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SCREENING OF *RHIZOBIUM* STRAINS ISOLATED FROM *VIGNA MUNGO* NATIVE TO RICE FALLOWS FOR THE PRODUCTION OF INDOLE ACETIC ACID

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ABSTRACT

A total of 19 Rhizobium strains (VM-1 to VM-19) isolated from the healthy root nodules of Vigna mungo native to rice fallows were screened for the production of Indole acetic acid (IAA). The strains could produce high amount of IAA when grown in YEMA medium supplemented with L-tryptophan (1.5 mg/ml) for 54 h. But IAA production varied in different strains. The strain VM-13 was found to be potent producer of IAA among the strains tested. Among the carbon and nitrogen sources tested maximum amount of IAA production was observed when Glucose and L-asparagine were used as carbon and nitrogen sources. The crude IAA extracted from the strain VM-13 was confirmed by co-chromatography with standard IAA.

KEY WORDS

Black gram, Indole acetic acid, L-tryptophan, Rhizobium, Rice fallow.

INTRODUCTION

Leguminous plants are important both ecologically and agriculturally, as they are the main source of biological nitrogen fixation (BNF) through root nodule formation [1]. The black gram (*Vigna mungo* (L.) Hepper), a major source of protein (24%) is one of the important pulse crops of Andhra Pradesh. It also improves the soil fertility by fixing 38 kg N/ha/year in soil from atmosphere [2]. It is mainly cultivated in a cereal-pulse cropping system primarily to conserve soil nutrients and utilize the left over soil moisture particularly after rice cultivation.

For effective utilization of land and human resources the cultivation of rice fallow land is very important. Cultivation of legumes in rice fallows can conserve soil NO3 from potential loss and additionally capture atmospheric N through BNF [3]. The root nodules of legumes contained appreciable amounts of phytohormones which played a key role in triggering the initiation of root nodules, nodule development, maintenance and senescence [4, 5]. The ability to synthesize phytohormone is widely distributed among plant associated bacteria. The majority of bacteria (80%) isolated from plant rhizosphere are able to produce IAA [6] and up to 74% of rhizobial strains could produce IAA [7].

Indole acetic acid (IAA) is one of the most physiologically active auxins. It is a common product of L-tryptophan metabolism by several microorganisms [8, 9, 10]. Rhizobia are the first group of bacteria which are attributed to the ability of plant growth promoting Rhizobacteria (PGPR) to release IAA that can help to promote growth in plants [11]. The present work is taken up to screen different *Rhizobium* strains isolated from the root nodules of *Vigna mungo* for IAA production and to find out the cultural requirements for enhancing the IAA production.

MATERIALS AND METHODS

In the present study, 19 *Rhizobium* strains were isolated from the freshly collected healthy root nodules of *Vigna mungo* cultivated in rice fallows on Yeast Extract Mannitol Agar (YEMA) medium [12]. The identity of the isolates as *Rhizobium* was confirmed on the basis of morphological cultural and biochemical characteristics on YEMA by standard methods [13]. For IAA production bacterial cultures were grown in 100 ml of Erlenmeyer flasks containing 30 ml of YEM broth [14] supplemented with L-tryptophan at pH 7.0 in triplicate on a rotatory shaker for 54 h at 30±2°C. Bacterial growth was determined by taking optical density (OD) at 540 nm using a Spectrophotometer (Elico-Cl 157).

The broth cultures were centrifuged at 5000 rpm for 20 min and the cell free supernatant was analysed for IAA [15]. To the 10 ml of supernatant, 2 ml of Salkowsky's reagent was added and incubated for 30 min under darkness. The amount of IAA produced was estimated calorimetrically at 540 nm [16].

Effect of Incubation period on IAA production

To study the effect of incubation period on IAA production the *Rhizobium* strains were inoculated into L-tryptophan supplemented YEM broth and incubated at 30±2°C on rotatory shaker at 200 rpm for 72 h. Samples were withdrawn at 6 h interval and growth and IAA production were determined.

Effect of L-tryptophan concentration on IAA production

The ideal concentration of L-tryptophan for the production of maximum IAA by the *Rhizobium* strains was studied by inoculating the strains into YEM broth supplemented with different concentrations of L-tryptophan (0.5–2.0 mg/ml).

Effect of carbon and nitrogen sources

The effect of different carbon sources (1%) on IAA production by *Rhizobium* strains was studied by inoculating each strain into the L-tryptophan supplemented YEM broth omitting mannitol. The effect of different nitrogen sources (0.1%) was also studied in L-tryptophan supplemented YEM medium.

Extraction of crude IAA

The best IAA producing *Rhizobium* strain VM-13 was inoculated into 300 ml of YEM medium with most suitable supplements and incubated at 30 ± 2°C for 3days on rotatory shaker. After incubation, the biomass was separated from the supernatant by centrifugation at 10000 rpm for 30 min. The supernatant was acidified to pH 2.5 to 3.0 with 1N HCl and extracted twice with ethyl acetate. The solvent fraction was evaporated to dryness in a rotatory evaporator at 40°C. The residue was dissolved in methanol and kept at 20°C [17].

Confirmation of IAA using TLC

Extract containing crude IAA along with the standard IAA was plated on TLC plates (Merck) and run by using benzene, n-butanol and acetic acid (70:25:5). Spots with R_f values identical to authentic IAA were identified under UV-light (254 nm) by spraying the plates with Ehmann's reagent [18].

RESULTS

A total of 19 strains were isolated from the root nodules of *Vigna mungo.* Based on morphological, cultural and biochemical characteristics, the strains were identified as species of *Rhizobium* [19]. All the 19 strains could produce IAA. The amount of IAA produced varied from strain to strain and relatively high amounts were recorded from some *Rhizobium* strains (VM-5, VM-8, VM-12 and VM-13) incubated for 54 h. This could be due to better utilization of medium components for IAA production by these strains compared to other strains. All these strains initiated IAA production at the beginning of their growth and reached maximum at 54 h of incubation (**Figure 1A-D**). Among these four strains, the maximum amount of IAA was produced by the strain VM-13 (48.4 µg/ml). The effect of different concentrations of L-tryptophan revealed that maximum growth and IAA production were observed at 1.5 mg/ml L- tryptophan for all the strains (**Figure 2**).

Effect of different carbon sources (1%) on IAA production revealed that the *Rhizobium* strains vary in their utilization and production of IAA. The *Rhizobium* strain VM-13 produced highest amount of IAA in the medium with glucose as carbon source followed by VM-12, VM-5 and VM-8. Next to glucose mannitol was the preferred carbon source for VM-5, VM-12 and VM-13 (**Table 1**). Statistical analysis showed that the effect of different carbon sources on IAA production was significant. Effect of different nitrogen sources (0.1%) on IAA production revealed L-

asparagine as the best nitrogen source for IAA production followed by KNO3 (**Table 2**). Among the four strains tested, *Rhizobium* strain VM-5 produced highest amount of IAA (48.1 µg/ml). The strain VM-13 produced relatively high amount of IAA in medium with KNO_3 as nitrogen source followed by L-asparagine. Statistical analysis showed that the effect of different nitrogen sources on IAA production was also significant. The production of IAA was also confirmed by co-chromatography of the sample along with standard IAA followed by spraying with Ehmann's reagent.

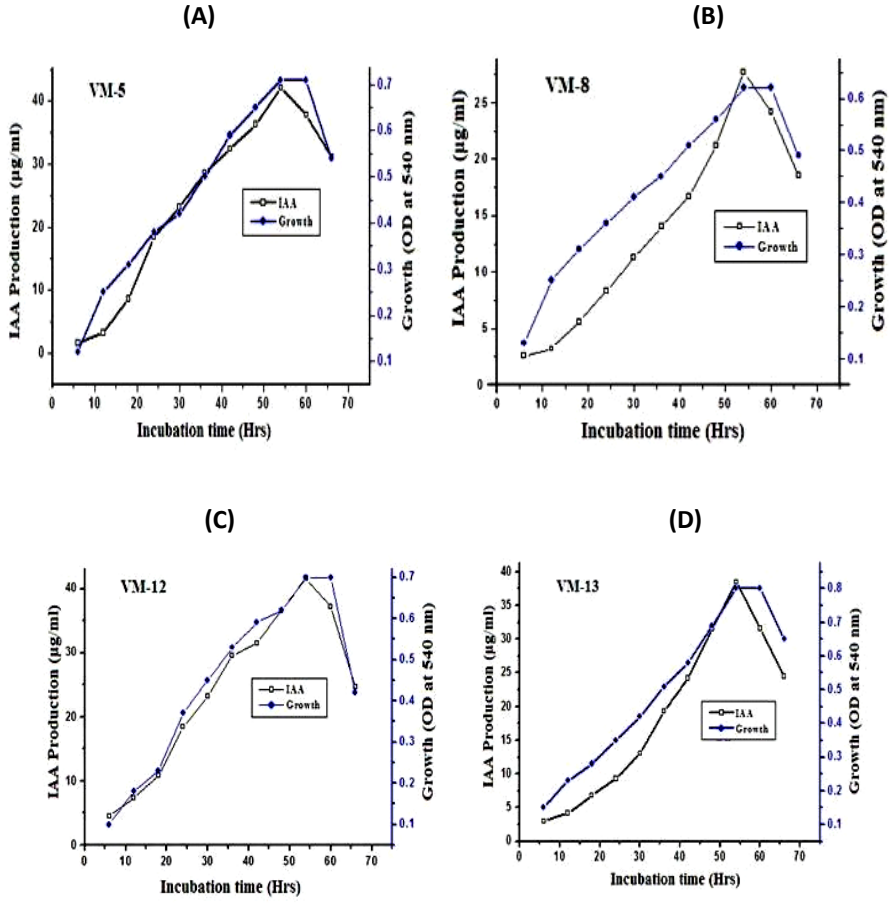

Figure 1: The effect of incubation period on growth and IAA production by *Rhizobium* strains

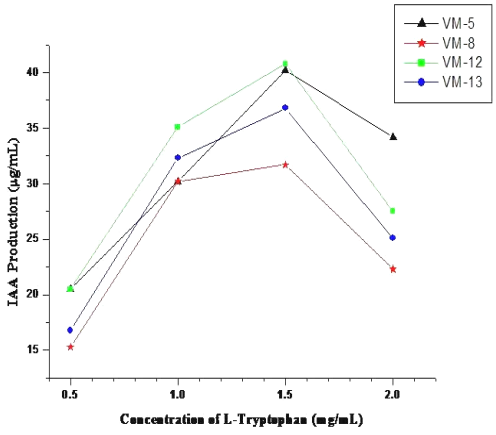

Figure 2: Effect of different concentrations of L-tryptophan on IAA production by *Rhizobium* strains

Table 1: IAA production (µg/ml) by *Rhizobium* strains

Carbon source*(1%)	VM-5	VM-8	VM-12	VM-13
Control	12.3	14.0	10.8	16.3
Mannitol	**40.7**	28.7	**42.3**	**36.8**
Glucose	**46.8**	**39.2**	**47.1**	**48.4**
Lactose	14.1	7.0	7.3	6.1
Galactose	17.3	17.6	23.8	12.7
Sucrose	31.1	37.1	35.4	36.3
Maltose	25.8	11.5	30.5	11.9

Each value is a mean of three replicates
***Significant at 5% between carbon sources (Fc = 4.568 Ft = 1.920)**

Table 2: IAA production (µg/ml) by *Rhizobium* strains

Nitrogen source*(0.1%)	VM-5	VM-8	VM-12	VM-13
Control	13.2	14.9	16.7	12.5
KNO_3	**45.1**	**40.4**	**41.2**	**47.4**
L-asparagine	**48.1**	**43.4**	**46.3**	**46.2**
$NaNO_3$	26.2	16.8	26.3	19.2
$(NH_4)_2SO_4$	20.2	15.0	17.7	6.4
NH_4Cl	12.3	10.0	25.5	20.2

Each value is a mean of three replicates
***Significant at 5% between nitrogen sources (Fc = 3.624 Ft = 2.630)**

DISCUSSION

The biomass as well as IAA production gradually increased up to 54 h. The decline in IAA production after 54 h might be due to release of IAA degrading enzymes like IAA oxidase and peroxidase in the medium by the bacteria [20, 8]. The isolates preferred L-tryptophan for maximum IAA production. Among the different concentrations of L-tryptophan tested the maximum amount of IAA production was observed at1.5 mg/ml. In earlier reports the *Rhizobium* sp isolated from root nodules of *Dalbergia lanceolaria* produced high amount of IAA at 2.5 mg/ml L-tryptophan concentration [21], while the *Rhizobium* sp. from root nodules of *Roystonea regia* produced maximum amount of IAA at 3 mg/ml L-tryptophan concentration [22] indicating the variation in utilising L-tryptophan for IAA production by *Rhizobium.* Among the carbon sources tested, the maximum IAA production was observed in glucose containing medium this may be due to the better utilization of glucose compared to other carbon sources. Glucose as the best carbon source for IAA production was reported earlier for *Rhizobium* sp. From *Cajanus cajan* and *Rhizobium* sp. from *Vigna mungo* [8, 10]. Among the tested nitrogen sources the maximum IAA production was observed in L-asparagine containing medium. L-asparagine as the best nitrogen source for IAA production by *Rhizobium* sp. from *Phaseolus mungo* was also reported by Ghosh et al [23].

The production of IAA was also confirmed by co-chromatography of the sample along with standard IAA followed by spraying with Ehmann's reagent showed the similar R_f value (0.83).

CONCLUSION

From this study it is clear that *Rhizobium* strains differ significantly in IAA production. The ability of *Rhizobium* strains to produce IAA in tryptophan supplemented medium suggested the possibility that the symbiont was responsible for higher content of root nodules. The IAA production is one of the beneficial aspects of the *Rhizobium* – legume symbiosis.

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