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Indole Acetic Acid production by fluorescent *Pseudomonas* isolated from the rhizospheric soils of *Malus* and *Pyrus*

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Abstract
Fluorescent *Pseudomonas*, a major component of rhizobacteria, promote the plant growth through their multifarious activities. In the present investigations, thirty strains of fluorescent *Pseudomonas* were isolated from the rhizosphere of apple and pear plants of their normal and replant sites and found that the count of *Pseudomonas* strains were more in normal site as compare to replant site. They were screened for auxins production (indole acetic acid or IAA) and it was found that the strains isolated from normal sites produced more auxins (7-30 µg/ml) as compared to the isolates of replant site (1-4µg/ml). Four strains *viz* PN-4-SAN, PN-10-SAN, AN-2-NAG and AN-4-NAG were selected on the basis of their higher auxin production. The maximum production of IAA was observed at 72 h incubation period at pH 7.0 under shaken condition at 28°C. The highest IAA was produced by strain AN-2-NAG (30 µg/ml) and PN-4-SAN (30 µg/ml) isolated from *Malus* (Apple) and *Pyrus* (Pear) rhizosphere soil, respectively. An attempt was made to extract, purify and evaluate IAA by thin layer chromatography and specific bioassay method. The IAA (Auxin) produced by both the isolates i.e. AN-2-NAG and PN-4-SAN showed Rf value of 0.81. The partially purified and extracted auxins were evaluated by bioassay. The auxins produced by isolates AN-2-NAG and PN-4-SAN showed highest increased in length of coleoptiles of avena. These isolates could be potential strains for bioinoculant production for apple and pear.

Keywords: *Pseudomonas*, PGPR, Indole acetic acid, Rhizosphere

INTRODUCTION

Fluorescent *Pseudomonas* species have emerged as largest and potentially most promising group of plant growth promoting rhizobacteria. Such microorganisms inhabiting rhizosphere of various plants are likely to synthesize and release auxins as secondary metabolites [1]. Auxin is a central regulator in many processes during plant growth development [2]. Bacterial IAA producers (BIPs) have the potential to interfere with any of these processes by input of IAA into the plant's auxin pool. Production of auxins i.e. indole acetic acid (IAA) is wide spread among *Pseudomonas* sp. Auxins induces additional root hair and/or lateral root formation [3].Thereby, enhancing the plant ability to take up nutrients from soil and increased yield. The use of such plant growth promoting rhizobacteria producing IAA is a new concept to solve the replant problem to some extent. The replant problem has become very serious problem in horticultural crops and it is distributed worldwide commonly encountered in establishing new orchards on old sites [4]. More specifically, the soil-borne fluorescent *Pseudomonas* has received particular attention because of their capacity to produce a wide range of enzymes and metabolites.

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MATERIAL AND METHOD

Isolation of fluorescent Pseudomonas species from the rhizosphere of Apple and Pear

Rhizospheric soil samples were collected from the normal and replant site of *Pyrus* (pear) and *Malus* (apple) orchids in Mandi district (Himanchal Pradesh), India. Fluorescent *Pseudomonas* was isolated upto its maturity level by dilution plate technique using Nutrient agar and King's B media.

Characterization and identification of selected bacterial isolates

Bacterial isolates were identified on the basis of morphological and biochemical characteristics according to the standard method described in Bergey's Manual of Systematic Bacteriology [5].

Screening of isolates for IAA (indole acetic acid) production

Pseudomonas sp. isolated from the rhizosphere soil of pear and apple orchards were screened out for the production of auxins [6].

Production and estimation of auxins

For production of auxins, test organisms were grown in nutrient broth for 72 h. at 28 ± 2^{0}C for *Pseudomonas* under shake conditions. Supernatant was prepared by centrifugation of cultures at 10,000 rpm for 20 minutes and was stored in deep fridge or at 4^{0}C. Quantitative measurement of auxins was done by colorimetric method [7] with slight modifications. Absorbance was measured at 535 nm. Concentration of auxins was estimated by preparing standard curve using pure indole acetic acid (IAA) as standard (10-100 µg/ml).

Effect of different media on the production of plant growth regulators by *Pseudomonas* sp. at different incubation period

The test organisms were grown in five different types of media ; succinate media, king's media, nutrient media, peptone water and trypticase soyabroth. Flasks were incubated at 28 ^{0}C for 0, 4, 8, 24, 48, 72 h under shaken conditions (90rpm). Supernatant were harvested by centrifugation at 10,000 rpm for 15 minutes at 4^{0}C and were used for estimation of auxins

Extraction and separation of auxins

Auxins were extracted and separated from supernatant by thin layer chromatography [6]. Acidified supernatant extracted with diethyl ether and partitioned with sodium bicarbonate. Extracted and concentrated fraction was dissolved in methanol. Methanol fraction (100 µl) spotted on silica gel-G plates and developed in isopropanol: water (30:20 v/v) for 12-14 h and sprayed with Salper reagent.

Evaluation of auxins by Avena coleoptile straight test

Coleoptiles (0.1 cm) length of 3 days old seedlings was dipped in Petri dish containing 1ml solution of test extracted solution, 1ml standard (IAA:10µg or 100µg) and 1ml water (blank) and was incubated at 28^{0}C for 48h in dark. Length of section was measured before and after the experiment.

RESULTS AND DISCUSSION

The growth of plant treated with IAA secreting PGPR is affected by the amount of IAA that the bacterium produces and the responses observed may vary from one species of plant to another. Thus PGPR facilitate plant growth by altering the hormonal balance within the affected plant [8]. The production of auxins also depends upon the strains and type of microorganisms and on their age. The maximum IAA was observed in the species of *Pseudomonas* species and *Bacillus* species [9, 10]. In present study, a total 17 *Pseudomonas* isolates were isolated from rhizosphere soil of pear plant; 14 from normal site and 3 from replant site and 13 *Pseudomonas* isolates were isolated from rhizosphere soil of apple plant; 10 from normal site and 3 from replant site up to its maturity level. On the basis morphology and biochemical tests, these isolates were identified as fluorescent *Pseudomonas. Pseudomonas* species were isolated and characterized to select and develop more efficient indigenous plant growth promoter to solve replant problem of apple and pear in old sites of orchards.

All the isolates of *Pseudomonas* isolated from the rhizosphere of normal site of apple and pear plants were found to produce auxins in range of 7-30 µg/ml (Table 1) while others isolated from replant sites showed poor response. The maximum auxins production was shown by *Pseudomonas* sp. by four isolates from apple and seven isolates from pear of fluorescent *Pseudomonas* sp. in the range of 24 µg/ml to 30µg/ml. All isolates differed statistically and significantly from each others in terms of production of auxins.

Table 1. Screening of fluorescent *Pseudomonas* for the production of auxins

Plant	*Pseudomonas* isolates	Auxins* (µg/ml)
Apple	AN-1-NAG	11.5
	AN-2-NAG	30
	AN-3-NAG	17.5
	AN-4-NAG	24
	AN-5-NAG	10
	AN-6-NAG	7.5
	AN-7-NAG	10
	AN-8-NAG	11
	AN-9-NAG	8.5
	AN-10-NAG	7
	AR-1-NAG	3
	AR-2-NAG	1
	AR-3-NAG	4
$CD_{0.05}$		1.43
Pear	PN-1-SAN	15
	PN-2-SAN	21.5
	PN-3-SAN	9
	PN-4-SAN	30
	PN-5-SAN	7.5
	PN-6-SAN	13
	PN-7-SAN	10
	PN-8-SAN	17
	PN-9-SAN	15
	PN-10-SAN	29
	PN-11-SAN	7.5
	PN-12-SAN	10
	PN-13-SAN	17.5
	PR-14-SAN	8.5
	PR-1-SAN	1
	PR-2-SAN	1
	PR-3-SAN	3
$CD_{0.05}$		1.40

The production of auxins also depends upon the type of microorganisms and strains and on their age. The maximum IAA was observed in the stationary phase of *Azotobacter* [11; 12] and other species of *Pseudomonas* species and *Bacillus* species [9]. The auxins type substances were detected by means of paper chromatography methods. In our studies these isolates produced auxins like substances in the stationary phase of growth i.e. at 72 hour of incubation period at 28°C for *Pseudomonas* sp. The results (Table 1) showed that production of auxins like substances by all the strains of *Pseudomonas* sp. ranges from 1 to 30 µg/ml. The homogeneity of the partially purified auxins were checked by thin layer chromatography (Fig 2). Auxins gave the maximum Rf value of 0.81. Pink spots corresponding to auxins or auxins like substances were visible when sprayed with Salper reagent (Table 2). Partially purified and extracted auxins evaluated by bioassay; avena coleoptile straight growth test and found increased length of coleoptiles. The auxins extracted samples from *Pseudomonas* sp. PN-4-SAN, PN-10-SAN, AN-2-NAG and AN-4-NAG showed the increase in length of avena coleoptile piece by 0.2, 0.25, 0.25, 0.2 cm respectively (Table 3). This increase in length of coleoptiles calculated from the dosage response curve of IAA. Because plants inoculated with bacteria received auxin continuously. Auxin in concentration more than 10-8 molar can stimulate the lateral root formation. Lateral branching in root and shoot systems represent a major determinant of plant architecture. Several lines evidence indicate that indole acetic acid is required at several stages of lateral root development [13 14, 15]. Our results showed with this method, auxin could be substituted by auxin producing rhizobacteria.

Table 2. Thin layer chromatographic analysis on Silica gel-G of partially purified bacterial plant growth regulators viz. auxins from *Pseudomonas* sp.

Plant growth regulators	Isolates	Solvent system	Spraying reagent	Color of spots	Rf value
Auxins	PN-4-SAN	Isopropanol: Water (30:20)	Salper	Pink	0.81
	PN-10-SAN	-do-	-do-	Pink	0.80
	AN-2-NAG	-do-	-do-	Pink	0.81
	AN-4-NAG	-do-	-do-	Pink	0.81

Table 3. Effect of partially purified auxins of *Pseudomonas* species on the length of Avena coleoptile of barley.

Partially purified auxins	Growth of coleoptile	
	Increased length (cm)	Auxins (µg/ml)
PN-4-SAN	0.20	40.00
PN-10-SAN	0.25	50.00
AN-2-NAG	0.20	50.00
AN-4-NAG	0.20	40.00
Standard	0.28	56.00

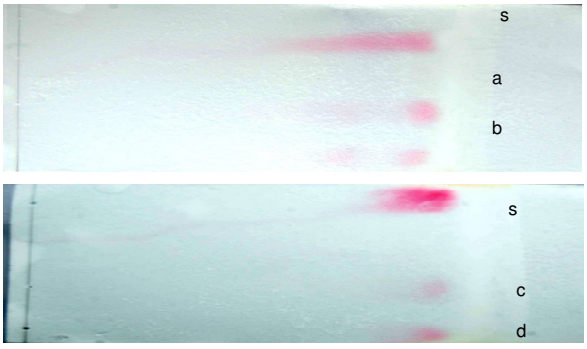

Fig 2. Thin layer chromatographic pattern on silica gel-G of partially purified auxins of *Pseudomonas* sp. PN-4-SAN(a), PN-10-SAN (b), AN-2-NAG (c), AN-4-NAG and standard (s) using Isopropanol:Water (30:20) solvent system and sprayed with Salper reagents.

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