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Isolation of Plant Growth Promoting Bacterial Species from Sorghum Bicolor Rhizospere Soil

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Abstract: ***Plant rhizosphere is known to be preferred ecological niche for soil microorganisms due to rich nutrient availability. Diverse group of microorganisms including Bacillus, Pseudomonas, Serratia, Acinetobacter, Enterobacter, Azatobacter spp. can be isolated and they enhancing the plant growth by different ways such as producing plant growth hormones, solubilize inorganic phosphate and prevent pathogens. Hence the present study is aimed to isolate the Plant Growth Promoting Rhizobacteria (PGPR) from the rhizosphere of Sorghum bicolor. In this study three effective strains such as Bacillus spp., Pseudomonas spp. and Klebsiella spp. were isolated and their Indole -3-Acetic Acid (IAA) producing ability were tested. Among these three strains Klebsiella spp. produced significantly higher amount of IAA when compared with other two strains and their plant growth promoting activity was studied using Sorghum bicolor.***

Keywords: PGPR, Indole -3-Acetic Acid, *Sorghum bicolor*

1. Introduction

The rhizosphere is the zone of soil surrounding the plant root where the biology and chemistry of the soil are influenced by the root. This zone is about 1mm wide, but has no distinct edge. Rather, it is an area of intense biological and chemical activity influenced by compounds exuded by the root, and by microorganisms feeding on the compounds [1]. Plant Growth Promoting Rhizobacteria (PGPR) are free-living bacteria and some of them invade the tissues of living plants and cause unapparent and symptomatic infections [2]. PGPR have the ability to protect above ground plant parts against viral, fungal and bacterial diseases by induced systemic resistance (ISR) [3]. PGPR was first defined by Kloepper and Schroth to describe soil bacteria that colonize the roots of plants following inoculation onto seed and that enhance plant growth. The following are implicit in the colonization process: ability to survive inoculation onto seed, to multiply in the spermosphere (region surrounding the seed) in response to seed exudates, to attach to the root surface, and to colonize the developing root system[4]. The PGPR have been divided into two groups: those involved in nutrient cycling and phytostimulation, and those involved in the bio control of plant pathogens. The PGPR-mediated processes involved in nutrient cycling include those related to non-symbiotic nitrogen-fixation, and those responsible for increasing the availability of phosphate and other nutrients in the soil [5, 6]. Plant morphogenic effects may also be a result the different ratios of plant hormones produced by roots as well as by rhizosphere bacteria [7]. Plant growth regulators participate in the growth and development of cells, tissues, organs, and in fact the entire plant. These compounds are active in plants in very minute amounts and their synthesis is extremely regulated. Plants not only produce phytohormones but also, numerous plant-associated bacteria both beneficial and harmful, produce one or more of these substances [8].

The first plant hormone we consider is auxin. Auxin deserves pride of place in any discussion of plant hormones because it was the first growth hormone discovered in plants, and much of the early physiological work on the mechanism of plant cell expansion was carried out in relation to auxin action. The term auxin is derived from the Greek word auxin which means to grow. Compounds are generally considered auxins if they can be characterized by their ability to induce cell elongation in stems and otherwise resemble Indole -3-Acetic Acid (IAA) (the first auxin isolated) in physiological activity. Auxins usually affect other processes in addition to cell elongation of stem cells but this characteristic is considered critical of all auxins and thus helps to define the hormone. Auxins were the first plant hormones discovered by Charles Darwin. Auxin was identified as a plant growth hormone because of its ability to stimulate differential growth in response to light stimulate. The in vitro bioassay in which auxin-containing agar blocks stimulated the growth of oat coleoptile segments led to the identification of IAA as the main naturally occurring auxin in plants. Applications of IAA or synthetic auxins to plants cause profound changes in plant growth and development [9]. IAA is chemically similar to the amino acid tryptophan which is generally accepted to be the molecule from which IAA is derived. Three mechanisms have been suggested to explain this conversion. Tryptophan is converted to indole pyruvic acid through a transamination reaction. Indole pyruvic acid is then converted to indole acetaldehyde by a decarboxylation reaction. The final step involves oxidation of indoleacetaldehyde resulting in indole acetic acid. Tryptophan undergoes decarboxylation resulting in tryptamine. Tryptamine is then oxidized and deaminated to produce indoleacetaldehyde. This molecule is further oxidized to produce indoleacetic acid [10]. Nowadays, under the modern agricultural practices, chemical fertilizers are used to boost the crop production. But the application of chemical fertilizers affects the total productivity of the crops and in the long run the soil becomes sterile and unfit for cultivation practices. Hence in order to enhance the fertility status of the soil, the natural way of feeding the soil with different types of organic inputs (composts, vermicomposts, Biofertilizers, farmyard manure etc.) has been developed so as to ensure sustained productivity. As plant root grow through soil they release water-soluble compounds such as amino acids, sugars and organic acid that supply food for the microorganism. In return, the microorganisms provide

nutrients for the plants. All this activity makes the Rhizosphere the most dynamic environment in soil. Because root are underground rhizosphere activity due to the food supply provided by the root exudates. Hence the present study is aimed to isolate the PGPR from their natural habitat and evaluate their plant growth promoting activity using *Sorghum bicolor*.

2. Materials and Methods

Soil samples from the rhizosphere of *Sorghum bicolor* plants were collected in a sterile polythene bag from the cultivation land in and around Gandhigram during December month. The soil sample was serially diluted and appropriate dilution was spread on nutrient agar plates. Plates were incubated at 28 ± 2°C for 24-48 hours. Predominant colonies were picked from these plates and maintained as pure cultures in respective media with periodic transfer to fresh media and stocked for further use. The biochemical tests were carried out to identifying the bacteria.

Screening of Bacterial Isolates for Indole-3-Aetic Acid (IAA) production

The organisms isolated form rhizosphere regions were identified and they were screened for their ability to produce IAA. Test bacterial culture was inoculated in the respective medium with tryptophan and incubated at 37º C for 2-3 days. After incubation period cultures were centrifuged at 8000rpm at 30 minutes. The 2ml supernatant was collected and mixed with 2 drops of orthophosphoric acid and 4ml Salkowoski reagent. (50 ml of 35% perchloric acid, 1 ml of 0.5 M ferric chloride). Incubate the tubes in dark room for half an hour. The tubes showed pink color indicates positive result for IAA production. Then the OD was measured at 530nm usind Spectronic 20D. The results were plotted in graph and they were compared with standard IAA.

Standard IAA Preparation

0.5g of indole-3-acetic acid is mixed with 100ml of distilled water. From this standard take (0.5, 1, 2, 3, 4 and 5ml) various concentrations up to 10ml for each concentration. Read the values using spectrophotometer at 530 nm. Optical density was noted and plotted in the standard graph.

Mass Multiplication

The pure culture of selected bacterial strain was grown in nutrient broth with tryptophan. This was called starter culture. Then they were added in appropriate broth for mass multiplication and the broth was incubated at 24-48hrs.

Seed Germination Study

Seedling bioassay of *Sorghum bicolor* was done using *Pseudomonas* sp., *Bacillus* spp. and *Klebsiella* sp. culture broth at various concentration (1ml, 2ml, 3ml, 4ml and 5ml) at different day intervals (5day,10dayand15day). Growth parameters such as germination percentage, root and shoot length, leaf length and fresh and dry weight of the plant were analyzed once in 5 days upto 15 days using standard procedures.

3. Results and Discussion

In the present study, isolation of bacterial cultures from the rhizosphere soil samples of *Sorghum bicolor* were made the rhizosphere harbors 32 different variety of PGPR among them only three were selected for further study based on IAA production. The bacterial isolates were identified based on the colony morphology, Gram's staining and other biochemical tests. The results are given in Table 1. Based on the biochemical test the isolates were identified as *Pseudomonas* spp., *Bacillus* spp. and *Klebsiella* spp. These bacterial isolates were mass multiplied for further study.

Table 1: Biochemical characteristics of bacterial isolates

Bacterial isolates	Biochemical characteristics												
	Gram's staining	Indole test	Methyl red test	Vogasprokaur test	Citrate test	Urease test	Catalase test	H_2S production test	Glucose utilization test	Gelatinhydrolsis test	Starch hydrolysis test	Nitrate reductase test	Identified bacterial isolates
Bacterial isolate 1	-	-	-	-	+	-	-	-	+	-	+	+	*Pseudomonas* sp.
Bacterial isolate 2	+	-	-	-	-	-	-	-	+	+	+	+	*Bacillus* sp.
Bacterial isolate 3	-	+	-	+	-	-	-	-	-	+	+	-	*Klebsiella* sp.

(+) positive, (-) negative

Screening of bacterial strains for IAA production:

All the isolates were screened for IAA production among them only three organisms showed IAA production ranging from 0.23 to 30.0 µg/ml. The results revealed that the *Klebsiella* spp. showed significantly higher quantity of IAA production. The quantities of IAA produced by three bacterial isolates are given in Table 2.

Table 2: IAA produced by bacterial isolates.

S.No	*Bacterial isolates*	*Optical density (nm)*	*IAA production µg/ml*
1	*Pseudomonas* sp	530	0.23
2	*Bacillus* sp	530	0.23
3	*Klebsiella* sp	530	0.30

These IAA producing cultures were tested for their plant growth promoting activity using *Sorghum bicolor*. The *Sorghum bicolor* seeds were surface sterilized using 5%

mercuric chloride solution for 2-3 min. The growth study of *Sorghum bicolor* was done in vermiculate supplemented with nutrient solution (T0), *Pseudomonas* spp. (T1) *Bacillus* spp. (T2) and *Klebsiella* spp. (T3) culture broth. The study was carried out at the period of 10 days. The major growth parameters such as germination percentage, root and shoot length, leaf length and fresh and dry weight of the whole plant were measured once in five day intervals. The plants inoculated with *Pseudomonas* sp. showed the highest growth at 5ml concentration on 10th day compared with the plants inoculated with *Bacillus* spp. and *Klebsiella* spp. the results are shown in Table 3, 4 and 5. Similar kind of experiment was carried out by other authors and the effect of *Pseudomonas* spp. and *Azotobacter* sp. isolates on root elongation was evaluated at the different concentrations of tryptophan, i.e. 0, 1, 2, and 5 mg/ml. Without tryptophan, the root elongation of germinating seeds of *S. aculeata* and *V. radiata* was highest with *Azotobacter* isolate Azs9, followed by Azs1 and Azs6, compared to the control [11].

Table 3: Evaluation of root and shoot length, leaf length, fresh and dry weight of *Sorghum bicolor* grown in vermiculate supplemented with *Pseudomonas* spp. (culture broth) at five different concentrations (1, 2, 3, 4 and 5ml) on 5d and 10d intervals

Concentration of bacterial (*Pseudomonas* Spp.) culture broth	Plant growth parameters									
	Root Length (cm)		Shoot length (cm)		Leaf Length (cm)		Fresh Weight (g)		Dry Weight (g)	
	5 d	10 d	5 d	10 d	5 d	10 d	5 d	10 d	5 d	10 d
Control	5.30±2.64	9.53±4.44	10.00±1.37	13.80±1.04	6.52±1.32	9.40±1.32	0.10±0.01	0.20±0.02	0.01±0.02	0.02±0.06
1ml	5.60±1.15	9.93±1.25	10.50± 1.32	14.50±0.86	6.83±0.76	10.50±1.33	0.15±0.06	0.30±0.01	0.01±0.01	0.03±0.05
2ml	6.60±3.51	11.28±4.16	11.60±1.15	14.60±1.44	7.50±0.55	11.60±1.20	0.20±0.09	0.40±0.02	0.02±0.01	0.04±0.02
3ml	7.80±2.46	11.33±1.52	12.30± 3.18	14.80±1.52	7.83±0.76	11.80±1.25	0.29±0.03	0.50±0.06	0.02±0.005	0.05±0.05
4ml	8.00±1.00	12.63±2.75	13.60± 1.76	16.30±0.76	7.83±1.04	12.30±1.80	0.35±0.08	0.60±0.04	0.03±0.02	0.06±0.07
5ml	8.50±0.86	13.83±2.36	14.40±1.15	18.30±1.09	8.33±0.57	12.50±4.35	0.45±0.04	0.70±0.02	0.04±0.01	0.07±0.01

*Results were shown in Mean ± Standard Deviation

Table 4: Evaluation of root and shoot length, leaf length, fresh and dry weight of *Sorghum bicolor* grown in vermiculate supplemented with *Bacillus* spp. (culture broth) at five different concentrations (1, 2, 3, 4 and 5ml) on 5d and 10d intervals.

Concentration of bacteria (*Bacillus* Spp.) culture broth	Plant growth parameters									
	Root Length		Shoot length		Leaf Length		Fresh Weight		Dry Weight	
	5 d	10 d	5 d	10 d	5 d	10 d	5 d	10 d	5 d	10 d
Control	6.16±0.76	6.29±1.89	9.13±1.02	10.5±0.28	5.1±0.76	4.58±0.28	0.10±0.01	0.15±0.02	0.01±0.02	0.01±0.06
1ml	6.36±1.32	7.75±1.75	9.16±1.04	10.4±2.29	6.5±0.5	7.66±1.52	0.13±0.06	0.20±0.01	0.01±0.01	0.02±0.05
2ml	7.16±1.25	7.30±2.92	10.3±1.80	11.8±1.52	7.5±0.5	7.84±1.52	0.17±0.09	0.27±0.02	0.01±0.01	0.03±0.02
3ml	7.66±0.57	8.51±2.25	11.5±0.86	12.6±3.54	7.3±0.86	8.18±2.75	0.23±0.03	0.37±0.06	0.02±0.005	0.03±0.05
4ml	8.35±2.64	8.75±2.75	11.66±1.52	12.1±1.04	7.6±0.76	8.31±1.44	0.30±0.08	0.48±0.04	0.03±0.02	0.04±0.07
5ml	8.66±0.57	11.11±6.33	12.3±3.51	13.6±0.86	7.8±0.28	8.43±1.89	0.37±0.04	0.59±0.02	0.03±0.01	0.05±0.01

*Results were shown in Mean ± Standard Deviation

Table 5: Evaluation of root and shoot length, leaf length, fresh and dry weight of *Sorghum bicolor* grown in vermiculate supplemented with *Klebsiella*spp. (culture broth) at five different concentrations (1, 2, 3, 4 and 5ml) on 5d and 10d intervals.

Concentration of bacteria (*klebsiella* Spp.) culture broth	Plant growth parameters									
	Root Length		Shoot length		Leaf Length		Fresh Weight		Dry Weight	
	5 d	10 d	5 d	10 d	5 d	10 d	5 d	10 d	5 d	10 d
Control	4.70±3.21	11.1±3.40	6.41±2.75	15.3±1.25	3.85±0.5	7.66±1.52	0.10±0.01	0.14±0.02	0.01±0.02	0.01±0.06
1ml	4.76±1.60	11.3±4.20	6.52±6.06	15.5±0.5	4.52±0.52	8.23±1.32	0.12±0.06	0.20±0.01	0.01±0.01	0.02±0.05
2ml	4.81±1.04	11.3±3.12	6.84±1.52	16.3±2	4.52±0.57	8.66±1.52	0.15±0.09	0.25±0.02	0.01±0.01	0.03±0.02
3ml	5.02±2.08	12.5±1.73	8.26±1.21	16.5±1.80	4.84±1.52	9.66±1.75	0.20±0.03	0.33±0.06	0.02±0.005	0.03±0.05
4ml	5.71±1.15	12.8±1.72	8.31±2.32	16.5±3.04	4.86±1.12	9.66±2.22	0.27±0.08	0.45±0.04	0.02±0.02	0.04±0.07
5ml	5.91±1.25	12.5±2.64	8.59±0.28	17.5±3.04	5.05±1.15	10.12±2.64	0.35±0.04	0.57±0.02	0.03±0.01	0.05±0.01

*Results were shown in Mean ± Standard Deviation

4. Conclusion

In the present study the effective plant growth promoting bacteria such as *Bacillus* spp., *Pseudomonas* spp. and *Klebsiella* spp. were isolated and their growth promoting activity were tested. Such type of study is necessary as it advocates that use of PGPR as inoculants or biofertilizers is an efficient approach to replace chemical fertilizers and these PGPR isolates may be used as biofertilizers to enhance the growth and productivity of the food crops. It is very

economical way to fertilize the soil without any deleterious effect to the environment.

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