11. Production and Characterization of IAA from Rhizospheric Pseudomonas RSML 24

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Abstract

Indole-3-acetic Acid (IAA) is member of auxin family, a natural auxin produced by plants, algae, mosses, lichens and a diverse group of microorganisms also. Bacteria belonging to the genera Azospirillum, Pseudomonas, Xanthomonas, and Rhizobium as well as Alcaligenes faecalis, Enterobacter cloacae, Acetobacter diazotrophicus and Radyrhizobium japonicum have been shown production of auxins(IAA) which help in stimulating plant growth. Pseudomonas species have emerged as potentially most promising group of plant growth promoting rhizobacteria (PGPR).

Pseudomonas isolates obtained from the rhizosphere of pomegranate were found to produce significant amount of indole acetic acid (IAA) when grown in a IAA Production medium The total of 7 Pseudomonas colonies were isolated and by using Kovac's reagent for preliminary screening. Prepared Standard graph of IAA was prepared as by using different IAA concentrations by Salkowski's Reagent.IAA concentrations in incubated broth culture supernatants by centrifugation using the method of Salkowski's Reagent.

Out of 7 isolates RSML1 RSML2 and RSML 24 Showed excellent production in IAA Production medium. The amount of IAA by Isolates RSML1, RSML2 and RSML24 produced significant amount of IAA at 48 hour of growth i.e., 09.2 µg/ml, 11.1 µg/ml & 21.2 µg/ml respectively.

The extracted sample characterized by Thin layer Chromatography using **Propanol: Water (8:2)** solvent it showed same Rf value (0.57) as like those obtained from Pseudomonas RSML 24.

Hence the present study was undertaken to isolate Pseudomonas RSML 24 from soil and to study IAA production under laboratory condition.

Key Word: IAA, Pseudomonas RSML 24, Pomegranate Rhizosphere.

Introduction

Indole-3-acetic Acid (IAA) is member of auxin family, a natural auxin produced by plants, algae, mosses, lichens and a diverse group of microorganisms also. Plant growth-promoting rhizobacteria (PGPR) are free-living, soil-borne bacteria, which promotes growth of the plant either by direct or indirect manner (1, 2). The direct mechanisms involve nitrogen fixation, phosphorus solubilization, and HCN production.

Bacterial Production of phytohormone like auxins, cytokinins, gibberellins, and ethylene(2, 3, 4). Bacteria belonging to the genera Azospirillum, Pseudomonas, Xanthomonas, and Rhizobium as well as Alcaligenes faecalis, Enterobacter cloacae, Acetobacter diazotrophicus and Radyrhizobium japonicum have been shown production of auxins(IAA) which help in stimulating plant growth (5). Pseudomonas species have emerged as potentially most promising group of plant growth promoting rhizobacteria (PGPR). Such Pseudomonas species inhabiting rhizosphere of various plants are likely to synthesize auxins as secondary metabolites [6].

Production of indole acetic acid (IAA) is wide spread among Pseudomonas sp. Auxins induces additional root hair formation [7].hence, enhancing the plant ability to absorb maximum nutrients from soil and increased yield.

The phytohormone IAA plays a role in plant growth and controlling many important physiological processes including cell division, elongation and differentiation to tropic responses, fruit development, senescence and responses to light and gravity (8, 9)

Hence the present study was undertaken to isolate Pseudomonas Sp. from soil and to study IAA production under laboratory condition from Pseudomonas RSML 24.

Materials and Method

Isolation of Pseudomonas isolates

The soil Pseudomonas was isolated from pomegranate agricultural field of shinde takli (26^0 18^0N $73^0$$01^0$E) located in Marathwada region, Maharashtra of India. Isolation of Pseudomonas was carried out by serial dilution technique followed by spread plate method using Pseudomonas isolation Agar (PIA) medium (Hi-Media, India). Inoculated plates were incubated at 28^0C for 72 hours in BOD incubator (Shital Scientific industries, India). Individual

Pseudomonas colonies were picked and further purified by sub-culturing on PIA media. The pigmentation and biochemical reactions were determined as described in Bergey's Manual of Determinative Bacteriology. All the 7 isolates of Pseudomonas were biochemically characterized for H2S production, citrate utilization, amylase, oxidase, urease, catalase and lipase production activity, indole production and pigment production

Stock culture was maintained by sub culturing at a regular monthly interval. After growing at pH 7.0 and 28 ^{0}C for 48 hours, the slants were preserved at −04^{0}C. From an actively growing stock culture, subculture was prepared on the fresh PIA slant and after 72 hours of incubation at 28^{0}C, was used as starting culture for IAA production.

Screening for bacteria producing indole acetic acid (IAA)

A total of 7 Pseudomonas colonies were isolated and by using Kovac's reagent for preliminary screening. (iso-amyl alcohol 150 mL, para-dimethylaminobenzaldehyde 10 mg., concentrated HCl 50 mL).Cultures were inoculated in tubes containing 5 mL of tryptone water (1.5 %, pH 7.5) and incubated at room temperature (28±2°C) for 48 hr. 0.5 mL of Kovac's reagent was added to each tube.

Preparation of standard graph of IAA

Standard graph of IAA was prepared as previous research by [10, 11, 12] using Different IAA concentrations are prepared as aqueous solution of IAA ranging from 10 microgram/ml to 100 micrograms/ml. To each 1 ml of the standard, 2ml of Salkowski's reagent contains 2% 0.5 M FeCl3 in 35% perchloric acid is added and taken readings after 30 minutes at 540 nm by UV-Visible spectrophotometer (Shimadzu 5000) Standard graph is prepared by plotting concentration of IAA in micrograms/ml Vs Optical Density at 540 nm.

Quantitative Estimation of IAA by Colorimetric assay method

IAA concentrations in incubated broth culture supernatants by centrifugation using the method of Salkowski's Reagent (12). After centrifugation of 1 ml of culture was added 2ml of Salkowski's reagent contains. Red color formation was quantified as the absorbance at a wavelength of 540 nm UV/VIS spectrophotometer (Shimadzu 5000).

Confirmation of IAA by using TLC:

Readymade TLC slide was used (Hi-Media). The extracted sample and standard IAA (10mg/100ml) were spotted on TLC plate. Solvent system **Propanol: Water (8:2)** was used. Chromatogram was developed with the Salkowski's reagent(13).

Effect of pH media on IAA Production

IAA production optimized the pH of medium by the different isolates: IAA production medium with 1g/100ml of tryptophan is adjusted to different pH as 5, 6, 7, 8 and 9. Media were inoculated with 24 hr old 5% inoculum .and incubated at 28^0C for 24 hrs. IAA production was studied by using Salkowaski reagent method after 24hrs.[13]

Resultand Discussion

A total of 7 isolates of fluorescent Pseudomonas sp. were isolated from rhizospheric soil and then identified on the basis of biochemical tests and sugar fermentation behavior as escribed in Bergys Manual of Determinative Bacteriology (Table 1).

Biochemical Observations: Table 01 Biochemical Characterization of rhizospheric Pseudomonas isolates

Characters	**Pseudomonas isolates**						
	RSMLB1	**RSML24**	**RSMLB3**	**RSMLB4**	**RSML1**	**RSML2**	**RSML23**
Pigmentation	+	-	+	-	+	-	-
H_2S production	+	+	+	+	+	+	+
Amylase	+	-	-	+	+	+	+
Urease	+	+	+	+	-	+	-
Catalase	+	+	+	+	+	+	+
Indole	+	+	-	+	-	+	+
Citrate utilization	+	+	+	+	+	+	+
MR	+	+	+	+	+	+	+
VP	-	-	-	-	-	-	-
Nitrate Reduction	-	-	-	-	-	-	-
Mannitol	A	A	A	A	A	A	A
Glucose	A	AG	A	A	A	A	AG
Maltose	A	A	A	AG	A	A	A
Sucrose	AG	AG	AG	AG	AG	AG	AG
Xylose	A	A	A	A	A	A	A

Identified strain	Pseudomonas sp.	Pseudomonas sp.	Pseudomonas sp.	Pseudomonas sp.	Pseudomonas sp.	Pseudomonas sp.	Pseudomonas sp.

A :Acid production, **AG** : Acid & Gas production, + :Test positive & - : Test negative

Screening for bacteria producing indole acetic acid (IAA)

Appearance of a pink coloration indicated the presence of indole derivatives. Out of 7 Pseudomonas isolates obtained from the rhizosphere of pomegranate only 03 isolates produced indole-acetic acid in IAA Medium (Fig-2).

Standard graph of IAA

Indication of Straight-line graph shows direct proportion between concentrations of IAA and the extent of Pink colour developed. Rf values(0.57) of the standard IAA produced and IAA produced by the selected isolates showed same value. Hence production of IAA by the organisms was confirmed.

IAA Production

The amount of IAA produced varied from 09.2 to 21.2 µg/ml of supernatant in different isolates. Isolates RSML1, RSML2 and RSML24 produced significant amount of IAA at 48 hour of growth i.e., 09.2 µg/ml, 11.1 µg/ml & 21.2 µg/ml respectively.

Maximum amount of IAA production was observed in Pseudomonas isolates RSML24 i.e. 21.2 µg/ml.

Fig1:Extraction of IAA

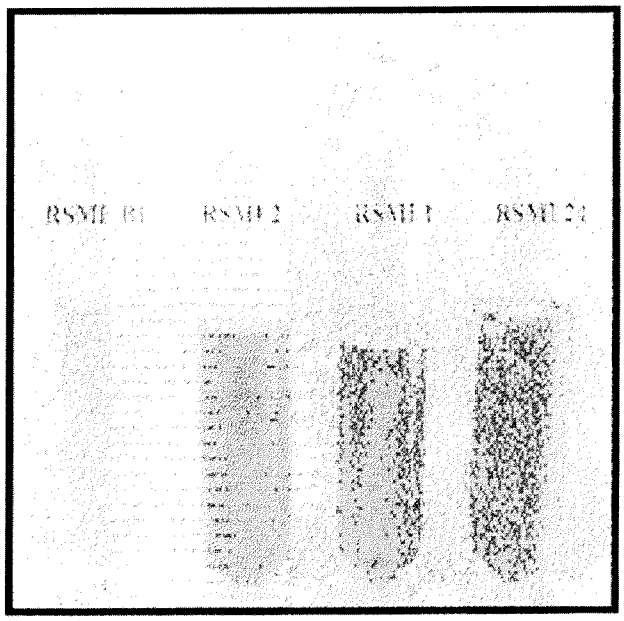

Fig2:Screening of *Pseudomonas* Isolates For IAA Production

Fig 2 : IAA detection by TLC Method

TLC Confirmation of IAA: Silica gel thin layer chromatography (TLC) technique was used to in purification, separation and possible identification of natural and synthetic indole derivatives.[11] separation showed Rf value of standard IAA 0.55,0.58,0.56. The same Rf value was obtained from IAA produced by the isolates. Standard IAA showed R_f value of 0.57. [11]

Conclusion

In this study, it is clear that rhizospheric Pseudomonas isolates has the ability to produce a significant amount of IAA in an IAA Production medium. Pseudomonas RSML24 isolates showed significant production of IAA was recorded 21.2 µg/ml. Similarly, RSML1 and RSML2 was able to produce IAA in a concentration of 09.2 & 11.1 µg/ml respectively. The significance of the study confirmed as the potential of these IAA producing isolates prevent environmental pollution by avoiding excessive applications of chemical fertilizers to cultivated fields.

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