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ORIGINAL ARTICLE

INDOLE-3-ACETIC ACID PRODUCTION AND ENHANCED PLANT GROWTH PROMOTION BY INDIGENOUS BACTERIAL SPECIES

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ABSTRACT: In the present study 28 bacterial strains from black gram (*Vigna mungo)* rhizosphere soil were isolated. Among them three predominant bacterial strains were identified using morphological and biochemical characteristics as *Pseudomonas* spp. *Bacillus* spp. and *Proteus* spp. The identified bacterial strains were screened for IAA production. Among them one bacteria that produced significantly higher quantity of IAA compared to other strains. Then the plant growth promoting activity of bacterial that is *Pseudomonas* spp. culture broth was tested at different concentrations (1, 2, 3, 4, and 5ml) and at different day intervals (5th and 10th d). The growth parameters such as root length (3.90±0.19cm), shoot length (15.50±0.81cm), fresh weight of the whole plant (0.29±0.00g) and dry weight of the whole plant (0.03±0.05g) have significantly enhanced by *Pseudomonas* spp. culture broth at 5ml concentration on 10th day compared to (1, 2, 3, 4, 5ml and in the control and on 5th day). The plant growth promoting efficiency of IAA separated from *Pseudomonas* spp. at different concentrations (1, 2, 3, 4 and 5ml) were tested using black (*Vigna mungo)* the crude IAA at 5ml concentration have significantly increased the root length (7.40±0.19cm), shoot length (17.50±0.82cm), fresh weight of the whole plant (0.58±0.00g) and dry weight of the whole plant (0.05±0.01g) compared to (1, 2, 3, 4ml and in the control and on 5th day).
KEYWORDS: Black gram *(Vigna mungo)*, Rhizosphere soil, *Pseudomonas* spp.

INTRODUCTION

Plant growth-promoting rhizobacteria (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 (Kloepper and Schroth, 1978).

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 nonsymbiotic nitrogen-fixation, and those responsible for increasing the availability of phosphate and other nutrients in the soil (Bashan and Holguin, 1998; Kennedy *et al.*, 2004).

Fluorescent *pseudomonads* (PGPR) are a group of beneficial microorganisms, which vigorously colonize roots and provide beneficial effects to plant development. Microbial formulations are carrier-based preparations containing beneficial microorganisms in a viable state intended for seed or soil application. They are designed to improve soil fertility and help plant growth by increasing their numbers and thus their biological activity in the root environment (Ahamed *et al.*, 2005). The PGPR have been known to directly enhance plant growth by a variety of mechanisms, namely fixation of atmospheric nitrogen that is transferred to the plant, production of siderophores that chelate iron and make it available to the plant root, solubilization of minerals such as phosphorus and synthesis of phytohormones. The inoculation of seeds with PGPR is known to increase nodulation, nitrogen uptake, and growth and yield response of crop plants (Dorosinsky and Kadyrov *et al.*, 1975).

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

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ability to induce cell elongation in stems and otherwise resemble indole -3- acetic acid (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 stimuli. The in vitro bioassay in which auxin-containing agar blocks stimulated the growth of oat coleoptile segments led to the identification of indole-3-acetic acid (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 (Bonner and Bandurski, 1952).

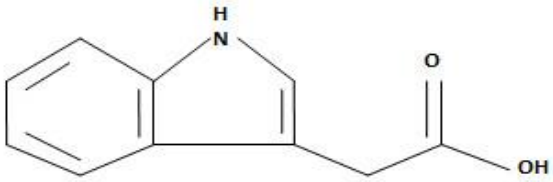

Fig 1: Structure of indole-3-acetic acid (IAA)

Structurally each IAA consists of indole nucleus and an acetic acid side chain. All the indole auxins are converted into IAA during the metabolism. The pH has a significant effect on the amount of IAA production. Vitamins also play a role in the regulation of IAA synthesis production of IAA increased with an increase in tryptophan concentration (Patten and Glick, 1996). 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 indole acetic acid (Jisc, 2012).

MATERIALS AND METHODS

Soil samples from the rhizosphere of *Vigna mungo* plants were collected in a sterile polythene bag from the cultivation land in and around Gandhigram Rural Institute-Deemed University, Gandhigram, Dindigul District, Tamilnadu, and South India 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 for identifying the bacteria.

2.1. 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 8000 rpm for 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 colour indicates positive result for IAA production. Then the OD was measured at 530nm using Spectronic 20D. The results were plotted in graph and they were compared with standard IAA.

2.2. Standard IAA Preparation

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

2.3. 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.

2.4. Seed Germination Study

Seedling bioassay of *Vigna mungo* was done using *Pseudomonas* spp., culture broth and crude IAA separated from culture broth at various concentration (1, 2, 3, 4 and 5ml) and at different day intervals (5day and 10day). Growth parameters such as root and shoot length, and fresh and dry weight of the whole plants were analysed once in 5 days up to 10 days using standard procedures.

RESULTS AND DISCUSSION

In the present study, isolation of bacterial cultures from the rhizosphere soil samples of *Vigna mungo* were made, the rhizosphere harbors 28 different variety of PGPR among them only one strains was 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 *Bacillus* spp., *Proteus* spp. and *Pseudomonas* spp.; these bacterial isolates were mass multiplied for further study.

3.1. Screening of bacterial strains for IAA production

All the three isolates were screened for IAA production among them *Pseudomonas* spp. only produced 0.67 µg/ml IAA compare to *Bacillus* spp. (0.42 µg/ml) and *Proteus* spp. (0.19 µg/ml). The results revealed that the *Pseudomonas* spp. showed significantly higher quantity of IAA production. The quantities of IAA produced by three bacterial isolates are given in Table 2. Similar observations for IAA production have been reported by other researchers. Lee and Knowles reported 20-90.8mg/L of IAA whereas *Azotobacter chrococcum, Azotobacter vinelandii, Pseudomonas aeruginosa, Pseudomonas putida, Serratia* sp. and *Klebsiella pneumonia* strains shown to produce IAA from 3.5 mg/L to 32.2mg/L (Lee and Knowles, 1970). *Azospirillum brasilense* produced 26.1µg/ml of IAA (Crozier, 1988). These IAA producing cultures were tested for their plant growth promoting activity using *Vigna mungo*. The *Vigna mungo* seeds were surface sterilized using 5% mercuric chloride solution for 2-3 min. The growth study of *Vigna mungo* was done in vermiculate supplemented with *Pseudomonas* sp. (T1) culture (broth) at different concentrations (1, 2, 3, 4 and 5ml) and crude IAA separated from *Pseudomonas* sp. (T2) at different concentrations (1, 2, 3, 4 and 5ml). The study was carried out at the period of 10 days. The major growth parameters such as root and shoot length and fresh and dry weight of the whole plant were measured once in five day intervals. The growth parameters such as root length (3.90±0.19cm), shoot length (15.50±0.81cm), fresh weight of the whole plant (0.29±0.00g) and dry weight of the whole plant (0.03±0.05g) have significantly enhanced by *Pseudomonas* spp. culture broth at 5ml concentration on 10th day compared to 1, 2, 3, and 4 ml and in the control and on 5th day the results are given in Table 3 and 4. The plant growth promoting efficiency of crude IAA separated from *Pseudomonas* spp. at different concentrations (1, 2, 3, 4 and 5ml) on black gram (*Vigna mungo)* have significantly increased the root length (7.40±0.19cm), shoot length (17.50±0.82cm), fresh weight of the whole plant (0.58±0.00g) and dry weight of the whole plant (0.05±0.01g) at 5ml concentration on 10th day compared to 1, 2, 3 and 4ml and in the control and on 5th day the results are given in table 5 and 6. Similar kind of experiment was carried out by Moor, (1989) and the effect of *Pseudomonas* spp. and *Azotobacter* spp. isolates on root elongation was evaluated at the different concentrations of tryptophan, i.e. 0, 1, 2, 3, 4 and 5 mg/ml. Without tryptophan, the root elongation of germinating seeds of *S. aculeata* and *V. radiata* was highest with *Azotobacte*r isolate Azs9, followed by Azs1 and Azs6, compared to the control (Moor, 1989). Findings of earlier workers who postulated that reported that the *Pseudomonas* and *Azotobacter* spp. cultured in the medium supplemented with 1 and 2 mg/ml of tryptophan enhanced the root elongation of *Sesbania aculeate* and *Vigna radiata*. At a 5 mg/ml tryptophan concentration these microbes, decreased the root elongation in both *S. aculeata* and *V. radiata.* Such an influence caused by both the isolates, indicated that tryptophan supplementation at a 5 mg/ml concentration is toxic and did not promote root elongation (Ahmad *et al.*, 2005).

Table 1: Biochemical characteristics of bacterial isolates

Bacterial strain	Biochemical characteristics												
	Gram staining	Indole test	Methyl red test	Vogas proskauer test	Citrate test	Urease test	Catalase test	H_2S reduction test	Glucose utilization test	Gelatin hydrolsis test	Starch hydrolysis test	Nitrate reductase test	Identified bacterial isolates
Bacterial isolate 1	+	-	-	-	-	-	-	-	+	+	+	+	*Bacillus* spp.
Bacterial isolate 2	-	+	+	-	-	+	+	+	+	+	-	+	*Proteus* spp.
Bacterial isolate 3	-	-	-	-	+	-	-	-	+	+	+	+	*Pseudomonas* spp.

Table 2: IAA produced by bacterial isolates

S. No	Bacterial Strains	Optical density (nm)	IAA production µ g ml
1.	*Pseudomonas* sp.	530	0.67
2.	*Bacillus sp.*	530	0.42
3.	*Proteus sp.*	530	0.19

Table 3: Evaluation of root length, shoot length, fresh and dry weight of black gram grown in vermiculite supplemented with *Pseudomonas* spp. (culture broth) at five different concentrations (1, 2, 3, 4 and 5ml) on 5d and in the control

S. No	Concentration of bacterial culture	Plant growth parameters			
		Root Length	Shoot length	Fresh Weight of the whole plant	Dry Weight of the whole plant
1	Control	1.10±0.152	8.10±0.152	0.21±0.00	0.02±0.00
2	1ml	1.46±0.057	11.70±0.20	0.22±0.00	0.02±0.00
3	2ml	1.80±0.152	12.40±0.36	0.23±0.00	0.03±0.00
4	3ml	2.50±0.169	13.20±0.57	0.23±0.00	0.03±0.00
5	4ml	2.80±0.172	13.70± 0.60	0.24±0.00	0.03±0.01
6	5ml	3.30±0.185	14.40±0.68	0.25±0.00	0.03±0.05

Table 4: Evaluation of root length, shoot length, fresh and dry weight of black gram grown in vermiculite supplemented with *Pseudomonas* spp. (culture broth) at five different concentrations (1, 2, 3, 4 and 5ml) on 10d and in the control

S. No	Concentration of bacterial culture	Plant growth parameters			
		Root Length	Shoot length	Fresh Weight of the whole plant	Dry Weight of the whole plant
1	Control	2.30±0.25	11.70±0.95	0.22±0.00	0.02±0.00
2	1ml	2.50±0.16	13.80±0.67	0.23±0.00	0.02±0.00
3	2ml	2.90±0.17	14.20±0.69	0.24±0.00	0.03±0.00
4	3ml	3.50±0.18	14.80±0.74	0.26±0.00	0.03±0.00
5	4ml	3.70±0.19	15.10±0.75	0.27±0.00	0.03±0.01
6	5ml	3.90±0.19	15.50±0.81	0.29±0.00	0.03±0.05

Table 5: Evaluation of root length, shoot length, fresh and dry weight of black gram grown in vermiculite supplemented with Crude IAA separated from *pseudomonas* sp. at five different concentrations (1,2,3,4,and 5ml) on 5d and in the control

S. No	Concentration of Crude IAA	Plant growth parameters			
		Root Length	Shoot length	Fresh Weight of the whole plant	Dry Weight of the whole plant
1	Control	1.10±0.15	7.20±0.15	0.22±0.00	0.02±0.00
2	1ml	4.90±0.16	8.10±0.16	0.29±0.00	0.02±0.00
3	2ml	5.10±0.17	9.20±0.17	0.30±0.05	0.03±0.00
4	3ml	5.50±0.17	10.30±0.20	0.38±0.05	0.03±0.00
5	4ml	5.90±0.17	10.70±0.21	0.42±0.06	0.04±0.01
6	5ml	6.30±0.19	12.40±0.25	0.48±0.06	0.04±0.01

Table 6: Evaluation of root length, shoot length, fresh and dry weight of black gram grown in vermiculite supplemented with Crude IAA separated from *pseudomonas* sp. at five different concentrations (1,2,3,4,and 5ml) on 5d and in the control

S. No	Concentration of Crude IAA	Plant growth parameters			
		Root Length	Shoot length	Fresh Weight of the whole plant	Dry Weight of the whole plant
1	Control	5.40±0.25	11.70±0.95	0.23±0.01	0.02±0.02
2	1ml	5.80±0.17	12.90±0.65	0.34±0.00	0.03±0.00
3	2ml	6.40±0.18	13.80±0.71	0.36±0.00	0.03±0.00
4	3ml	6.60±0.18	14.20±0.75	0.47±0.00	0.04±0.00
5	4ml	6.90±0.19	15.90±0.79	0.48±0.00	0.04±0.01
6	5ml	7.40±0.19	17.50±0.82	0.58±0.00	0.05±0.01

CONCLUSION

Agriculture soil study ways to make soils more productivity. They classify soils and test them to determine whether the nutrients vital to plant growth. Such nutritional substances include compounds of nitrogen, phosphorus, and potassium. If a certain soil is deficient in these substances, fertilizers may provide them. In the present study the effective plant growth promoting bacterial species (*Pseudomonas* spp.) was isolated and their growth promoting activities 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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