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Isolation & screening of indole acetic acid (IAA) producing rhizobacterial strains

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Abstract
The production of Indole acetic acid (IAA) is the main feature of rhizospheric bacteria that stimulates plant development. IAA is a frequent metabolic product of L-tryptophan metabolism. The present study focuses on screening and determining IAA-producing microorganisms from rhizospheric soil samples of crops wheat, maize, and Soyabean. The motive of the research was the application of IAA as a biofertilizer for the above-selected crops. Two bacterial isolates were identified from above rhizospheric soil as IAA producers. Production and Qualitative analysis of IAA was accomplished by use of centrifugation and Salkowaski's reagent respectively. The effect of IAA as a biofertilizer was further checked by a pot assay to examine the growth of wheat, maize, and Soyabean plants.

Keywords: indole acetic acid (IAA), rhizospheric soil, biofertilizer, salkowaski's reagent, crops

Introduction
The rhizosphere is the small area of soil nearby the plant roots which influences the secretion of roots by associated soil microorganisms [1]. This soil is rich in microorganisms and should be explored for obtaining PGPR (Plant Growth- Promoting Rhizobacteria) which can be useful for the development of bio inoculants for the growth and yield of agricultural crop plants [2]. Agriculture is the key source of subsistence for India's population [3]. Different crops like sugarcane, Soyabean, wheat, maize, jowar, rice, pulses, etc are highly produced crops in India [4]. Maharashtra is the prime and major state in agriculture. The leading crops include wheat, Soyabean, cotton, tur, and Soyabean [5]. The coordination between microorganisms and plants is well-known for its advantageous effect [6]. The free-living soil bacteria, isolated from the rhizospheric part of plants are referred to as Plant Growth Promoting Rhizobacteria (PGPR) [7]. PGPR prevents the leaching of nutrients and enhances the obtainability of nutrients in the rhizospheric region of plants surrounding the soil [8]. Regulating nutritional and hormonal balance, making plants resistant to pathogens and dissolving nutrients are some of the important mechanisms of PGPR [9]. Many bacteria feed on sloughs of plant cells, proteins, and carbohydrates released by plant roots in the soil [10]. Many rhizospheric bacteria like *Pseudomonas, Bacillus, Rhizobium,* and *Azotobacter* show a significant role in the production of IAA [11]. Naturally occurring plant hormone IAA is considered in a class of auxin [12]. Auxin is the chief governor of plant development and growth. Indole 3 butyric acid (IBA) is another type of natural auxin present in plants [13]. IAA participates in almost every feature of the growth of plants and development like the formation and development of the embryo, tissue differentiation, cell division, response to light, strengthening shoot height, and root length, increasing the division of cell stem, elongation of coleoptiles, apical dominance, and increase in the rooting, vascular differentiation, and development of fruit [14]. Concentration and production of IAA can be influenced by factors like media conditions, temperature, pH, strain, and availability of nutrients [15]. IAA is released in greater quantities in the residence of tryptophan microorganisms [16]. In recent years, biofertilizers have come out as a powerful substitute for chemical fertilizers because of their non-toxic, easy to use, cost-effective and eco-friendly nature [17]. Biofertilizers are microbial compositions that help plants for growth when applied to soil or seed by uptake of nutrients [18]. The use of IAA as a biofertilizer is a boon to farmers. Significant production of IAA by PGPR has been broadly confessed [19]. *Azospirillum brasilense* in wheat promotes the number and height of side roots of wheat seedlings [20]. In many bacteria and plants, IAA is a metabolic product obtained from tryptophan by two mechanisms like Trp independent and Trp dependent pathways [21]. Conversion of tryptophan to IAM (Indole-3-acetamide) by enzyme tryptophan-2-monooxygenase and then a breakdown of IAM to IAA (Indole acetic acid) by enzyme IAM-hydrolase is carried out in Trp dependent pathway [22]. This study aimed to isolate and characterize IAA-producing bacteria from rhizospheric soil samples and then check the effect of IAA as a biofertilizer on wheat, maize, and Soyabean crops.

Materials and Methods
1. Sampling and Physico-chemical analysis
Different rhizospheric soil of wheat, maize, and Soyabean was collected from the village of Sanvatsar, Jawalke, and Kopargaon, near Ahmednagar district, Maharashtra, India. Four Physico-chemical properties of the soil

sample were analyzed: pH, texture, temperature, and NPK(Nitrogen, Potassium, Phosphate) content [23]. The soil was kept in polythene bags for further study and biofertilizer preparation [24].

2. Screening for IAA producing microorganisms

The rhizospheric soil of wheat, maize, and Soyabean samples was serially diluted in sterile saline and each aliquot was spread on a sterile Nutrient Agar plate respectively [25]. Each plate was re-streaked to get an isolated colony. Two broths were used - Nutrient broth with tryptophan (Tryptone broth) and nutrient broth without tryptophan was inoculated with an isolated colony from a sterile Nutrient agar plate and was allowed to incubate for 3 days at 37^0 C.

3. Isolation and Identification of screened isolate

Isolates dependent on morphological observation and biochemical tests were identified. These tests include; the indole test, citrate utilization test, vogus Proskauer test, methyl red test, Sugar fermentation tests, Gram staining, motility, endospore staining, catalase, oxidase, amylase, etc [26, 27].

4. Estimation and Quantitative analysis of IAA

For estimation of IAA, both the broth were compared for turbidity. At 5,000 rpm for 20 minutes the broth was centrifuged. The derived supernatant was named crude IAA. Analysis of produced crude IAA was carried out by using Salkowaski’s reagent. The cell-free broth was collected, 1 ml of it was mixed with 2 ml of Salkowaski’s reagent and incubated in dark for 20 min at 37^0 C [28].

5. Use of crude IAA as biofertilizer

Estimated crude IAA was used as a biofertilizer. Seeds of Wheat, Maize, and Soyabean were treated with 2 ml of crude IAA aseptically for 2 min. Treated seeds were further used in pot assay.

6. Pot assay

The result of IAA as a biofertilizer was analyzed by using a pot assay. 50 gm of agricultural soil was placed on each platter of the pot tray [28, 29]. Seeds of soya, wheat, and maize were planted separately on pot trays. In each pot tray, 25% of pots were sown with treated IAA seeds, and 25% of pots were sown with untreated seeds. Plants were watered regularly. The growing crops were often examined by measuring shoot length and root length for plant growth [29, 30]. Test plants with prepared IAA as a biofertilizer were compared with materialistic biofertilizers.

Results and Discussion

1. Physico-chemical analysis

The major physicochemical characteristics of the agricultural field of Wheat, Maize, and Soyabean were studied. The observations are given in Table 1. Overall the pH of the soil was found to be slightly alkaline between the range of 7.5 to 8.12, the temperature in the range between 28 to 37^0 C, and the texture mostly slit. The alkaline pH of soil contains a high amount of ions like magnesium, calcium, and sodium. Slight alkaline pH of soil indicates difficulty in peptization of soil.

Table 1: Physico-chemical analysis of soil

Place of Collection	Crop	pH	Texture	Temperature in degree Celsius	Accessible nitrogen kg ha^{-1}	Accessible phosphate kg ha^{-1}	Accessible potassium kg ha^{-1}
Sanvatsar	Wheat	7.5	Loam	28	205	40	209
Jawalke	Maize	8.2	Silt	37	198	53	199
Kopargaon	Soyabean	8.12	Silt	37	460	52	250

Fig 1: Different Rhizosphere soil samples of Soyabean, Wheat, and Maize.

2. Screening for IAA producing microorganisms

Isolated colonies were obtained on sterile nutrient agar plates from rhizospheric soil of Wheat, Maize, and Soyabean. Nutrient broth with tryptophan shows high turbidity which indicates bacterial growth to a higher extent as compared to nutrient broth without tryptophan after incubation of 3 days at 37^0 C.

Fig 2: Nutrient broth with tryptophan and Nutrient broth without tryptophan showing comparative bacterial growth

3. Isolation and Identification of screened isolate

Two bacterial isolates were confined as IAA producers from 3 soil rhizospheric soil samples based on morphological and biochemical characteristics. (Table 2) The isolates were coded as I-1 and I-2 (from the Soyabean, wheat, and maize rhizosphere soil sample). From the observed results and by comparing it with Bergey's manual of determinative of bacteriology *Bacillus mycoides* was identified as an isolate for a colony coded as I-1 from Soyabean Rhizosphere soil sample and *Bacillus spp* was tentatively recognized as an isolate for a colony coded as I-2 from wheat and maize rhizospheric soil sample.

Table 2: Morphological and biochemical features of isolates from rhizospheric soil

Sr. No	Test	I -1	I-2
1	Media	Tryptone broth	Tryptone broth
2	Gram - Character	+	+
3	Shape and arrangement	Rod	Rod
4	Cultural characteristics		
	Size	1-2 mm	1 mm
	Shape	Irregular	Irregular
	Pigmentation	-	-
	Margin	Entire	Entire
	Elevation	Convex	Convex
	Opacity	Opaque	Transparent
	Consistency	Smooth	Smooth
	Motility	Nonmotile	+
	Endospore Staining	+	+
5	Sugar fermentation and Acid production test		
	Glucose	-/+	+/+
	Maltose	-/+	+/+
6	Biochemical test		
	Indole Production	-	-
	Methyl Red Test	+	-
	Vogus Proskauer Test	-	-
	Citrate Utilization test	+	+
7	Enzyme		
	Catalase	+	+
	Oxidase	-	-
	Amylase	+	+

I-1: 1st Isolate, I-2: 2nd Isolate

Fig 3: Isolated colonies from rhizospheric soil grown on a sterile nutrient agar plate

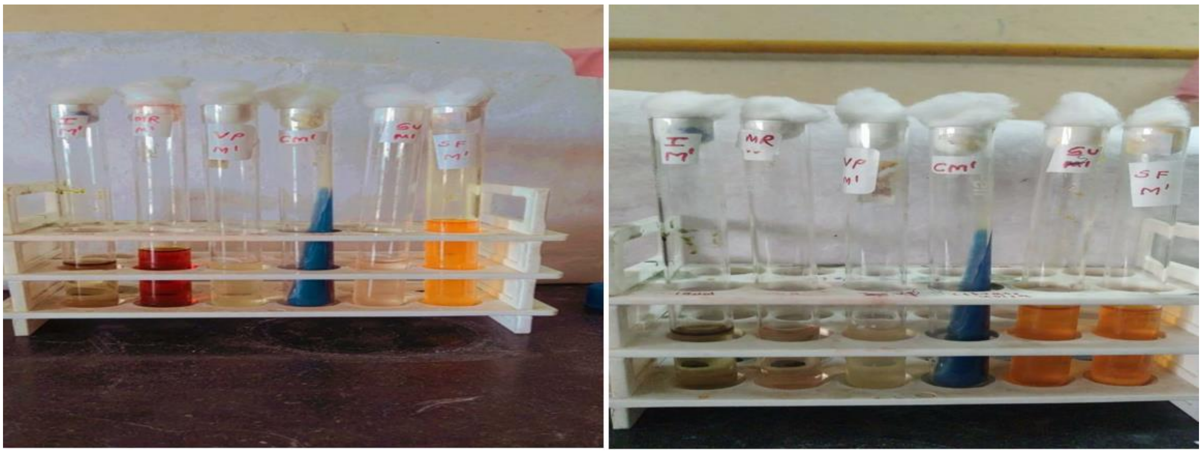

Fig 4: IMViC and sugar test for I-1 and I-2

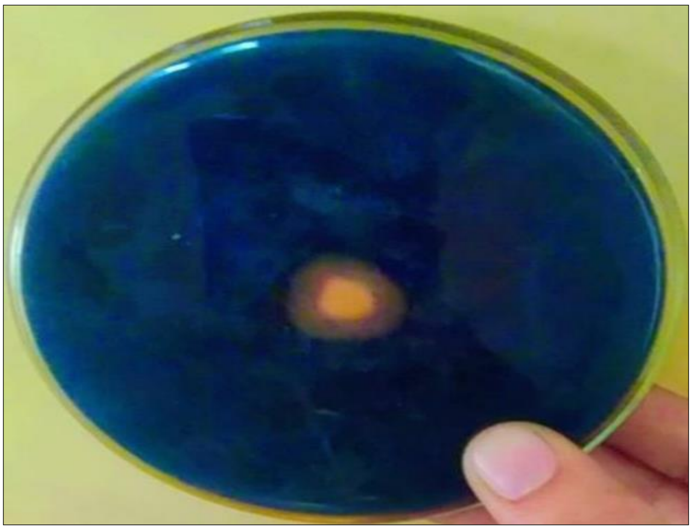

Fig 5: Amylase test showing zone of hydrolysis

4. Estimation and Qualitative analysis of IAA: appearance

Estimation of IAA was checked by centrifugation of both the broths. After centrifugation, 2 ml of Salkowski's reagent was added to 1 ml of cell-free broth. The appearance of pink color in Tryptone broth showed the presence of IAA when compared with nutrient broth without tryptophan.

Fig 6: Trypton broth tube showing IAA production against negative control.

5. Use of crude IAA as a biofertilizer

After 15 days of plantation maximum development was seen in all three plants with treated seeds in comparison to plants with untreated seeds.

Fig 7: Effect of IAA as biofertilizer on wheat, maize, and Soyabean crops sequentially

6. Pot assay

After 15 days crops were noticed for their external characteristics like root and shoot length. Maximum growth was observed in root and shoot height of all three crops with treated seeds in contrast to crops with untreated seeds. The IAA as a biofertilizer showed a remarkable effect on plant growth. The greatest boom was seen in the root and shoot length of the maize crop besides wheat and soyabean.

Table 3: Result of IAA as a biofertilizer on Root and Shoot length of Soyabean, Maize, and Wheat crop

Sr. No.	Crops	Specimen	Average Root Length	Average Shoot Length
1	Soyabean	Control	13	8
		Test	6	5.5
2	Maize	Control	60	17
		Test	33	11.5
3	Wheat	Control	26.5	8.5
		Test	14	3.3

Table 4: Variation between IAA as a Biofertilizer and Materialistic fertilizer

Sr. No.	Crops	Specimen	Average Root Length	Average Shoot Length
1	Soyabean	Test BF	13	8
		Test MF	15.5	10.2
2	Maize	Test BF	60	17
		Test MF	61.8	19.2
3	Wheat	Test BF	26.5	8.5
		Test MF	28.1	11

Test BF: Biofertilizer, MF: Materialistic fertilizer

A comparison was also performed between IAA-treated seeds as a biofertilizer with materialistic fertilizer for three crops. According to data (Table 4), it can be concluded that IAA as a biofertilizer is productively efficient with materialistic fertilizers.

Fig 8: Effect of IAA on the root, and shoot height of Soyabean, Maize, and wheat crops respectively in contrast to control.

Conclusion

It is clear from this study that rhizospheric bacteria are a rich source of IAA found in soil. Identified isolates can uptake tryptophan provided in the medium and produce IAA. It is concluded that IAA as a biofertilizer showed efficient growth in Wheat, Maize, and Soyabean crops.

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