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# Amminocyclopropane-1-carboxylate (ACC) Utilization and Indole-3-acetic Acid (IAA) Production by Sesbania rostrata rhizobial isolates

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ABSTRACT: In present ere agriculture is facing challenges due to low nitrogen availability therefore high amount of chemical fertilizers are used. Symbiotic association occurs by *Rhizobium* that causes the growth of shoots and roots nodules in legumes and nitrogen fixation for improving fertility of soil. Therefore, selection of the appropriate strain is significant for enhancing biological nitrogen fixation. The current study was intended to isolate and characterize rhizobial strain from root and shoot nodule of *Sesbania rostrata* plants on selective yeast extract mannitol agar (YEMA) medium. On the basis of morphological properties, 26 isolates were recognized as rhizobia. These possessed a smooth texture and convex slope and were spherical, milky white, mucoid in shape. All microbial isolates were identified as Gram-negative, rodshaped, and motile upon microscopic analysis. Biochemical depiction of rhizobia showed that all rhizobia were able to produce IAA however, 65% of rhizobial isolates could utilize ACC. These rhizobia have plant growth promoting traits so used as a biofertilizer and use of chemical fertilizer is reduced.

Keywords: ACC, Symbiotic, Rhizobia, Sesbania rostrata, Indole-3-acetic acid (IAA).

#### INTRODUCTION

For sustainable agricultural practices symbiotic associations between legume and rhizobia are very essential. In this view, the phytohormone ethylene plays a vital function in inhibiting the nodulation process during nodule formation (Schaller, 2012). In addition it also influences several other plant developmental cues, together with a variety of stress responses that prevent plant growth. Gaseous plant hormone ethylene formed endogenously by every bigger plants and is known being among the best crucial molecules for regulating plant development (Bleecker and Kende 2000 and Lin et al., 2009). Ethylene is produced from the ACC present in the root exudates by the enzyme ACC oxidase. It is key phytohormone, however more formed ethylene in stressful situation can effect in the inhibition of plant growth or death, particularly for seedlings. When ethylene is produced in the high concentration produces defoliation and other cellular activities, in a decline in crop performance resulting (Bhattacharyya and Jha, 2012). It is involved in a number of phases of symbiosis too, together with the early reaction to nodule development, bacterial nod factors, abscission and senescence (Csukasi et al., 2009 and Patrick et al., 2009). Though, a number of bacteria can reduce harmful effect of ethylene levels through act of the enzyme ACC deaminase, which degrades ACC to ammonia and  $\alpha$ -ketobutyrate, both of which the microbe or the plant can metabolize (Glick, 2014). ACC deaminase not acting an essential function in the nodulation procedure however can adapt the persistence of nodules (Nascimento et al., 2016). An inducible enzyme, ACC deaminase's synthesis, is stimulated by the presence of its substrate (Singh et al., 2015). Adding of ethylene precursor ACC to plant roots barren nodulation in Medicago truncatula (Penmesta and Cook, 1997), whilst the adding of the ethylene inhibitor AVG (L-α-aminoethoxyvinyl-glycine) improved the quantity of nodules in pea (Nukui et al., 2000). Bacteria exhibiting 1-aminocvclopropane-1-carboxvlic acid (ACC) deaminase activity, which inhibits the biosynthesis of ethylene in higher plants, promote plant growth through the degradation of ethylene precursors, such as ACC (Sarapat et al., 2020). The ACC deaminase bacteria offer drought tolerance by adaptable plant ethylene levels. In addition to plant adaption mechanisms, plant growth-promoting rhizobacteria (PGPR) can enhance salt tolerance in plants via ion production of antioxidants, ACC homeostasis, deaminase, phytohormones, extracellular polymeric substance (EPS), volatile organic compounds, accumulation of osmolytes, activation of plant antioxidative enzymes, and increase of nutrients uptake (Riseh et al., 2021).

Growth phytohormone indole-3-acetic acid (IAA) being considered the most significant agent of auxin class (Ludwig-Muller et al., 1993 and Serban et al., 2017). Tryptophan and indole derivatives are familiar precursors for usual biosynthetic pathways. The rhizosphere region isolated microorganisms of different crops have capability to generate IAA as minor metabolites because of plentiful supply of substrates. As a regulator of many biochemical functions, such as cell division, elongation, selectivity and tropic responses, fruit maturity, and senescence, IAA plays a critical role in the development and proliferation of plants. They can also prevent abscission of leaves, flowers and fruits. IAA synthesis by microorganism has been recognized from prolonged period. IAA produced by bacteria contributes to circumvent the host defense by derepressing the IAA signaling in the plant. IAA in addition has a straight consequence on bacterial survival and its resistance to plant defense (Spaepen et al., 2007). Eighty percent of the microbes from various crops' rhizosphere can synthesize and produce auxins as secondary metabolites (Patten and Glick, 1996). IAA formation via indole-3-acetic aldehyde and indole-3pyruvic acid is found in a majority of bacteria like saprophytic species of the genera "Pseudomonas and Agrobacterium; Erwinia herbicola: certain representatives of Rhizobium, Bradyrhizobium, Klebsiella, Azospirillum and Enterobacter" (Ahemad and Khan 2012). The production of indole-3-acetic acid (IAA) is an essential tool for rhizobacteria to stimulate and facilitate plant growth (Lebrazi et al., 2020)

# MATERIALS AND METHODS

**Isolation of native rhizobia.** Big sized, healthy, intact and pink nodules were chosen for separation of bacteria from *Sesbania rostrata* root as well as stem. An aseptic rod was used for crushing every apparent outside nodule in a Petri dish. YEMA medium enriched with 0.0025 percent (w/v) Congo red as an indicator was spread on Petri plates with a single loopful of the nodule suspension and cultured at  $28\pm2^{\circ}$ C for four days. At the end of incubation stage, the rhizobial colonies showed slight or no Congo red absorption appears white and mucoid. They were chosen out by a sterilized inoculating loop and were supplementary purified via streak plate method. The most important isolates were maintained on YEMA slants at 4°C in refrigerator for additional description (Vincent, 1970).

Amminocyclopropane-1-carboxylate (ACC) utilization. Some of the bacteria can utilize the substrate 1-amminocyclopropane-1-carboxylate as sole nitrogen resource by using enzyme ACC deaminase. The ACC utilization of different bacteria can be checked on minimal medium plates supplemented with 3mM ACC (Dworkin and Foster 1958). Actively growing log phase cells of different rhizobia were spotted on such medium plates. The expansion of different rhizobia on ACC enriched media plates was observed after 3-5 days of incubation at  $28\pm2^{\circ}$ C. The different rhizobia showing superior expansion on ACC supplemented medium plates were characterized as ACC utilizers and presumed to have ACC deaminase activity. The expansion on minimal medium plates was used as control to compare the growth on plates supplemented with ACC.

# Production of indole-3-Acetic Acid (IAA)

Estimation of Indole-3-acetic acid was done by Salkowski's method (Mayer, 1958).

#### Reagents

(1) Salkowski's reagent- 1 ml of 0.05M FeCl<sub>3</sub> in 50 ml of 35% of perchloric acid (HClO<sub>4</sub>).

(2) Indole-3-acetic acid stock solution- 100 mg ml<sup>-1</sup> in 50% ethanol.

Rhizobia were grown in flasks with 25 ml of yeast extract mannitol broth enriched by 0.1 g  $L^{-1}$  DL-tryptophan. These flasks were incubated at  $28\pm2^{\circ}$ C. Fourth days, two ml of culture broth was centrifuged at 7,000 rpm for 2 min. and IAA was determined in culture supernatant using following procedure:

Salkowski's reagent was added to 2 ml of culture supernatant, mixed, and shaken, then let to stand at room temperature for 30 minutes to acquire a pink color, which was measured on spectrophotometer at 530 nm. Standard was pure indole-3-acetic acid.

### **RESULTS AND DISCUSSION**

A total of 26 rhizobia were isolated from Sesbania rostrata (Root as well as stem nodules) and stored at 4°C for further studies. Enzymes such as ACC deaminase are formed via the variety of strains and its production is typically associated to free-living bacteria/rhizobacteria, a few fungi and members of Stramenopiles. Bacteria and fungi that convey ACC deaminase can lesser the impact of a range of diverse stresses that influence plant enlargement and progress. ACC deaminase considered as important PGP traits for rhizobacteria and endophytic bacteria (Glick, 2014). ACC deaminase making may be beneficial in the nodulation practice thus enhance the nitrogen supply for legume plants because of an added efficient nodulation. The enzyme ACC deaminase lowers plant ethylene levels is one of the key mechanisms employed by plant growth promoting bacteria to help plant development under stress conditions. Screening of all Sesbania rostrata rhizobia for ACC utilization was carried out on minimal medium plates supplemented with 3mM ACC. Log phase actively grown cells were spotted on the ammonium sulphate  $(2 \text{ gL}^{-1})$  as control medium plate and on ACC (3 mM) supplemented medium plate. These plates were incubated for 3-4 days at 28±2°C and compared the development of rhizobia on plates supplemented with both N source i.e. ammonium sulphate and ACC. It was observed that all rhizobial isolates tested showed development on

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minimal medium supplemented with ammonium sulphate as compared to ACC supplemented plates which showed growth of 65% rhizobia, indicating that only these rhizobial isolates have ACC deaminase activity (Table 2, Plate 1 and Fig. 1). Likewise, Singh et al. (2017) reported that forty nine percent of the rhizobia from pigeon pea showed growth on ACC supplemented plates. On ACC-supplemented plates, 38.9% of Pseudomonas strains exhibited good growth, according to Khandelwal and Sindhu's (2013) research. To reduce drought stress in Vigna mungo L. and Pisum sativum L., Saikia et al., (2018) investigated the effects of the consortium with three rhizobacteria that produce ACC-deaminase: Ochrobactrum pseudogrignonense RJ12, Pseudomonas spp. RJ15, and Bacillus subtilis RJ46. The consortium treatment considerably enhanced the treated plants' dry weight, dry shoot length, and seed germination %. According to Saleem et al., (2018), inoculated velvet bean plants with plant growth promoting rhizobia (PGPR) that included the ACC deaminase enzyme during a drought environment grew more quickly than untreated plants. Compared to

uninoculated plants, ethylene emission from the roots and foliage of inoculated velvet bean plants was noticeably lower. Therefore, PGPR that exhibit ACC deaminase activity shield plants against growth suppression caused by drought, excessive salt, nematodes, bacterial and fungal diseases, flooding, anoxia, metals, and organic pollutants (Gamalero and Glick, 2015). Endophytic bacterial strains of Bacillus subtilis LK14 has revealed major scenario of ACC deaminase (448.3  $\pm$  2.91 nM  $\alpha$ -ketobutyrate mg<sup>-1</sup> h<sup>-1</sup>) (Khan et al., 2016). Many researchers reported that around 50-55% of abiotic stress tolerant rhizobial isolates obtained from diverse legumes like pigeonpea, guar, mungbean and mothbean have been found to be good ACC deaminase producers (Kuldeep, 2013; Dhull et al., 2016 and Mondal et al., 2017). Belimov et al. (2019) also reported that R. leguminosarum bv. viciae 1066S exhibiting ACC deaminase activity increased shoot biomass, nodulation, nitrogen fixation, water use efficiency (WUE), and nutrient uptake in pea plats exposed to water deficit conditions.

Sr. No.	Sesbania species	Name of rhizobial isolates	No. of rhizobial isolates
1.	<i>Sesbania rostrata</i> (root nodulating)	SRKe(i)/r, SRKe(ii)/r, SRTn/r, SRMa/r, SRUd/r, SRPr/r, SRKr(i)/r, SRKr(ii)/r, SRKr(iii)/r, SRSn/r	10
2.	Sesbania rostrata (stem nodulating)	SRKe(i)/s, SRKe(ii)/s, SRKe(iii)/s, SRTn/s, SRMa/s, SRMa(i)/s, SRUd/s, SRPr/s, SRKr(i)/s, SRKr(ii)/s, SRKr(iii)/s, SRHn/s, SRMg/s, SRHs/s, SRSn/s, SRHg/s	16
		Total	26

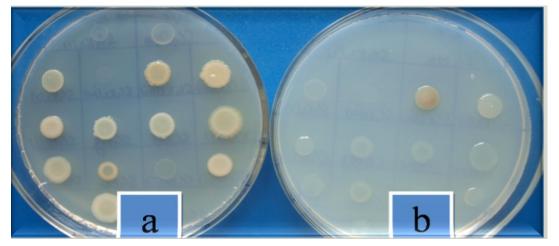


Plate 1: ACC deaminase activity by some of the *Sesbania rostrata* rhizobial isolates on minimal media plate (a) and ACC supplemented plate (b).

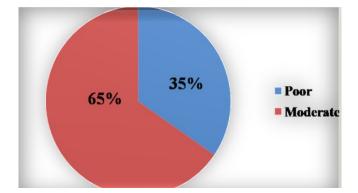


Fig. 1. Categorization of Sesbania rostrata rhizobial isolates for ACC utilization.

IAA production is prevalent amongst environmental bacteria that inhabit soils. Therefore, all 26 rhizobial isolates were tested for IAA production. All rhizobial isolates were found to be IAA producers, though, their production quantity wide-ranging significantly from 0.45 to 22.77 µg/ml. Out of 26 isolates 23, 31 and 46% were poor, moderate and excellent IAA producers, respectively. Maximum IAA production was observed by the rhizobial isolates SRTn/s (22.77 µg/ml), while the isolate SRHg/s (0.45 µg/ml) showed minimum IAA production (Table 2, Fig 2 and Plate 2). It was observed that all isolates showed IAA production however, their production amount varied considerably. Indole acetic acid was produced by 26 Rhizobial strains isolated from Sesbania sesban (L.) Merr. in different zones of Andhra Pradesh, according to Sridevi and Mallaiah (2007), but only five strains produced the most significant amount in YEM broth supplemented with L-tryptophan. Maximum amount (28.0 µg/ml) of IAA was produced by Rhizobium strain 13 after seventy two hours of incubation. Mohite (2013) also reported that out of ten IAA producing isolates, five isolates were selected as efficient producers from rhizosphere bacteria. Comparable, outcome were also observed by Khalid et al., (2004) who categorised the in vitro IAA production by rhizobacteria in three major groups: low (1 to 10  $\mu$ g/ml), medium (11 to 20  $\mu$ g/ml) and high producers (21 to 30 µg/ml). Compared to control plants, seedlings inoculated by IAA-producing bacteria produced more shoot biomass, longer roots, and more colonization (Etesami et al., 2014). Dhull et al., (2016) also reported that all the 54 clusterbean rhizobia were found to be IAA producers, though, their production quantity varied considerably. Boora (2016) also reported that most of the abiotic stress tolerant pigeon pea rhizobia were good IAA producer having different levels of IAA production. Similarly, Subha (2018) reported that all the native rhizobial isolates from different legumes crop showed IAA production, however their production amount varied considerably from 1.62 to 12.3 ug/ml. IAA secretion was estimated of the three Rhizobium isolates (Rf3, Rf11 and Rf12), the maximum amount of IAA was found in yeast extract mannitol medium supplemented with 500µg/ml L-tryptophan. Lebrazi et al. (2020) isolated eighty rhizobial bacteria isolated from root nodules of Acacia cyanophylla grown in different regions of Morocco were firstly screened for their ability to produce IAA. Then, IAA production by a combination of isolates and the inoculation effect on the germination of Acacia cyanophylla seeds was studied using the best performing isolates in terms of IAA production.

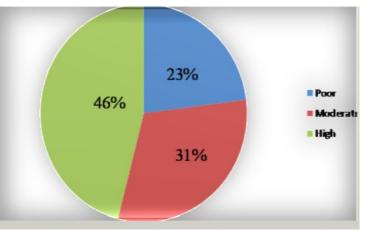


Fig. 2. Categorization of Sesbania rostrata rhizobial isolates for IAA production.

Sr. No.	Sesbania species		
51.110.	-	IAA-production (µg/ml)	ACC utilization
		rata (root nodulating)	
1.	SRKe(i)/r	16.80	+
2.	SRKe(ii)/r	7.66	-
3.	SRTn/r	1.84	+
4.	SRMa/r	15.77	+++
5.	SRUd/r	7.96	++
6.	SRPr/r	13.48	++
7.	SRKr(i)/r	22.19	++
8.	SRKr(ii)/r	0.63	+
9.	SRKr(iii)/r	6.75	-
10.	SRSn/r	4.28	+++
	Sesbania rostr	ata (stem nodulating)	
11.	SRKe(i)/s	9.26	-
12.	SRKe(ii)/s	14.72	-
13.	SRKe(iii)/s	15.95	+++
14.	SRTn/s	22.77	-
15.	SRMa/s	9.47	++
16.	SRMa(i)/s	8.38	-
17.	SRUd/s	10.95	++
18.	SRPr/s	12.40	+++
19.	SRKr(i)/s	14.02	+++
20.	SRKr(ii)/s	2.35	+
21.	SRKr(iii)/s	4.37	++
22.	SRHn/s	10.86	+
23.	SRMg/s	5.73	+++
24.	SRHs/s	15.01	-
25.	SRSn/s	7.90	-
26.	SRHg/s	0.45	-

Table 2: Characterization of Sesbania rostrata rhizobial isolates for plant growth promoting (PGP) traits.



Plate 2: IAA production by some of the Sesbania rostrata rhizobial isolates.

## CONCLUSION

All *Sesbania rostrata* rhizobial isolates were able to produce IAA and however, 65% of rhizobial isolates could utilize ACC (1-aminocyclopropane-1carboxylate). So, these isolates have plant growth promoting traits which is useful for present as well as upcoming crops for improving nitrogen.

#### **FUTURE SCOPE**

These isolates have tremendous potential in near future <sup>F</sup> to be used as biofertilizers in salt affected, alkaline and waterlogged field conditions, which will not only *Singh et al.*, *Biological Forum – An International Journal* 

improve nitrogen availability also having plant growth promoting ability in *Sesbania rostrata* so act as nitrogen reserve for next crop.

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