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Production of Indole Acetic Acid (IAA) from Endophytic Bacteria Isolated from *Saussurea Obvallata*

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ABSTRACT

Endophytes are plant beneficial bacteria that thrive inside plant and improve plant growth under normal and challenging condition as they contain growth regulators. Endophytes are capable of synthesizing bioactive compounds which protect plants against pathogens. Endophytic bacteria can improve plant health by targeting pest and pathogens by antibiotic, hydrolytic enzymes production and creating nutrient limitations. *Saussurea obvallata* is a species of flowering plant considered as medicinal herb in Tibetan medicine. The leaves of *Saussurea obvallata* were selected for isolation of endophytic bacteria. A total number of three endophytic bacteria were isolated and they were designated as IS-1, IS-2, IS-3. All the three isolates were screened for their ability to produce IAA using Salkowski's reagent. The two isolates showing IAA producing ability were subjected to IAA production using Nutrient broth tryptophan medium Isolate IS-2 was found to be producing 380 µg/ml amount of IAA in presence of 7 µg/ml tryptophan in the medium. The endophytic bacterial isolate IS-2 which has been tentatively identified as *Micrococcus luteus* and found promising isolate for IAA production.

Key words: *Endophytes, Saussurea Leaves, Nurient agar, Indole Acetic Acid, Tryptophan*

Introduction

Endophytes are the organisms that can live between living cells. The word endophyte means 'in the plant. The term endophyte was coined by Wilson (1995) to distinguish epiphytic organisms living on the surface of plants. Endophytes belong to diverse taxa such as bacterial, fungal, archaeal, Protista, etc. Endophytes defined as organisms isolated from surface, sterilized explants or from within plant tissue and do not produce harm to host plant. (Hallman *et al*., 2011). Endophytes are plant beneficial bacteria that thrive inside plants and improve plant growth under normal and challenging conditions. Many of them capable of synthesizing bioactive compounds, which protect them against pathogen. Indirectly entophytic bacteria can improve plant health by targeting pest and pathogens with antibiotic, hydrolytic enzymes, nutrient limitation. The interest in entophytic bacteria has increased as they colonize, the internal tissues of the in host plant and improve plant tolerance to various abiotic stress factors and can protect plant from various pathogenic microbes. (Malfanova *et al*., 2011).

Plant growth promoting endophytes differ from biocontrol strains in that they don't inhibit pathogens but increase plant growth through the improved cycling of nutrients and minerals such as nitrogen, phosphates and other nutrients. Endophytes also promote plant growth by a number of similar

mechanisms these include phosphate solubilization activity (Verma *et al*., 2001) Indole acetic acid production (Lee *et al.,* 2004). Indole acetic acid also known as IAA, is a heterocyclic compound that is aphytochrome called auxin. IAA has many different effects as all auxins do, such as inducing cell elongation and cell division with all subsequent results for plant growth and development. On larger scale, IAA serves as signaling molecule necessary for development. *Saussurea obvallata* (Brahma kamal), the state flower of Uttarakhand, India is an endemic herb of the Himalayan region (encompassing the Indian Himalayan Region, Northern Burma and Southwest China). The plant is distributed at an altitudinal range of 3000-4800 m (Pant and Semwal, 2013). Brahma Kamal is used for the preparation of traditional medicines by the local people in Tibet and other places including Garhwal Himalayas. It is well known that indigenous rhizobacteria exert beneficial effects on plants productivity and sustenance of soil health through their capacity for phosphate solubilization (Behrooz *et al.,* 2019), production of indole aceticacid (IAA) (Selvakumar *et al*., 2008), ammonia, HCN and cell wall degrading enzyme production (Tsegaye *et al.,* 2019).

The purpose this research is to isolate and identify IAA producing promising endophytic bacteria.

Materials and Methods

Collection of samples

Leaves of *Saussurea obvallata* were collected from garden of KIMSDU, Karad.

Isolation of endophytic bacteria from *Saussurea obvallata*

Surface sterilization: The leaves of *Saussurea obvallata* were thoroughly washed with running tap water. They were then immersed in 75% ethanol for 2 min and surface sterilized in 0.1 % mercuric chloride for 1 min and finally washed with sterile distilled water.

Isolation of Endophytic bacteria

Surface sterilized samples were cut with sterilized knife and forceps. Then inner extract was collected for isolation of indole acetic acid producing bacteria using nutrient agar medium. The medium contains: peptone 1 g, NaCl 0.5g, Beef extract 0.3g, distilled water 100 ml, pH 7.2, Agar-Agar 2.5. A loopful of sample was streak inoculated on nutrient agar medium and kept for incubation at 30 ^{0}C for 48 hr. The well isolated colonies were selected for morphological and cultural characterization. Total 03 isolates were obtained from leaves of *Saussurea obvallata*. The isolates were further checked for IAA production.

Morphological and cultural characterization of isolates

Morphological and cultural characterization was done on the basis of colony size, shape, color, margin, opacity, consistency, elevation, motility and gram staining and endospore staining.

Biochemical characterization of bacterial isolates

Biochemical tests like production of catalase, oxidase, uera hydrolysis, nitrate reduction, arginine hydrolysis, starch hydrolysis, casein hydrolysis, gelatin liquification, lipase, H2s production, IMVic test and some carbohydrate fermentation tests like glucose, mannitol, lactose , maltose, fructose, arabinose were used (Aneja, 2001).

Screening of bacterial isolates for Indole acetic acid (IAA) production

All obtained isolates were screened for IAA production. The test bacterial culture were inoculated in nutrient broth and incubated at 28± 2 ºC for 6 days, cultures were centrifuged at 8000 rpm for 15 min. 2 ml of supernatant was mixed 2 drops of orthophosphoric acid and 4 ml of Salkowaski's reagent. Development of pink color indicates IAA production (Kamnev *et al*., 2001).

Production and extraction of crude IAA

Single bacterial colonies of isolates found to be positive were inoculated in 200 ml nutrient broth and incubated at 28± 2 ºC for 1 week on a rotary shaker. Bacterial cells were separated from the supernatant by centrifugation at 8000 rpm for 15 min, supernatant was acidified to pH 2.5 to 3.0 with 1N HCL and extracted twice with ethyl acetate and fraction was evaporated at 4 ºC.

Confirmation of product (IAA) by Thin layer Chromatography

Thin layer chromatography slide was prepared with silica gel and calcium carbonate. Benzene: propanol: acetic acid (70:25:5) was used as solvent system. The extracted sample and standard IAA were spotted on TLC plates spots with Rf values identical to stan-

dard IAA were identified by spraying the plates with Ehrlich reagent (Mohite, 2013; Ehamann, 1977).

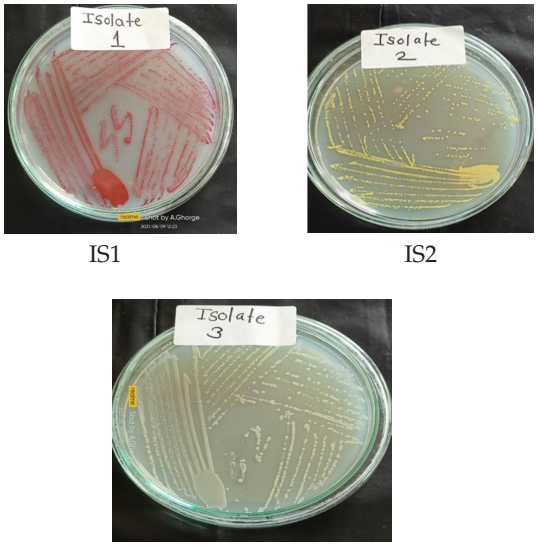
Results and Discussion

Isolation and identification of endophytic bacteria

Three bacterial isolates were isolated as IAA producer from the leaves of *Saussurea obvallata* and were coded as IS1, IS2 and IS3. The isolates were identified based on morphological observation and biochemical characterization (Table 1) Bergy's Manual of Determinative of Bacteriology was used as a reference to identify the isolates (MacFaddin, 2000). The isolates were identified as *Micrococcus roseus*, *Micrococcus luteus* and *Bacillus subtilis.*

Characterization of IAA production potential

IAA production was checked with the use of Salkowaski's reagent (Ehmann, 1977) from the 3 isolates the IAA production was found to be positive in isolate IS2 and IS3. IAA production when compared between isolate IS2 and IS3 the production was more in isolate IS2 (Fig. 1). Isolate IS2 was more suitable for IAA production. The isolate IS2 showed 380 µg/mL amount of IAA and isolate IS3 showed 280



IS1 IS2

IS3

Photoplate 1. Colonial MorphologyofIsolates

Table 1. Morphological, cultural and biochemical characterization of IAA producing endophytic bacteria

Characteristics	Results of Isolates		
	IS-1	IS-2	IS-3
Size	1mm	1mm	2mm
Shape	Circular	Circular	Circular
Color	Red	Yellow	White
Margin	Entire	Entire	Entire
Elevation	Flat	Flat	Convex
Opacity	Opaque	Opaque	Opaque
Consistency	Moist	Moist	Moist
Gram nature	Gram positive cocci	Gram positive cocci	Gram positive short rods
Motility	Non-motile	Non-motile	Motile
Endospore	-	-	+
Biochemical test			
Catalasetest	+	+	+
Oxidasetest	+	+	+
Ureahydrolysistest	-	+	+
Nitratereductiontest	+	-	+
Starchhydrolysistest	+	+	+
Caseinhydrolysistest	+	+	+
Gelatinliquefactiontest	+	+	-
Lipase production test	+	-	+
H_2S production test	-	-	+
Indole production test	-	+	-
Methyl red test	-	+	+
VP test	-	+	+
Citrate utilization test	-	+	+

(+ =positive test) (- = negative test)

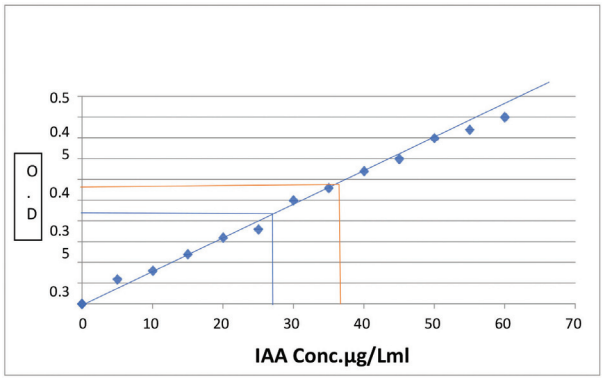


Fig. 1. IAA production by bacterial isolate IS2 and IS3

µg/mL IAA production.

Detection of IAA by Thin layer chromatography

Purified IAA sample was compared with standard IAA on TLC chromatograms. TLC of ethyl acetate extract showed pink colored spot at the Rf corresponding to the standard IAA (0.93) as shown in Fig. 2. It confirmed IAA producing potential of isolate.

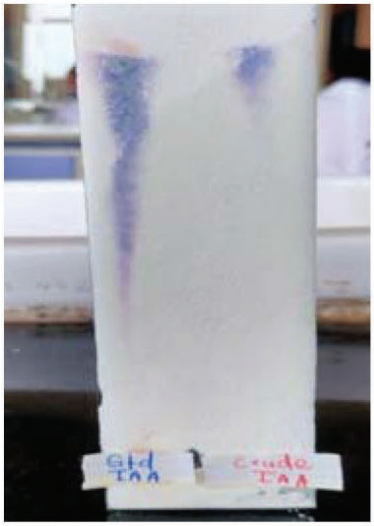


Fig. 2. TLC analysis of IAA production by Isolate IS-2

Effect of L-Tryptophan concentration on IAA production

L-Tryptophan is generally considered as an IAA precursor because of its addition to IAA producing bacterial culture enhances IAA biosynthesis (Hasuty, Choliq and Hidayat, 2018). The best isolate IS2 showing maximum yield was subjected for IAA production. Maximum IAA production was found

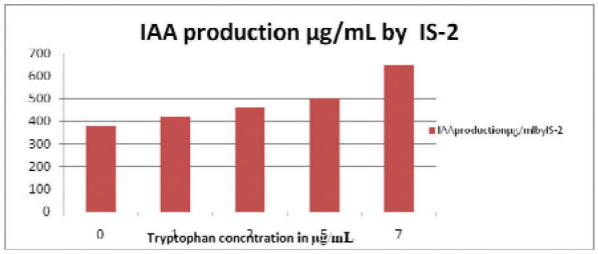


Fig. 3. Effect of tryptophan on production of IAA

in medium amended with 7 mg/ml tryptophan concentration and yield was found to be 650 µg/ml (Fig. 3). Thus yield of IAA was increased upon addition of L-tryptophan in the medium. Hence this medium was found useful for further IAA production studies.

Conclusion

From this study, it is clear that endophytic bacteria isolated from the leaves of *Saussurea obvallata* can provide a rich source of indole acetic acid (IAA) production and has ability to produce a significant amount of IAA in a tryptophan medium. Overall three isolates were identified among which isolate IS2- *Micrococcus luteus* showed the maximum production of IAA and is promising isolate for IAA, followed by IS3 and hence it is promising isolate. It is concluded that presence of such growth promoting endophytic bacteria accountable for beneficial effect on growth of plant. Further studies on optimization and finding of media and environmental factors (pH, temperature, incubation period etc) will lead to development of better IAA producing isolates and will help to reduce the use of chemical fertilizers.

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Conflicts of Interest

The authors declare that there are no conflicts of interests.

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