

## A Study on the Biopotentials of Bacterial Strains Isolated from Paddy Rhizosphere

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Three bacterial strains *Bacillus* sp, *Proteus* sp, and *Pseudomonas* sp isolated from paddy rhizosphere soil were screened for IAA production. All the three bacterial isolates were able to produce IAA. These isolates were also tested for IAA production at different pH and in different time intervals. *Pseudomonas* sp was able to produce higher amount of IAA at pH 7(12.81 $\mu$ g/ml) and on 7 d (12.78 $\mu$ g/ml). The bioassay result reveals that the crude IAA extract from *Pseudomonas* sp can promoted the germination percent (90), vigour index (495) shoot (3.8cm) and root length (10.0cm) in paddy, *Oryza sativa* in T9 on 7<sup>th</sup> day compared to other treatments and in control.

**Key words:** Rhizosphere, Bacterial isolates, IAA.

Rice is a staple food for a large part of the world's human population. In India it is grown all over the country. A number of research has been carried out on increasing rice production in different parts of India. In the recent times work related to the use of plant growth regulators (PGR) is rising up and use of microbial plant growth promoters would help in preventing stress caused by chemical PGRs. Plant growth promoting rhizobacteria (PGPR) are considered to promote plant growth directly or indirectly. PGPR can exhibit variety of characteristics responsible for influencing plant growth. The common traits include production of

plant growth regulators (auxin, gibberellin, ethylene etc.). HCN and antibiotics. Indole acetic acid (IAA) is one of the most physiologically active auxins. IAA is a common product of L-tryptophan metabolism by several microorganisms including PGPR (Lynch 1985).

### MATERIAL AND METHODS

#### Isolation and identification of bacterial isolates from paddy rhizosphere soil

Rhizosphere soil was collected from the paddy fields of Athoor region, Dindigul District, Tamil Nadu, India and the bacterial strains from this soil were isolated by serially diluting the soil sample and plating on the Nutrient agar medium. From among the colonies that developed, three bacterial colonies which showed better growth were isolated and identified based on Gram's staining character and various biochemical tests such as indole test, methyl red test, Vogues Proskauer test, citrate test, urease test, glucose

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utilization test, catalase test, H<sub>2</sub>S production test, gelatin hydrolysis, starch hydrolysis, casein hydrolysis and nitrate reduction test (Cappuccino and Sherman, 1992).

#### Screening of bacterial isolates for Indole Acetic Acid (IAA) production

The three isolated bacterial strains were grown in Nutrient broth supplemented with tryptophan (500- µg/ml) medium without tryptophan served as control and centrifuged at 10,000 rpm for 15 minutes. 2ml of the supernatant was mixed with 2 drops of orthophosphoric acid and 4 ml of Salkowski reagent (50 ml of 35 percent perchloric acid and 1 ml of 0.5M FeCl<sub>3</sub> solution) (Salkowski, 1985). Development of pink color indicated the production of IAA. (Ivanova *et al.*, 2001).

#### Determination of IAA production at different pH and at different time intervals

The pH of Nutrient agar medium was adjusted from 3 to 10 with the help of 1N sodium hydroxide or 0.1 N hydrochloric acid and 1ml of each bacterial strain was inoculated and incubated for 24 h at 37<sup>o</sup> C. After incubation the cultures were centrifuged and IAA produced by the bacterial isolates was measured spectrophotometrically at 530 nm. For optimization of days the cultures were inoculated in Nutrient broth supplemented with tryptophan and kept for incubation. At different day intervals i.e. from 1 day to 7 day the IAA produced by the three bacterial isolates were measured spectrophotometrically at 530 nm (Ivanova *et al.*, 2001). The concentration of IAA produced by each bacterial culture at various pH and at various day intervals was determined by comparing them with a standard curve of pure indole-3 acetic acid.

#### Bioassay

##### Percentage germination of *Oryza sativa*

*O. sativa* seeds were germinated for a week under aseptic conditions on a filter paper moistened with pure culture of the three selected bacterial isolates i.e. *Bacillus* sp, *Proteus* sp and *Pseudomonas* sp at three different concentrations i.e. 1ml, 3ml and 5ml. The percentage germination was calculated using the following formula.

$$\text{Germination (\%)} = \frac{\text{No of Seeds germinated}}{\text{Total no of seeds}} \times 100$$

#### Vigour index

*Oryza sativa* seeds were surface sterilized and planted in paper cups containing sterilized vermiculate. IAA crude extracts prepared by centrifugation the three bacterial cultures grown in Nutrient agar medium were separately poured into the cups containing the paddy seeds at 1ml, 3ml and 5ml concentrations. After a week the shoot and root lengths of the seedlings were measured and the vigour index was calculated using the given formula (Balki & Anderson, 1973).

$$\text{Vigour Index} = \text{Germination \%} \times \text{seedling length}$$

## RESULTS AND DISCUSSION

The results of the Gram staining and various biochemical tests carried out for the three selected bacterial isolates are given in Table 1. Based on their characteristics they were identified as *Bacillus* sp, *Proteus* sp and *Pseudomonas* sp.

The results of the screening of the three selected isolates for IAA production showed the development of pink colour which indicated a positive reaction for the production of IAA by all the three isolates. Studies on optimization of pH for IAA production showed a highest yield at a pH of 7 (12.81µg/ml) by *Pseudomonas* sp as shown in Table 2. Studies on optimization of days showed a higher rate of IAA production on the 7 day (12.78µg/ml) by *Pseudomonas* sp as shown in Table 3. work carried out by (Hutcheson and Kasuge, 1985 and Magie *et al.*, 1963) also showed variation in the level of IAA production by *Pseudomonas* sp isolated from soybean rhizosphere at various pH. The evaluation of germination percentage and vigour index of *O. sativa* seeds indicated that crude IAA extract of *Pseudomonas* sp at 5ml concentrations can enhance the germination percentage (90) and vigour index (495) as shown in Table 4. In the control (uninoculated) the percentage of germination was 40 and vigour index was 60. (Balki & Anderson, 1973), have also observed similar kind of results in their studies in soybean plants. Among the three bacterium tested *Pseudomonas* sp promoted higher shoot length (3.8cm) and root length (10.0cm) at 5 ml concentrations on 7th day in *O. sativa* when compared to the other two organisms and in

**Table 1.** Gram's staining and biochemical characteristics of the three bacterial isolates collected from the paddy rhizosphere soil

Tests carried out	Bacterial isolate 1	Bacterial isolate 2	Bacterial isolate 3
Gram's staining	+	-	-
Indole test	+	+	+
Methyl red test	-	+	-
Voges Proskauer test	-	-	-
Citrate utilization test	-	-	+
Urease test	-	+	-
Catalase test	-	+	-
H <sub>2</sub> S production test	-	+	-
Utilization of glucose	+	+	+
Gelatin hydrolysis	+	+	+
Starch hydrolysis	+	-	+
Casein hydrolysis	+	+	+
Nitrate reduction	+	+	+
Identification result (name of the bacteria)	<i>Bacillus</i> sp	<i>Proteus</i> sp	<i>Pseudomonas</i> sp

+ - Positive;                      - -Negative

**Table 2.** IAA production by bacterial isolates at various pH

Bacterial isolates	IAA production (µg/ml) by bacterial isolates							
	3	4	5	6	7	8	9	10
Control	5.10	6.11	7.20	8.12	9.11	8.10	7.21	6.20
<i>Bacillus</i> sp	8.20	9.10	10.13	11.40	12.48	11.50	10.20	9.10
Control	3.20	4.10	5.14	6.17	7.30	6.50	5.70	4.50
<i>Proteus</i> sp	7.17	8.28	9.31	10.38	11.01	10.50	9.70	8.40
Control	6.13	7.21	8.11	9.10	10.07	9.51	8.71	7.51
<i>Pseudomonas</i> sp	8.11	9.13	10.50	11.79	12.81	11.91	10.81	9.71

**Table 3.** IAA production by bacterial isolates at various day intervals

Bacterial isolates	IAA production (µg/ml) by bacterial isolates						
	1d	2d	3d	4d	5d	6d	7d
Control	1.20	2.34	3.59	5.20	6.21	7.33	9.20
<i>Bacillus</i> sp	5.10	6.50	7.69	8.92	9.58	11.23	12.5
Control	1.00	1.98	2.90	3.71	4.88	5.91	7.50
<i>Proteus</i> sp	4.00	5.50	6.79	7.83	8.92	9.99	11.00
Control	1.50	2.79	3.93	5.77	6.93	8.22	10.00
<i>Pseudomonas</i> sp	5.55	6.79	7.99	9.10	10.00	11.79	12.78

**Table 4.** Evaluation of germination percentage and vigour index of *Oryza sativa* seed treated with various concentration of the crude extract of IAA produced by *Bacillus* sp, *Proteus* sp and *Pseudomonas* sp(7d)

Treatment	Control	T1	T2	T3	T4	T5	T6	T7	T8	T9
Germination percentage	40	60	70	80	50	60	65	55	70	90
Vigour index	60	144	264	294	95	144	169	121	245	495
T-Control										

T1-Seeds applied with 1 ml of crude IAA of *Bacillus* sp  
T2- Seeds applied with 3 ml of crude IAA of *Bacillus* sp  
T3- Seeds applied with 5 ml of crude IAA of *Bacillus* sp  
T4- Seeds applied with 1 ml of crude IAA of *Proteus* sp  
T5- Seeds applied with 3ml of crude IAA of *Proteus* sp  
T6- Seeds applied with 5 ml of crude IAA of *Proteus* sp  
T7- Seeds applied with 1 ml of crude IAA of *Pseudomonas* sp  
T8- Seeds applied with 3 ml of crude IAA of *Pseudomonas* sp  
T9- Seeds applied with 5 ml of crude IAA of *Pseudomonas* sp

**Table 5.** Estimation of root and shoot length of *Oryza sativa* grown at various concentrations of the crude IAA produced by *Bacillus* sp, *Proteus* sp, and *Pseudomonas* sp (7d)

Name of the isolate	Root length (cm)			Shoot length (cm)		
	Concentration of the crude IAA extract					
	1ml	3 ml	5 ml	1 ml	3 ml	5 ml
Control	1.2	1.2	1.2	1.2	1.2	1.2
<i>Bacillus</i> sp	2.2	3.0	3.2	6.5	8.2	9.6
<i>Proteus</i> sp	2.0	2.7	3.5	6.8	7.2	8.6
<i>Pseudomonas</i> sp	2.4	2.8	3.8	6.7	7.3	10.0

control. Similar kind of observations were made by (Vasudevan *et al.*, 2002).

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