strongly adhering soil particles. The root itself is a part of the system, because certain micro-organisms, the endophytes, are able to colonize root tissues (Barea *et al*., 2005).

Different plant has different microbial communities in their rhizosphere. It mainly consists of plant growth promoting rhizobacteria and mycorrhizae. The rhizosphere's microbial activity is crucial for plant health because it aids in nutrient uptake and provides defence against plant pathogen. Plant-microbe interactions, governed by root exudates via the chemotactic response of the microbes toward root-secreted organic compounds, play an important role in root colonization and biological control (antifungal, antibacterial, and antiviral) activities (Hassan *et al*., 2019). The soil compartment immediately surrounding the plant root contains a significantly higher number of microorganisms than non-rooted bulk soil. A subset of rhizosphere bacteria that can colonize the root environment is referred to as rhizobacteria.

Beneficial root colonizing rhizosphere bacteria , the PGPR are defined by three intrinsic characteristics;

1. They must be able to colonize the root.
2. They must survive and multiply in microhabitats associated with the root surface in competition with other microbiota.
3. They must promote plant growth.

The PGPR participate in many important ecosystem processes such as the biological control of plant pathogens, nutrient cycling and seedling growth. PGP mechanisms have been grouped traditionally into direct and indirect mechanisms. The difference between the two is not always evident, indirect mechanisms, as a general rule, are those that happen outside the plant, while direct mechanisms are those that occur inside the plant and directly affect the plant’s metabolism (Goswami *et al*., 2016). Direct mechanisms such as fixation of atmospheric nitrogen, solubilization of minerals (phosphorus, zinc), production of siderophores and synthesis of plant

growth hormones (IAA, cytokinin, gibberellins, abcisic acid). An indirect mechanism involves the biological control of plant pathogens and deleterious microbes, through the production of antibiotics, lytic enzymes, and hydrogen cyanide.

PGPRs are considered an eco-friendly alternative to hazardous chemical fertilizer. The use of PGPRs as biofertilizers is a biological approach toward the sustainable intensification of agriculture. An ideal PGPR strain should fulfil the following criteria;

1. It should be highly rhizosphere-competent and eco-friendly.
2. It should colonize the plant roots in significant numbers upon inoculation.
3. It should be able to promote plant growth.
4. It should exhibit a broad spectrum of action.
5. It should be compatible with other bacteria in the rhizosphere.
6. It should be tolerant of physicochemical factors like heat, desiccation, radiations, and oxidants.
7. It should demonstrate better competitive skills over the existing rhizobacterial communities (Basu *et al*., 2021).

2. MATERIALS AND METHODS

2.1 Sample collection and soil analysis

Rhizospheric soil samples were collected from *Oryza sativa* (rice) plants from Udwada and Segvi, *Cajanus Cajan* (pigeon pea) plants from mograwadi Valsad, *Muasceae sapientum* (banana) plants from mograwadi Valsad, *Trigonella foenum-graecum* (fenugreek) plants from Bilimora, *and Corindrum sativum* (coriander) plants from Bilimora and agriculture soil from Bartad. Soil samples were collected at a depth 5–10 cm. Samples were collected in bags and stored at low temperature. Further, physical and chemical properties were performed. Soil physical properties include color, texture, structure, consistence, moisture, porosity and water holding capacity. Soil chemical properties include pH, organic carbon, calcium, chloride, calcium and magnesium concentration.

2.2 Isolation of bacteria from the rhizospheric soil

For isolation of rhizospheric bacteria, soil suspension was prepared. For soil suspension 1 gm of soil was weighed and transferred into 10 ml sterile distilled water. On pre-prepared Luria bertani agar plates, soil suspension was streaked by four flame method or zigzag method. Plates were incubated at ambient temperature for 24 – 48 hours. After incubation colony characteristics were recorded. Gram staining was also performed for bacterial isolates obtained from Luria Bertani agar plate.

2.3 Analysis of isolates for extracellular enzyme activities

2.3.1 Cellulase enzyme activity

The isolates were spot inoculated on carboxy methyl cellulose agar plate and incubated for 2-3 days at ambient temperature. After incubation plates were flooded with gram's iodine. A clear halo zone around the colony indicates cellulose production (Modi *et al*., 2017).

2.3.2 Amylase enzyme activity

The isolates were spot inoculated on starch agar plate and incubated for 2-3 days at ambient temperature. After incubation plates were flooded with gram's iodine. A clear halo zone around the colony indicates amylase production (Pranay *et al*., 2019).

2.3.3 Protease enzyme activity

The isolates were spot inoculated on milk agar plate and incubated for 2-3 days at ambient temperature. After incubation a clear halo zone around the colony indicates protease production (Modi *et al*., 2017).

2.3.4 Lipase enzyme activity

The isolates were spot inoculated on tributyrene agar plate and incubated for 2-3 days at ambient temperature. After incubation a clear halo zone around the colony indicates lipase production.

2.3.5 Pectinase enzyme activity

The isolates were spot inoculated on pectin agar plate and incubated for 2-3 days at ambient temperature. After incubation plates were flooded with gram's iodine. A clear halo zone around the colony indicates pectinase production (Kabir *et al*., 2019).

2.3.6 Chitinase enzyme activity

The isolates were spot inoculated on chitin agar plate and incubated for 2-3 days at ambient temperature. After incubation a clear halo zone around the colony indicates chitinase production (Kamil *et al*., 2007).

2.4 Analysis of isolates for plant growth promoting activities

2.4.1 Determination of Indole 3- Acetic Acid

The production of indole acetic acid was assayed by using Salkowski method. Production of pink colour indicates the IAA production (Modi *et al*., 2017).

2.4.2 Phosphate solubilization

The isolates were inoculated on Pikovskaya's agar plates by spot inoculation method. Plates were incubated at ambient temperature. The appearance of transparent halo zones around the bacterial colonies indicated the phosphate solubilizing activity of the isolates (Ahmad *et al*., 2008).

2.4.3 Nitrogen Fixation

Screening of nitrogen fixing isolates was done on Jensen medium, the medium devoid of nitrogen source. Isolates were inoculated on medium by zigzag inoculation method and those isolates were able to grow, who were able to fix nitrogen. Colonies were observed on plates after 48-72 hours of incubation.

2.4.4 Catalase activity

Catalase test was performed by taking a drop of 3 % hydrogen peroxide was added to 48 hours old bacterial colony on a clean glass slide and mixed using a sterile tooth-pick. The effervescence indicated catalase activity (Kumar *et al*., 2012).

2.4.5 Ammonia production

Overnight grown isolates cultures were inoculated in 10 ml peptone nitrate broth and incubated at 30° C for 48 hours in incubator shaker. After incubation 0.5 ml of Nessler's reagent was added. The development of faint yellow to dark yellow or brown color indicated the production of ammonia (Ahmad *et al*., 2008).

2.4.6 Zinc solubilization

The isolates were inoculated on Bunt and Rovira medium agar plates by spot inoculation method. The plates were incubated at ambient temperature for 24-48 hours. This medium containing ZnO as the zinc source. The appearance of transparent halo zones around the bacterial colonies indicated the zinc solubilizing activity of the isolates (Tamboli, 2019).

2.5 Seed germination assay

Effect of the isolates on germination 3 seeds of *Triticum aestivum* (wheat) were first surface sterilized with the help of 0.1% $HgCl_2$ for 3 minutes followed by washing with water to remove mercury traces then seeds were inoculated with each isolate were incubated in petriplates on two layers of moistened filter paper. For control, seeds treated with water instead of bacterial suspension. Maintain sufficient moisture for germination and plates incubated at ambient temperature for 7 days. After incubation length of roots and shoots were measured. Graph was plotted and statistical analysis was done using MS Excel software (Kumar *et al*., 2012).

2.6 Pot assay

For pot assay, the soil used was repeatedly sterilized. Then soil was transferred to the plastic container. The seed of *Vigna radiata* (mung bean) and *Vicia faba* (broad bean) were first surface sterilized with the help of 0.1% $HgCl_2$ for 3 minutes followed by washing with water to remove mercury traces. Seeds and inoculum (broth of isolates) were added. Plants were watered daily for 7 to 14 days. After the incubation roots and shoots of plant were measured. Graph was plotted and statistical analysis was done using MS Excel software.

2.7 Analysis of isolates for biocontrol activities

2.7.1 HCN production

Isolates were streaked on nutrient agar medium containing 4.4 g per liter of glycine. A Whatman filter paper No. 1 soaked in 0.5% picric acid solution in 2% sodium carbonate was placed inside the lid of a plate. Plates were sealed with parafilm and incubated at ambient temperature for 4 days. Development of light brown to dark brown color indicated HCN production (Kumar *et al*., 2012).

2.7.2 Antimicrobial activity

To carry out the antimicrobial activity supernatant is required. For supernatant, isolates were inoculated in Luria Bertani broth and incubated at ambient temperature for 48 hours on rotary shaker. After incubation broth was centrifuged to separate out supernatant and it was used to carry out antimicrobial activity. Agar well diffusion assay was used for antimicrobial activity experiment, in which nutrient agar plates were streaked (by help of sterile swab) with test organism like *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and for antifungal activity sabouraud's agar plates were streaked with test fungi like against *Penicillium sp*., *Aspergillus sp*. and *Fusarium sp*.. With the help of sterile cup borer wells are created on the agar plate, supernatant was added in wells. Antibacterial activity plates were incubated at ambient temperature for 24 hours and antifungal activity plates were incubated ambient temperature for 3-5 days. After incubation plates were observed for zone of inhibition**.**

3. RESULT AND DISCUSSION

Total seven different soil samples were collected from different sites which are shown in **Table 1**. Results of physical properties of soil are shown in **Table 2**. Results of chemical properties of soil are shown in **Table 3**. From the seven different samples total 19 isolates were isolated. Out of 19 isolates, 5 isolates were gram negative short rod occurring in singly and pair; 5 isolates were gram positive long rod occurring in chain and pair; 6 isolates were gram positive short rod occurring in pair and singly; 3 isolates were gram positive cocci occurring in bunch and singly.

Table 1 Sample collection sites

Sample No.	Collection sites
A1	Rhizosphere of rice plant, Udawada
A2	Rhizosphere of rice plant, Segavi
A3	Rhizosphere of banana plant, Valsad
A4	Rhizosphere of bean plant, Valsad
A5	Rhizosphere of fenugreek plant, Bilimora
A6	Rhizosphere of coriander plant, Bilimora
A7	Agriculture soil, Bartad

Table 2 Results of physical properties of soil

Properties	A1	A2	A3	A4	A5	A6	A7
Color	Light brown	Light brown	Brown	Black	Black	Black	Brown
Texture	Slit	Slit	Loam	Loam	Loam	Loam	Slit
Structure	Aggregated	Aggregated	Granular	Granular	Granular	Granular	Aggregated
Consistency	Non sticky	Non sticky	Non sticky	Slightly sticky	Slightly sticky	Slightly sticky	Non sticky
Moisture content (%)	28	24	13	20	33	29	18
Porosity (%)	25	20	31	20	28	29	43
Water holding capacity (%)	55	60	50	64	56	44	40

Table 3 Results of chemical properties of soil

Properties	A1	A2	A3	A4	A5	A6	A7
pH	6.5	6.5	7.0	6.5	6.5	6.5	6.5
Organic carbon (%)	60	50	78	69	54	50	66
Calcium (mg/ml)	60	70	90	80	120	100	130
Calcium and Magnesium (mg/ml)	90	110	130	160	170	250	100
Chloride (mg/ml)	177	177	168	115	159	141	106

3.1 Result of isolates for extracellular enzyme activities

Rhizosphere microorganisms release extracellular enzyme for the initial degradation of high molecular polymer such as cellulose, chitin, lignin and protein. Lytic enzymes such pectinase and chitinase are active against soil fungi. Isolates were screened for cellulase, amylase, protease, lipase, pectinase and chitinase enzyme production. Results of extracellular enzyme activities of isolates are shown in **Table 4**. Isolates B3, B5, B8 and A2 gave highest zone of hydrolysis for cellulase enzyme production. Isolate B3 gave highest zone of hydrolysis for amylase enzyme production. Isolate S4 gave highest zone of hydrolysis for protease enzyme production. Isolate A2 gave highest zone of hydrolysis for lipase enzyme production. Isolate A5 gave highest zone of hydrolysis for pectinase enzyme production. Isolate B2, B6, A2, A5 and S1 gave highest zone of hydrolysis for chitinase enzyme production. **Table 4 Results of extracellular enzyme activities of isolates**

Isolates	Zone diameter (mm)					
	Cellulase	Amylase	Protease	Lipase	Pectinase	Chitinase
B1	13	12	15	7	20	39
B2	17	30	18	12	32	**40**
B3	**20**	**35**	-	20	35	35
B4	-	10	35	-	-	25
B5	**20**	30	30	15	29	35
B6	15	29	-	12	25	**40**
B7	9	25	30	19	15	33
B8	**20**	25	30	16	35	34
B9	12	24	28	-	10	35
A1	15	-	-	15	29	34
A2	**20**	29	30	**25**	35	**40**
A3	-	-	15	-	20	-
A4	-	-	18	-	32	27
A5	17	10	30	23	**38**	**40**
S1	17	26	35	20	30	**40**
S4	18	25	**38**	20	33	38
U1	19	30	30	15	33	**40**
U3	15	23	-	-	25	29
U4	13	20	22	20	35	32

3.2 Result of isolates for plant growth promoting activities

Isolates were characterized for plant growth promoting activities such as indole 3- acetic acid, phosphate solubilization, nitrogen fixation, ammonia production, zinc solubilization.

3.2.1 Determination of Indole 3- Acetic Acid

IAA is type of natural auxin. Auxin is plant growth promoting hormone which is directly connected to the elongation factor of cell. If IAA is more so cell elongation is also more. This hormone is also produced by some rhizospheric bacteria so they increase the root colonization. If root is well developed so plant easily uptake nutrient and water from the soil. Out of 19, isolates 10 isolates gave positive result for IAA. Isolates B2, B3, B4, B5, B8, B9, A2, A4 and A5 gave positive result for indole 3- acetic acid test. Results of IAA production of isolates are shown in **Table 5**.

3.2.2 Phosphate solubilization

Phosphorus is macronutrient for plant development. Phosphorus is present in soil but in insoluble form. Phosphate solubilizing bacteria convert insoluble to soluble form of phosphorus. Out of 19 isolates, 18 isolates gave zone of solubilisation. Isolate B9 showed maximum clear zone of 33 mm. Results of phosphate solubilization of isolates are shown in **Table 5**.

3.2.3 Nitrogen fixation

Nitrogen is also macronutrient for plant growth. Nitrogen is present in atmosphere but only prokaryotic microorganisms have ability to fix atomospheric nitrogen to nitrate or ammonia. Out of 19 isolates, 17 isolates were grown on nitrogen deficient medium. All isolates gave positive result for nitrogen fixation except isolate A3 and A4. Results of nitrogen fixation by isolates are shown in **Table 5**.

3.2.4 Catalase activity

Out of 19 isolates, 15 isolates gave positive result for catalase test. Results of catalase test by isolates are shown in **Table 5**.

3.2.5 Ammonia production

Out of 19 isolates, 12 isolates gave positive result for ammonia production test. Isolates B1, B2, B3, B5, B6, B7, B8, B9, A2, S1, and S4 gave positive result for ammonia production. Results of ammonia production of isolates are shown in **Table 5.**

3.2.6 Zinc solubilization

Zinc is micronutrient for plant growth. Out of 19 isolates, 17 isolates gave zone of zinc solubilization. Isolates B4 and B7 showed maximum clear zone of 20mm. Results of zinc solubilization of isolates are shown in **Table 5.**

Table 5 Results of plant growth promoting activities of isolates

Isolates	Indole 3 - Acetic Acid	Nitrogen fixation	Catalase activity	Ammonia production	Zone diameter (mm)	
					Phosphate solubilization	Zinc solubilization
B1	-	+	+	+	21	17
B2	+	+	+	+	19	10
B3	+	+	-	+	15	16
B4	+	+	+	-	19	**20**
B5	+	+	+	+	20	13
B6	+	+	+	+	20	15
B7	-	+	+	+	21	**20**
B8	+	+	-	+	12	10
B9	+	+	+	+	**33**	13
A1	-	+	+	+	25	14
A2	+	+	+	+	12	13
A3	-	-	+	-	20	19
A4	+	-	-	-	22	15
A5	+	+	-	-	20	10
S1	-	+	+	+	15	12
S4	-	+	+	+	20	11
U1	-	+	+	-	13	-
U3	-	+	+	-	25	15
U4	-	+	+	-	-	-

3.3 Result of seed germination assay

Out of 19 isolates, three isolates did not give much effect on shoot and root. Isolates A3 and U3 did not give more effect on root as compared to control. Isolate A4 did not give more effect on shoot as compared to control. Isolate U4 and S1 gave maximum effect on seed germination. Effect of isolates on *Triticum aestivum* (wheat) seed germination is shown in **Figure 1**.

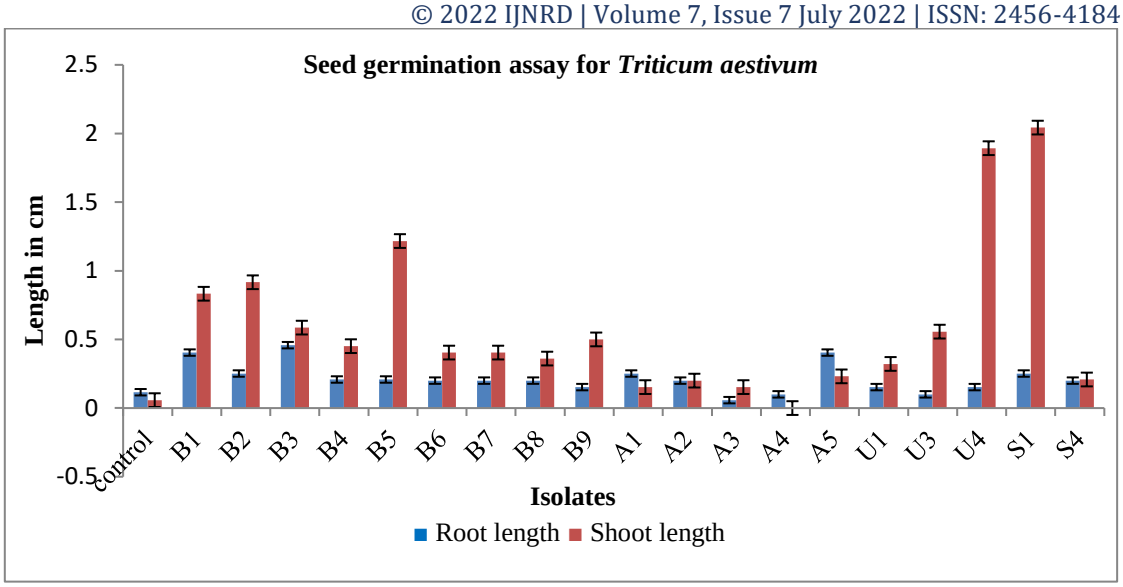

Figure 1 Result of effect of isolates on *Triticum aestivum* (wheat) seed germination

3.4 Result of pot assay

In case of *Vigna radiata* (mung bean)**,** Out of 19 isolates four isolates did not give more effect on growth of plant as compared to control. Isolates B4, B5, B7 and A3 did not give more effect on growth of plant as compared to control. Isolates B8, B9, A4, A5 and S1 gave maximum effect on growth of plant. Effect of isolates on growth of *Vigna radiata* (mung bean) seeds are shown in **Figure 2.** In case of *Vicia faba* (broad bean), Out of 19 isolates four isolates did not give more effect on growth of plant as compared to control. Isolates B5, B7, A3 and S4 did not give more effect on growth of plant as compared to control. Isolates B2, B8, B9, A4 and U4 gave maximum effect on growth of plant. Effect of isolates on growth of *Vicia faba* (broad bean) seeds is shown in **Figure 3**.

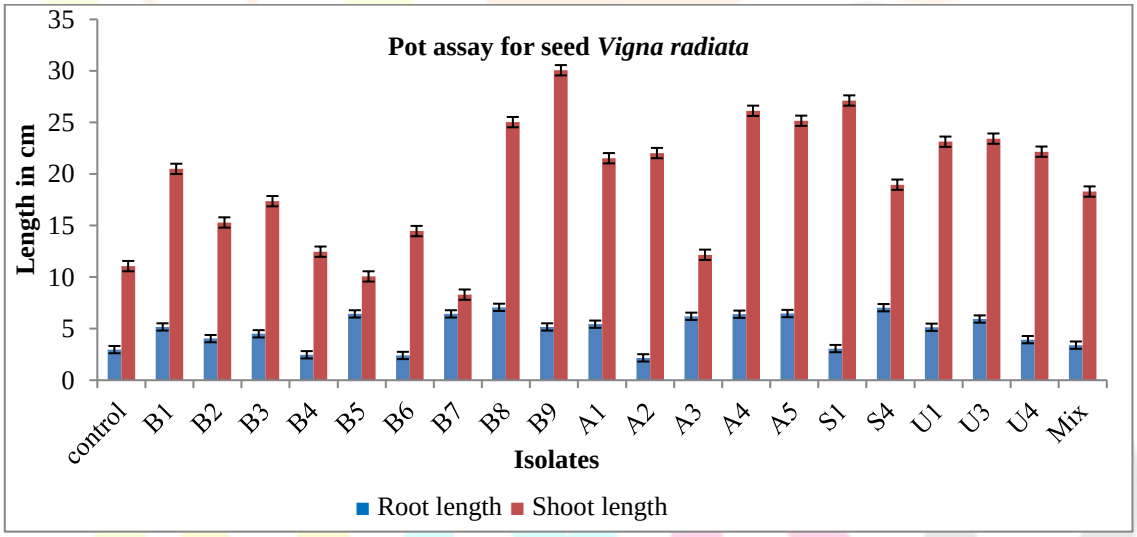

Figure 2 Result of effect of isolates on growth of *Vigna radiata* (mung bean) seeds

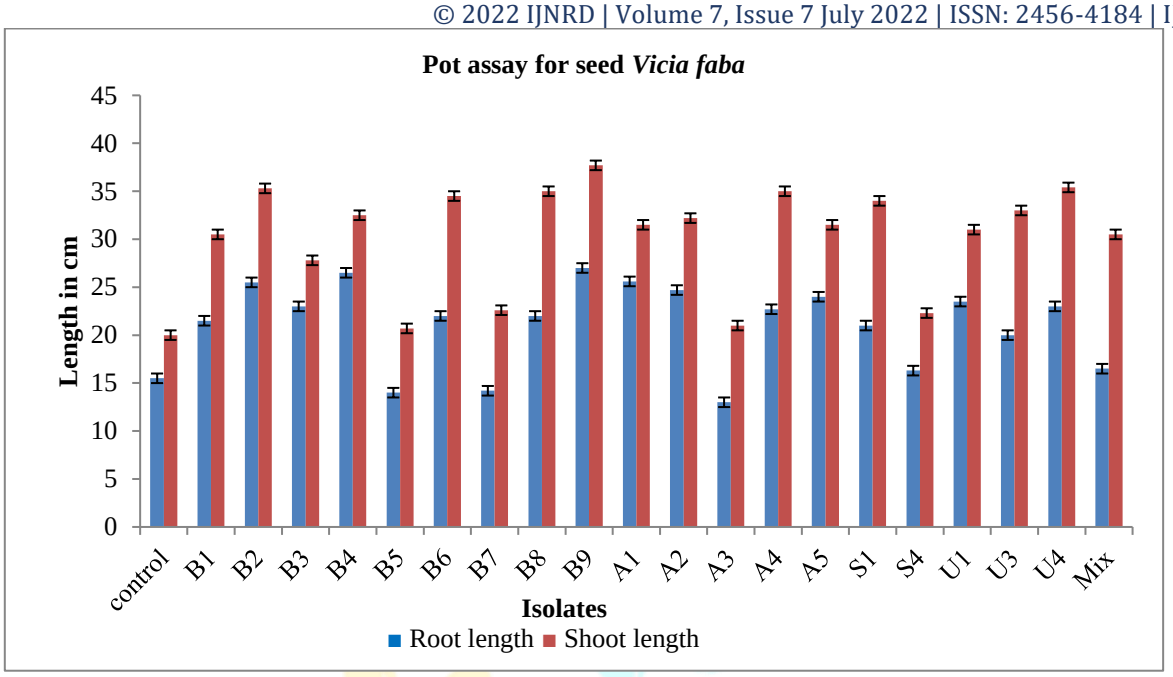

Figure 3 Result of effect of isolates on growth of *Vicia faba* (broad bean) seeds

3.5 Result of isolates for biocontrol activities

Isolates were tested for biocontrol activities such as HCN production and antimicrobial activities.

3.5.1 HCN production

HCN is volatile compound which is produce by bacteria in a small amount against plant pathogens. Out of 19 isolates, only 5 isolates B5, B7, B9, S4 and U4 gave positive result for HCN production.

3.5.2 Antimicrobial activity

Isolate A1 gave positive result for antibacterial against *E.coli* as test bacteria and isolate B2 gave positive result for antifungal against *Penicillium sp.* as test fungi.

4. CONCLUSION

From rhizospheric soil sample of different sites, bacteria were isolated showed different extracellular enzyme activities, plant growth promoting activities and biocontrol activities. Seed germination and pot assay were also carried out to check efficiency of isolates as biofertilizer. The present study indicates that isolates obtain in this study have good ability to produce extracellular enzyme and plant growth promoting activities as compared to biocontrol activities.

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