INDOLE ACETIC ACID PRODUCTION BY SALT TOLERANT FREE LIVING BACTERIA ASSOCIATED WITH WHEAT RHIZOSPHERE

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Abstract: Plant growth promoting rhizobacteria (PGPR) are known to influence plant growth by various direct or indirect mechanisms. In search of efficient PGPR strains with high activity of IAA , a total of 58 isolates belonging to *Pseudomonas, Azotobacter* and *Bacillus* were screened for plant growth promoting trait ie. indole acetic acid (IAA).The eighteen isolates (nine *Azotobacter*, six *Pseudomonas* and three *Bacillus*) were evaluated for quantitative IAA production. All the *Azotobacter* isolates shown to produce higher range (95.60-175.20 µg/ml) of IAA, while *Pseudomonas* produced (44.40- 95.00 µg/ml) IAA. More interestingly all *Bacillus* isolates also shown high potential of producing in the range of 95.60-170.20 µg/ml of IAA. The isolate Azt5, Bc1 and Bc3 tolerated 7% NaCl concentration.

Keywords: PGPR, Wheat Rhizosphere, Indole acetic acid, Salt tolerance

INTRODUCTION

The growth of many microorganism in the rhizospheric region depends upon the root exudates released by the plants (Bais *et al.*, 2006). Interactions between plant and microbes are intensely studied and especially those that benefit plant growth. The PGPRs may benefit the host by causing plant growth promotion or biological disease control. PGPR activity has been reported in strains belonging to several genera such as *Azotobacter, Pseudomonas, Azospirillum, Acetobacter, Burkhalderia* and *Bacillus* (Fisher *et.al* 2007, Sachdeva *et.al* 2009, Agrawal *et.al* 2011). PGPR can exhibit a variety of characteristics responsible for influencing plant growth. The common traits include production of growth regulators such as auxin, giberellin, ethylene siderophores, ammonia, Phosphorus solubilization, HCN & antibiotics etc. (Wahyudi *et.al.,* 2011, Etesami et.al 2009 and Ahmad *et.al.,* 2008).The Growth regulator especially IAA (Indole-3-Acetic Acid), often affects the root systematic features such as primary root growth side root formation and root hairs. Auxins are a group of herbal hormones while IAA is the most important of them (Khakipur *et.al* 2008).The isolating of native strains adapted to the environment and their study may contribute to the formulation of an inoculants to be used in region crops. The different stage of life cycle of wheat consists of elongation, flowering stage, fruiting stage and ripening, fruiting stage It is found that rate of roots exudates released by the root of the wheat at flowering stage is higher as compared to other stages, hence greater microbial biota and activity is expected during this stage. Thus present study has the view to investigate native PGPR free living bacteria, associated with rhizosphere of wheat during flowering stage to evaluate their ability to produce IAA.

MATERIAL AND METHOD

Quantitative Screening of rhizobacteria for Indole Acetic acid

The IAA production was assayed by the modified method as described by Laper and Scrowth (1986). Bacterial cultures were grown for 48 h (*Pseudomonas, Bacillus*), and 72 h (*Azotobacter)* on their respective media at 28^0C on rotary shaker. Fully grown cultures were centrifuged at 10000 rpm for 15 min. The 2 ml of supernatant was mixed with 2-3 drops of O-phosphoric acid and 4 ml of salkouski reagent solution (1 ml of $FeCl_3$ 0.5M mixed in 50ml of 35% $HClO_4$). The samples were incubated for 25 min at room temperature. The development of pink color was observed and optical density was taken at 530nm with help of spectrophotometer. The concentration of IAA produced by cultures was measured with the help of standard graph of IAA obtained in the range of 20-200 microgram per ml.

Salt tolerance

The pure cultures of all isolates were streaked on nutrient agar medium, containing 3% to 7% NaCl concentration. Control plates with NaCl amendment were also kept for observation for all strains. All plates were incubated at 30 ^{0}C for 48 hours and observed for the presence or absence of the growth.

RESULTS AND DISCUSSION

Quantitative screening of rhizobacteria for Indole Acetic acid:

Total of 18 selected rhizobacterial isolates of *Pseudomonas* (nine), *Azotobacter* (six) and *Bacillus* (three) were tested for quantitative IAA production . The production of IAA was recorded highest in isolates of *Azotobacter*, followed by *Bacillus* and *Pseudomonas* respectively. Among *Azotobacter* isolates , Azt-4 and Azt-7 produced highest amount

(175.20 µg/ml) of IAA followed by Azt-1>Azt3 >Azt-6. However, *Pseudomonas* rhizobacterial isolates produced IAA in the range of 44.40-95.60 µg/ml in the broth culture medium (Table-1). Wahyudi *et al*. (2011) reported that *Bacillus* spp. Cr4 produced 86.82 mg/l IAA in culture medium supplemented with L Tryptophan while 32.80 µg/ml IAA production was reported by Ahmad *et al.*, 2004. The findings of present investigation are outstanding in reference to earlier reports.

Salt tolerance

The present study shown that out of 18 selected strains, Azt-5, Bc-1 and Bc-3 tolerated even 7% NaCl concentration. All the rhizobacterial strains were able to grow at 3% NaCl concentration except Azt4 and Ps-1(Table-2). Rangarajan *et al.*, 2002 screened *Pseudomonas* strains for salt tolerance, out of 256 strain, only 36 strains could grow at 4.5% NaCl concentration and no strain was able to grow at 6% NaCl concentration.

Table 1. Production of Indole Acetic acid (IAA) by selected Rhizobacterial isolates grown in respective medium

SN.	Isolate	IAA Production µg/ml
1	Azt1	130.15
2	Azt2	114.66
3	Azt3	114.66
4	Azt4	175.20
5	Azt5	95.60
6	Azt6	114.60
7	Azt7	175.20
8	Azt8	114.60
9	Azt9	114.60
10	Ps1	95.00
11	Ps2	79.60
12	Ps3	44.40
13	Ps4	66.20
14	Ps5	79.60
15	Ps6	79.60
16	Bc1	95.60
17	Bc2	170.20
18	Bc3	161.00

Table-2 Antibiotic sensitivity and salt tolerance of selected rhizobacterial test isolates grown in respective medium.

S.N.	Isolate	NaCl concentration (%)				
		3	**4**	**5**	**6**	**7**
1	Azt1	++	-	-	-	-
2	Azt2	++	-	-	-	-
3	Azt3	+	-	-	-	-
4	Azt4	-	-	-	-	-
5	Azt5	+++	++	+	+	+
6	Azt6	++	-	-	-	-
7	Azt7	++	+	-	-	-
8	Azt8	++	+	-	-	-
9	Azt9	+	+	+	-	-
10	Ps1	-	-	-	-	-
11	Ps2	+	+	-	-	-
12	Ps3	+	+	-	-	-
13	Ps4	+	+	-	-	-
14	Ps5	+	-	-	-	-
15	Ps6	+	-	-	-	-
16	Bc1	+++	+++	+++	+++	+
17	Bc2	+++	+	-	-	-
18	Bc3	+++	+++	++	++	+

Azt= *Azotobacter*, Ps= *Psedomonas*, Bc= *Bacillus*, Incubation period 36 hours
+++= maximum growth , ++= medium growth, + poor growth, - = no growth

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