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RESEARCH ARTICLE

INDOLE ACETIC ACID AND GIBBERELLIC ACID PRODUCING *Pseudomonas and Bacillus* FROM THE RHIZOSPHERE OF RICE *(Oryza Sativa L.)*

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ABSTRACT

In the present investigation, plant growth promoting bacteria such as Pseudomonas and Bacillus were isolated from the rhizosphere soil of rice (Oryza sativa L.). Bacillus is a group of gram positive, aerobic or anaerobic bacteria widely found in soil and water. Bacillus is a common free - living species found in all types of soil and environment. Pseudomonas is gram negative rod shaped and polar flagellated bacteria found in most of the environment. During the isolation studies about ten different Bacillus and ten different Pseudomonas isolates were isolated and characterized based on biochemical studies. The isolated Bacillus and Pseudomonas isolates were designated as PGPBB1-PGPBB10 and PGPBP1-PGPBP10 respectively. All the twenty isolates of Bacillus and Pseudomonas were studied and screened for the IAA and GA3 production. In the production of IAA and GA3 all the isolates of pseudomonas recorded much significant values compared with Bacillus isolates from the rhizosphere soil of rice.

INTRODUCTION

PGPB - Plant Growth Promoting Bacterial organisms are known for their beneficial role played on the plant life through nitrogen fixation, Phosphorous solubilization, Zinc, Silicon solubilization and growth promoting substances production. The growth promoting substances like Indole acetic acid (IAA) and gibberellic acid (GA_3) played vital role in the mitotic cell division, elongation, growth and development of plants, The rhizosphere is the area in and around root zone and its adhered soil particles known to attract majority of PGPR and other bacterial organisms through secreting root exudates, rhizodeposits and fraction of humus. These organic substances rich in nutrients, vitamins, minerals, carbohydrates, amino acids, organic acids, as well as other growth related compounds, which are known to attract all kinds of microorganisms. The root exudates normally released in to the rhizosphere, and microorganisms were well known to be chemo attracted and move towards root exudates. If it may be a PGPR organisms it will colonize and multiply both in the rhizosphere, rhizoplane and in some cases some organisms enters into the plant parts as endophytes (Kloepper *et al.,* 1989).

MATERIALS AND METHODS

Estimation of Indole Acetic Acid (IAA) by Pseudomonas isolates: About 100 ml of King's B broth was prepared in Erlenmeyer's flask and sterilized. Freshly prepared, filter sterilized L-tryptophan solution was added to each flask to a final concentration of 100 mg^{-1}. The flask were added with 1.0 ml culture broth of each Pseudomonas isolates and incubated at 37°C in the dark for seven days. After incubation, the culture was centrifuged at 6000 rpm for 5 min to remove the bacterial cells. The supernatant was brought to pH 2.8 with 1 N HC1. Fifteen ml of the acidified supernatant was taken in 100 ml conical flask and to it equal volume of diethyl ether was added and incubated in dark for 4 h. IAA extraction was done at 4°C in a separating funnel using diethyl ether. The organic phase was discarded and the solvent phase was pooled and evaporated to dryness. To the dried material, two ml of methanol was added, pooled and the IAA present in the methanol extract was determined using the method of Gorden and Paleg (1957). To 0.5 ml of the methanol extract, 1.5 ml of distilled water and four ml of Salper's reagent (1.0 ml of 0.5 M $FeCl_3$ in 50 ml of 35 per cent perchloric acid) were added and incubated in dark for one hour. The intensity of pink colour developed was read at 535 nm in a spectrophotometer. From a standard curve prepared with known concentrations of IAA, the quantity of IAA in the culture filtrate was determined and expressed as µg 25 ml of *culture* medium.

Estimation of Indole Acetic Acid (IAA) by *Bacillus* isolates

About 100 ml of Pikovskaya's broth was prepared in Erlenmeyer's flask and sterilized. Freshly prepared, filter sterilized L-tryptophan solution was added to each flask to a final concentration of 100 mg l"1. The flask were added with

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1.0 ml culture broth of each Bacillus isolates and incubated at 37°C in the dark for seven days, then the IAA produced by Bacillus isolates was determined by the procedure mentioned the above chapter.

Estimation of Gibberellic Acid (GA_3) by *Pseudomonas* isolates: About 100 ml of King's B broth was reported in Lrlenmeyer's flask and sterilized. The flask was added with 1.0 ml culture broth of each Pseudomonas isolate and incubated at 37°Cin the dark for seven days. After seven days of incubation, the culture was centrifuged at 8000 rpm for 10 min to remove the bacterial cells. Fifteen ml of the culture was pipetted out separately into the test tubes and two ml of zinc acetate solution was added.

Preparation of reagents

Zinc acetate solution: A quantity of 21.9 g of zinc acetate was dissolved in 80 ml of distilled water and one ml of glacial acetic acid was added and the volume was made upto 100 ml with distilled water.

Potassium ferrocyanide solution: A quantity of 10.6 g of potassium ferrocyanide was dissolved in 100 ml of distilled water. After two min, two ml of potassium ferrocyanide solution was added and centrifuged at 8000 rpm for 10 min. Five ml of supernatant was added to five ml of 30 per cent hydrochloric acid and the mixture was incubated at 27°C for 75 min. The blank was prepared with five percent hydrochloric acid. Absorbance was measured at 254 nm in a UV-VIS spectrophotometer. From the standard graph prepared by using gibberellic acid solutions of known quantities, the amount of GA_3 produced by the culture was calculated and expressed as pg 25 ml broth.

Estimation of Gibberellic acid (GA_3) by *Bacillus* isolates: About 100 ml of Pikovskaya's broth was reported in Erlenmeyer's flask and sterilized. The flask was added with 1.0 ml culture broth of each Bacillus isolates and incubated at 37°Cin the dark for seven days. Then the GA_3 produced by each isolate was determined by the procedure described in the earlier chapters in materials and methods.

RESULT AND DISCUSSION

Among the PGPB isolates of Bacillus and *Pseudomonas,* all the ten isolates of Pseudomonas recorded much significant values in the production of both IAA and GA_3 compared with the other isolates of *Bacillus*. All the ten isolates of Pseudomonas produced IAA and GA_3 and quantity were ranged from 20. 10µg Per 25 ml to 66.00µg per 25 ml of broth for the IAA. Among the ten *pseudomonas* isolates, the isolate PGPBP-6 recorded maximum value to the range of 66.00µg per 25 ml compared with the other nine isolates. In GA_3 production the value ranged from 3.00µg to 7.5 µg per 25ml. *Bacillus* isolates also recorded appreciable values on IAA and GA_3 production, the values were ranged from 18.00µg per 25 ml and GA_3 production on the value were 1.76 µg to 5.60 µg per 25ml. Further, PGPB isolates performed better in the lab condition on IAA and GA_3 production, whereas future studies are needed to exploit potentiality of *Pseudomonas* and *Bacillus* under different environmental conditions. The results of the present findings in accordance with the findings of Dobbelaere *et al.* 1999. In the estimation IAA all the twenty isolates from *Pseudomonas* and *Bacilllus* recorded appreciable values, whereas *Pseudomonas* isolates recorded much significant values on IAA protection compared with *Bacillus* isolates, the values ranged from 20.01 to 66.00 µg/25 ml of broth for *Pseudomonas.* In the case of *Bacillus* the recorded values in the range of 18.00 to 24.96 µg/25ml of broth.

Table 1. Screening of *Pseudomonas* isolates for Indole acidic acid and Gibberellic acid production

Name of the isolates	1AA (µg) 25ml of broth	GA_3 (µg) 25ml of broth
PGPBP1	31.04	6.28
PGPBP2	29.00	3.89
PGPBP3	34.66	4.50
PGPBP4	20.10	3.00
PGPBP5	66.00	7.56
PGPBP6	54.02	6.48
PGPBP7	60.60	7.00
PGPBP8	50.10	5.99
PGPBP9	47.00	3.21
PGPBP10	52.04	5.44
S.Ed	1.50	0.10
CD (P=0.05)	3.20	0.29

Table 2. Screening of *Bacillus* isolates for Indole acidic acid and Gibberellic acid production

Name of the isolates	IAA (µg) 25ml of broth	GA_3 (µg) 25ml of broth
PGPBB1	20.60	2.88
PGPBB2	24.66	3.33
PGPBB3	22.00	3.00
PGPBB4	18.00	1.76
PGPBB5	24.80	4.80
PGPBB6	24.96	4.38
PGPBB7	24.80	4.20
PGPBB8	20.86	2.66
PGPBB9	20.26	2.58
PGPBB10	20.86	3.11
S.Ed	1.44	0.12
CD (P 0.05)	2.99	0.49

The results are similar to the findings of Tien *et al.,*1994. In GA_3 production also *Pseudomonas* performed better and recorded much significant values compared with the isolates of *Bacillus*. The present result showed that the isolates of *Pseudomonas* were dominating in the production of growth promoting substances production like IAA & GA_3 and in on other hand *Bacilllus* showed and recorded much lesser values in the production of IAA & GA_3. The present findings are in the similar line of Baca *et al.,* 1994; Kloepper *et al.,* 1980.

Conclusion

The results of the present research clearly showed the efficiency of *Pseudomonas* and *Bacillus* on 1AA & GA_3 production, further these benefits should be screened on agricultural crops on different environments.

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