INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Indole acetic acid production from *Pseudomonas* species isolated from rhizosphere of garden plants in Amravati

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ABSTRACT

Variety of life forms in nature are continuously promoting each other and to a low degree inhibiting others. It is not possible for individual organism to survive independently in any habitat. Give and take phenomenon is a part of survival in such competitive environment. Though plants are capable of independent growth being autotrophic however growth of plants when surrounded by microbes is largely influenced as is popularly exemplified by nitrogen fixing bacteria. Besides providing elements in easily assimilable forms microbes also provide hormones essential for plant growth. Indole acetic acid (IAA) is one of those important hormone provided by microbes to plants. Species of *Pseudomonas* are undertaken for IAA production from local soil of Amravati. Species of *Pseudomonas* are widely distributed in nature with a range of industrial and agricultural potentials. Those *Pseudomonas* species were selectively obtained followed by screening for IAA production. Most efficient species were selected for further studies like optimization of growth conditions for *Pseudomonas,* comparative studies for IAA production and effect of IAA on seed germination. The study shows *Pseudomonas* is potential bacterium for IAA production. Further it was observed that within short period of time considerable enhancement in the germination was observed in case of seeds treated with IAA. Thus *Pseudomonas* species isolated from the rhizospheric soil sample would be helpful in increasing crop productivity.

KEYWORDS: IAA, *Pseudomonas,* comparative study, optimization.

INTRODUCTION

Considerable amount of carbon fixed by plants is secreted through root exudate is eventually utilized by bacteria that in turn help plants by acting as biofertilizers, rhizoremediators, photostimulators and stress controllers designated as plant growth promoting rhizobacteria[1]. The carbonaceous compounds released through root exudates includes sugars, polysaccharides, amino acids, aromatic acids, aliphatic acids, fatty acids, sterols, phenolics, enzymes, proteins, plant growth regulators and secondary metabolites. Secondary metabolites such as flavonoids of plants are released during seed germination[2].

Obvious to mention there are many reasons for plant-microbial affection. Beside extensively studied nitrogen fixation by nodule rhizobial interaction, other important and recently undertaken research fields include metal extraction, detoxification, drought resistant plants and salt resistant plants. Phosphate solubilization is one of the initial applications of rhizobacterial studies. With event of time other applications have been studied includes resistance to pesticides, antibiotics like phosphonomycin, salt tolerance and pH tolerance. The microbes surrounding plants consist of rhizobia,

mycorrhizae, endophytes and plant-growth promoting rhizobacteria (PGPR), plant pathogens and invertebrate herbivores.

Rhizobia were discovered in 19th century by Beijerinck named as *Bacillus radicicola*. Year later Frank renamed these bacteria as *Rhizobium*[3]. Hiltner (1904) discovered the region surrounding roots was much rich in bacterial population than other soil parts[4]. The ability to synthesize phytoharmone is popular among those plant associated bacteria i.e. 80% of the bacteria isolated from plant rhizosphere produce indole acetic acid (IAA) which are naturally occurring substances with indole nucleus possessing growth-promoting activity referred as auxin. Beside plant growth IAA also affects the central metabolism of bacteria as studied in case of *Sinorhizobium meliloti*[5]. Few significant works regarding microbial IAA are reviewed below.

Pseudomonas is extensively studied bacterium for IAA production which also posses additional positive capabilities helpful in growth of plants. Optimization of media for IAA production by *Pseudomonas* has been studied by Balaji et al, (2012). Carbon sources selected were glucose, starch, lactose, cellulose and glycerol of which glycerol was found to be the most productive carbon source for IAA production. Sources of nitrogen consisted peptone, soybean meal, yeast extract, beef extract and tryptone of which yeast extract could yield higher IAA levels[6]. Varying levels of IAA have been reported in different studies ranging from 50 µg/ml to 100mg/ml despite using *Pseudomonas* for IAA production. The best yield of 53 µg/ml was obtained using a medium containing lactose as principal carbon source and 0.2% peptone as nitrogen source[7]. Among several reports regarding optimization of media for IAA production includes a study carried by Apine et al, (2011) using *Pantoea agglomerans*. Simple meat extract 8 g/l and 1g/l of tryptophan was an optimized media for production of IAA. Within short period of time i.e. 48hrs IAA was produced. In vitro root induction on *Nicotiana tobacum* (leaf) explants was enhanced by IAA produced using *P. agglomerans*[8]. Similar studies for optimization of media to produce IAA from *Pseudomonas putida* was carried by Bharucha & Kamlesh, (2013). One of the medium consisted of sucrose 0.5 %, $(NH_4)_2SO_4$ 10 mg/ml and tryptophan 0.2 mg/ml at pH 7.5 whereas the time period required was 96h. The puirified IAA positively affected mustard growth[9].

Beside media components, physiological conditions and heavy metals also affect IAA production. Environmental conditions like temperature, availability of a carbon source and aeration does influence IAA production was studied by Ona et al, (2005)[10]. Depletion of carbon sources enhances IAA synthesis whereas aerobic conditions inhibit IAA synthesis. Heavy metals dose affect the growth of plants and also IAA production. Of the heavy metals selected to study the influence on production of IAA Hg was most inhibitory compared to Pb, Cd and Ba[11]. Comparative studies between *Azotobactor* and *Pseudomonas* were carried with respect to IAA production. It was found that low amount of IAA was produced by *Azotobactor* strains without tryptophan. In case of *Pseudomonas* it was observed that IAA production increased with increase in concentration of tryptophan from 1mg/ml to 5mg/ml. Unlike most reports *Pseudomonas* isolates showed inhibitory effects on root elongation of *Sesbania aculeata* and *Vigna radiata* compared to the control whereas the isolates of *Azotobacter* demonstrated stimulatory effects on both plants[12]. IAA production and nitrogen fixing abilities were studied from *Pseudomonas* and *Azotobactor* isolated from sugarcane growing areas. The nitrogen fixing abilities were confined to *Azotobactor* only. The nif gene was also isolated from *Azotobactor*. The nitrogen fixing isolates could produce low levels of tryptophan. The effect of those strains was studied on sorghum plant which showed enhancement in root length, root area and plant dry weight as compared to control[13].

Alternative bacteria for IAA production are *Arthrobacter*, *Streptomyces atrovirens*, *Klebsiella*, *Bacillus* and *Paenibacillus* species. Effcient IAA producing species of *Arthrobacter* have been isolated from aquatic fern also. Sufficient IAA was produced after two days which was tryptophan based[14]. Around 210 isolates were screened for IAA production of which 65.7% were found to produce IAA. Further, 12 efficient isolates selected contained an efficient IAA producer which belonged to *Streptomyces atrovirens* as studied by 16S rRNA gene sequencing. Tryptophan concentration of 5mg/ml was needed for highest yield of IAA. However the time period required for sufficient IAA production was 13 days[15]. Strain of *Klebsiella* was the most efficient strain screened through 216 isolates from rice rhizospheric soils in Northern Thailand by Chaiharn and Lumyong (2011)[16]. Aspects considered were phosphate solubilization and IAA production. This strain also had stimulatory effect on bean seedling. *Bacillus* and *Paenibacillus* isolated from the rhizosphere of pasture plants to study the effect of pH and metal on IAA production and phytase activity. Comparatively high IAA production was observed in case of *Paenibacillus* species cultivated in diluted medium rather than full strength medium. Low pH affected IAA production and phytase activity of *Bacillus* species. At a concentration of 10mM of Fe and Al both IAA production phytase activity inhibited significantly[17]. Among other popular studies includes

IAA production from *Xanthomonas axonopodis*, *Rhizobium* species, *Bradyrhizobium japonicum*, *Azotobacter* sp, *Vibrio* species, *Bacillus amyloliquefaciens*, *Streptomyces* sp, *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae*[18,19,20,21,22,23,24,25]. Among the rare reports regarding presence of *Escherichia* and *Staphylococcus* around rhizosphere includes a study by Ali et al, (2009). The potential of these bacteria for IAA production was carried along with widely studied bacteria for IAA production i.e. *Bacillus, Pseudomonas, and Micrococcus*. Positive correlation was observed between these bacterial auxin and growth of *Triticum aestivum*. There was enhancement in shoot length, number of tillers, spike length and seed weight of *Triticum aestivum*[26].

Cyanobacteria and endophytic bacteria are also suitable candidates for IAA production. In *Arthrospira platensis* it was observed that IAA was accumulated to the cells than it was released into the medium. The IAA produced had positive effect on lateral root formation in *Pisum sativum*[27]. *Curcuma alismatifolia* is popular flower crop from which endophytic bacteria that can fix N_2 and produce IAA were isolated. These bacteria were significant for reduction of propagation period and were characterized as *Sphingomonas pseudosanguinis*, *Bacillus methylotrophicus* and *Bacillus drentensis*. These bacteria were responsible for enhancement in plant growth and yield in terms of the plant height, plant weight and leaf area[28].

Bio and nanotechnology fields also focus on IAA production. Genetically engineered strain of *Pseudomonas fluorescens* was used for elevated synthesis of IAA. This strain increased root weight of cucumber by 17-36% compared to natural *Pseudomonas fluorescens* strain. However susceptibility of plants to pathogens remained unaltered[29]. *Pseduomonas fluorescens* species were used for overexpression of IAA by metabolic engineering[30]. Effect of nanoparticles on production of IAA by *Pseudomonas chlororaphis* was studied by Dimkpa et al, (2012)[3]. CuO nanoparticle enhances the production of IAA whereas ZnO nanoparticles had inhibitory effect.

Rhizospheric flora of crop plants varies according to season and variety of crops used which also affects potential of those bacteria for IAA production. On the other side rhizospheric flora of garden plants is stable from season to season which might be stable source of IAA producing bacteria. As *Pseudomonas* is versatile bacterium found in most habitats hence this study focuses on IAA production from *Pseudomonas* species.

MATERIALS AND METHODS

Knowing the fact that IAA is environmentally safe and has potential to replace chemical fertilizer, hence *Pseudomonas* sp from rhizospheric soils were undertaken for study. Soil samples were collected from garden of Sant Gadge Baba Amravati University, Amravati from specific depth of about 30cm which is known as the rhizospheric soil. This soil was collected in clean zip-lock plastic bags.

Isolation of *Pseudomonas*:

Those soil samples were serially diluted and were spread on cetrimide agar. After incubation colonies were observed on cetrimide agar. Characteristic colonies of *Pseudomonas* are because of fluorescent pigment. These isolates were confirmed by biochemical and morphological properties.

Screening of *Pseudomonas* for IAA production:

Individual isolates of *Pseudomonas* were further tested for IAA production. Around twenty isolates were taken for study. These were inoculated in nutrient broth with tryptophan and incubated at 28ºC-30^0C for 7 days with intermittent testing for IAA. Orthophosphoric acid (2drops) and Solawaski's reagent (4mL) were added to the test samples and were kept for 25 min for pink color development. Pink color is indication of IAA in the media. Out of 20 isolates only 15 were found positive for IAA production of which 10 were selected further depending on intensity of red coloration responsive to IAA production.

Comparative study of IAA produced from *Pseudomonas* species:

Standard graph was prepared using purified IAA. IAA absorbs maximally at 530 nm. The quantitative estimation of IAA produced by *Pseudomonas* was done spectrophotometrically at 530 nm. Comparative studies were carried afterwards. Prior to this, cultures were grown in nutrient media containing tryptophan and were incubated for 7 days. Incubation period was selected on the basis of periodic studies carried between 1 to 10 days for IAA production which was maximum at seventh day.

Optimization of pH and temperature:

The isolates were grown on nutrient broth at various pH and temperature. The microbial culture that displayed maximum turbidity at respective pH and temperature were chosen at its optimum pH and temperature.

Effect on seed germination:

Seeds were first sterilized with 0.2% $HgCl_2$ and kept

for 5min. These were again washed with distilled water and kept for 15 min. Filter paper was dipped in media containing IAA over which moth beans were placed. Care was taken to avoid dryness of filter paper. Similar set up was made using standard IAA and control with distilled water only.

RESULT AND DISCUSSION

Isolation of *Pseudomonas*:

Pseudomonas is gram negative, motile, oxidase and catalase positive bacterium with characteristic green colored growth. These were isolated on cetrimide agar. However pigment was more prominent on nutrient agar (Fig1). Properties of ten most significant bacteria have been shown in Table1 and 2.

Screening of *Pseudomonas* for IAA production:

Screening of *Pseudomonas* was done on the basis of red coloration formed in production media after addition of Solavaski reagent. The optical densities were measured at 530nm and out of 15 cultures 10 efficient cultures were selected for further studies (Fig2).

Comparative study of IAA produced from *Pseudomonas* species:

Standard graph was prepared using purified IAA. After growing cultures in tryptophan containing nutrient solution for seven days, IAA was estimated and comparative studies carried.

Culture D8 was the most efficient in producing IAA with a concentration of 81.8 µg/mL. IAA produced by D9 was 75.8 µg/mL followed by D7 i.e. 74.9µg/mL. Lowest IAA was produced by D1 i.e. 21.5 µg/mL. Intermediate IAA production was by D3-34.3 µg/mL, D4-63.2 µg/mL, D5-72.9 µg/mL, D10-70.7 µg/mL (Table3).

Optimization of physiological conditions for growth of *Pseudomonas*:

There was no growth or very less at pH5 and pH10. Most cultures displayed luxuriant growth at pH7 whereas culture D6 also grew well at pH9. Sufficient growth was observed at varied temperatures i.e 20ºC, 30ºC and 37ºC. Very few cultures showed less growth at temperature 45ºC (Table4 and 5).

Effect of IAA on seed germination:

Seeds were moistened with experimental, standard IAA and control which contained distilled water only. Considerable enhancement in the germination time was observed. In seeds kept with experimental IAA, germination was observed in 17h whereas for control this period was 24h.

CONCLUSION

Pseudomonas is a versatile bacterium used in many industrial and environmental applications. In this study the potential for indole acetic acid production from *Pseudomonas* is carried. Efficient indole acetic acid (IAA) producing *Pseudomonas* species were isolated. The phytohormone auxins play a central role in plant growth and development. IAA is environmentally safe, it has the potential to replace chemical fertilizers and thus enhances the plant growth and also considered suitable for the treatment of seeds. The effect of IAA on seed germination was studied, within short period of time, considerable enhancement in the germination time of moth beans was observed. Thus it is concluded that the *Pseudomonas* species isolated from the rhizospheric soil sample were found to be useful in the production of IAA and these species of *Pseudomonas* would be promising agents to help crop productivity.

Table 1
Cultural and morphological characteristics of IAA producing *Pseudomonas*

Culture code	Cultural characteristics						Morphological properties			Identified Bacterial species
	Colour	Size	Shape	Margin	Elevation	Opacity	Gram staining	Shape	Motility	
D1	Greenish	0.4 mm	Circular	Smooth	Convex	Opaque	-	SR	M	Pseudomonas sp
D2	Greenish	0.4mm	Circular	Smooth	Flat	Opaque	-	SR	M	*Pseudomonas sp*
D3	Greenish	0.2 mm	Irregular	Rough	Convex	Transluscent	-	SR	M	*Pseudomonas sp*
D4	Greenish	0.2mm	Circular	Rough	Convex	Translucent	-	SR	M	*Pseudomonas sp*
D5	Greenish	0.3 mm	Irregular	Rough	Flat	Translucent	-	SR	M	*Pseudomonas sp*
D6	Greenish	0.4 mm	Circular	Smooth	Convex	Translucent	-	SR	M	*Pseudomonas sp*
D7	Greenish	0.4mm	Circular	Smooth	Flat	Translucent	-	SR	M	*Pseudomonas sp*
D8	Greenish	0.4mm	Irregular	Smooth	Convex	Opaque	-	SR	M	*Pseudomonas sp*
D9	Greenish	0.2 mm	Circular	Smooth	Flat	Opaque	-	SR	M	*Pseudomonas sp*
D10	Greenish	0.3 mm	Circular	Smooth	Convex	Opaque	-	SR	M	*Pseudomonas sp*

M-Motile, - Negative, + Positive

Table 2
Biochemical characteristics of IAA producing *Pseudomonas*

Culture code	Biochemical characteristics																	Identified Bacterial species
	Catalase	Oxidase	Indol	MR	VP	Citrate	Arabinose	Xylose	Fructose	Dextrose	Lactose	Mannitol	Rhamnose	Salicin	Cellobiose	Sucrose	Malonate	
D1	+	+	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	*Pseudomonas species*
D2	+	+	-	-	-	+	+	-	-	-	-	+	-	+	-	-	-	*Pseudomonas species*
D3	+	+	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	*Pseudomonas species*
D4	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	*Pseudomonas species*
D5	+	+	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	*Pseudomonas species*
D6	+	+	-	-	-	+	+	-	-	-	-	+	-	+	-	-	-	*Pseudomonas* species
D7	+	+	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	*Pseudomonas species*
D8	+	+	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	*Pseudomonas species*
D9	+	+	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	*Pseudomonas species*
D10	+	+	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	*Pseudomonas species*

M-Motile, - Negative, + Positive

Table3
Production of IAA by ten efficient *Pseudomonas* species

Culture Designation	Optical Density	µg/mL
D1	0.209	21.5
D2	0.215	27.3
D3	0.289	34.3
D4	0.666	63.2
D5	0.704	72.9
D6	0.783	74.7
D7	0.786	74.9
D8	0.817	81.8
D9	0.793	75.8
D10	0.690	70.7

Table4
Optimization of pH for growth of *Pseudomonas*.

Culture Designation	pH5	pH7	pH9	pH11
D1	-	++	-	+
D2	-	++	-	-
D3	-	++	-	+
D4	-	++	-	+
D5	-	++	+	-
D6	-	++	++	-
D7	-	++	-	-
D8	+	++	+	-
D9	+	++	+	-
D10	-	++	+	-

Where: (++) = Luxuriant growth, (+) = Moderate growth, (-) = No growth.

Table5
Optimization of temperature for growth of *Pseudomonas*

Culture Designation	20º C	30ºC	37ºC	45ºC
D1	++	++	+	+
D2	++	++	+	-
D3	++	+	+	-
D4	+	+	++	+
D5	+	+	++	+
D6	+	++	++	-
D7	++	++	+	-
D8	++	++	+	-
D9	+	+	+	-
D10	+	+	+	-

Where: (++) = Luxuriant growth, (+) = Moderate growth, (-) = No growth.

Fig1
Characteristic growth of *Pseudomonas*

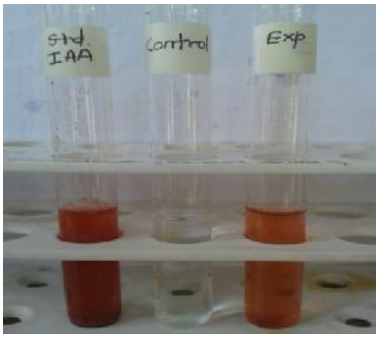

Fig 2
Estimation of IAA in Standard (Left), Control (Middle) and Experimental (right).

Fig 3
One of the photo showing germination of moth beans in control, experimental and standard IAA

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