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In Vitro Studies on IAA Producing Ability of Potassium Solubilizing Bacteria

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Abstract Experiment was conducted to evaluate the *in vitro* studies on indole acetic acid (IAA) producing ability of K solubilizing bacteria. Out of 75 isolates 28 isolates were selected as potential KSB based on zone of solubilization. Among the 28 isolates, 9 were from Lingasugar, 11 from Raichur and 8 from Deodurga taluk. Out of these isolates, KSBD-58 produced higher amount of IAA (15.13 µg ml^{-1}) which was significantly higher than all other isolates including the reference strain (13.23 µg ml^{-1} of IAA). Among 28 isolates nine were produce more than 10 µg ml^{-1} of IAA whereas 12 isolates produce more than five µg ml^{-1} and remaining eight isolates were produce less than five µg ml^{-1} of IAA.

Keywords KSB, *Frateuria aurantia*, IAA.

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Introduction

Potassium is one of the essential macronutrient and the most abundantly absorbed cation in higher plants. It plays an important role in the growth and development of plants. Potassium is critical to not only getting the crop off to a good start, but to supporting maturity. Potassium deficiency leads plant to develop weak roots, grow slowly, lodge easily, produce small seeds and lower yields.

Potassium is present in relatively large quantities in soils, ranging between 0.5 and 2.5% . However, in most cases only a small fraction of it is available to plants being the most abundant element on earth'scrust. Indian soils have sufficient quantity of potassium to support crops raised in it, but ironically, this potassium content in soil is in unavailable form (which accounts for 90 to 98% of total potassium in all soils) to plants, to resolve this problem the potassium solubilizing bacteria (KSB) would be a novel solution to convert insoluble form of soil potassium into soluble (available to plant) form through production of organic acids. Potassium solubilizing bacteria such as *Frateuria aurantia*, *Bacillus mucilagenosus* and *Bacillus edaphicus* are example of microorganisms that are used as potassium biofertilizer. One of the most important factors contributing to the efficiency of any biofertilizer is its plant growth promoting activity. *Bacillus* spp. are considered to be the helpful microorganisms that hold remarkable abilities for syn-

thesizing a vast array of beneficial substances [1]. *Bacillus* spp. having potent plant growth promoting traits such as indole acetic acid (IAA) and gibberllic acid (GA) production , phosphate solubilization, nitrogen fixation and biocontrol attributes like production of HCN, siderophore, hydrolytic enzymes and antibiotics have been isolated from soybean [2].

Material and Methods

Production of IAA by the isolates

All the isolates were subjected to qualitative analysis for the production of IAA [3]. Luria agar supplemented with 0.06% sodium dodecyl sulfate and one per cent glycerol was prepared and plated. The surface area of the agar medium was divided into squares of 2 cm × 2 cm by marking on the bottom of each plate. The overnight culture of each isolate grown on luria agar was spotted with sterile tooth pick in each square. The spotted plates were overlaid immediately with sterile disc of whatman no. 1 filter paper. Plates were incubated until the colonies reached the size of 0.5 to 2.0 mm in diameter. After an appropriate incubation period, the filter paper discs were removed from the plates and treated with Salkowaski's reagent (2% of 0.5 M $FeCl_3$ in 35% perchloric acid) by soaking in a petridish containing the reagent. The reaction was allowed to proceed until adequate color was developed.

Bacteria producing IAA were identified by the formation of characteristic red halo around the colony on filter paper. The isolates showing IAA production were further examined for the amount of IAA production as detailed below.

Quantitative estimation of IAA extraction

The overnight cultures of the isolates which showed the production of IAA in qualitative estimation were inoculated to 50 ml of sterilized Czapeck's solution and incubated at 37°C for seven days in dark. After incubation, the cultures were centrifuged at 6000 rpm for 20 minutes. The supernatant was collected in a conical flask and used for estimation of IAA.

Table 1. Production of IAA by local efficient isolates of potassium solubilizing bacteria.

Sl. No.	Isolate	IAA	IAA ($\mu g\ ml^{-1}$)
1	KSBL-2	+	2.24^{v}
2	KSBL-5	+	12.27^{d}
3	KSBL-8	+	3.40st
4	KSBL-9	+	11.37^{e}
5	KSBL-12	+	7.50^{kl}
6	KSBL-13	+	8.65^{i}
7	KSBL-18	+	5.36^{pq}
8	KSBL-23	+	10.01^{g}
9	KSBL-25	+	7.15^{l}
10	KSBR-28	+	2.72^{u}
11	KSBR-30	+	6.62^{m}
12	KSBR-31	+	6.08^{n}
13	KSBR-32	+	11.64^{e}
14	KSBR-35	+	3.53^{s}
15	KSBR-38	+	5.52^{op}
16	KSBR-41	+	12.96^{c}
17	KSBR-42	+	9.23^{h}
18	KSBR-44	+	7.81^{jk}
19	KSBR-46	+	4.65^{q}
20	KSBR-50	+	8.02^{j}
21	KSBD-51	+	10.52^{f}
22	KSBD-55	+	9.74^{g}
23	KSBD-58	+	15.13^{a}
24	KSBD-63	+	3.01^{t}
25	KSBD-64	+	4.93^{q}
26	KSBD-67	+	12.74^{c}
27	KSBD-72	+	5.89^{no}
28	KSBD-74	+	4.11^{r}
29	Reference strain	+	13.23^{b}
		SEm±	0.12
		CD at 1%	0.47

Estimation of IAA

Twenty five ml of the supernatant was collected and the pH was adjusted to 2.8 using IN HCl in a 100 ml conical flask. Equal volume of diethyl ether was added to it and incubated in dark for four hours. Extraction of IAA was done at 4 °C in a separating funnel using diethyl ether. The organic phase was discarded and the solvent phase was pooled and evaporated to dryness. To the dried material, two ml of methanol was added, pooled and the IAA present in the methanol extract was determined using the method of Gordon and Paleg [4]. To 0.5 ml of methanol, 1.5 ml of distilled water and four ml Sapler's reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% perchloric acid) were added and incubated in dark for

one hour. The intensity of pink color developed was read at 535 nm in a UV-visible spectrophotometer. From a standard curve prepared with known concentrations of IAA, the quantity of IAA in the culture filtrate was determined and expressed as µg ml^{-1} of the medium.

Results and Discussion

All the 28 isolates were examined for the production of IAA along with reference strain (*Frateuria aurantia*) on luria agar with SDS (0.06%) and glycerol (15). Based on the development of red color on the filter paper or green fluorescence under UV light, all the isolates including reference strain were positive for IAA production (Table 1). These isolates were further subjected for quantitative determination of IAA.

Quantitative determination of IAA produced by the isolates

The amount of IAA produced by 28 isolates along with reference strain (*Frateuria aurantia*) was determined at 10 DAI and results are presented (Table 1). The amount of IAA produced by different isolates ranged from 2.24 to 15.13 µg ml^{-1} broth and 0.08 to 3.05 µg ml^{-1} broth respectively. Among the isolates examined, KSBD-58 was found to produce the highest amount of IAA (15.13 µg ml^{-1} broth) which was significantly superior over the other isolates including the references strain (13.23µg ml^{-1} of IAA). Nine isolates including reference strain produced more than 10 µg of IAA ml^{-1} broth . The results obtained are in concordance with the findings of Archara [5] wherein she observed a IAA production of 16.50 µg ml^{-1} broth. Similar results were observed by Kumar et al. [6], wherein they observed an IAA production of 17 µg ml^{-1} broth.

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