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ORIGINAL ARTICLE

Non-rhizobial bacterial endophytes from chickpea nodules portraying Plant Growth promotion

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ABSTRACT
The present study is designed to study the non rhizobial endophytic bacteria from nodules of chickpea for their plant growth promoting traits. The nodules were collected and isolation was done and total 96 isolates were collected. All the 96 isolates were tested on NBRIP medium and 36 bacterial isolates shown clear halo yellow zone indicated the positive test for P-solubilization. Further these 38 isolates were screened for qualitative estimation of P-solubilization in 100ml of Pikovaskaya's broth with TCP (0.1%) as inorganic substrates with initial pH 7.0. P-solubilization activity of different isolates was measured at different time intervals (3, 6, 9 12 and 15 days). PSB isolates showed maximum amount of P-solubilization at 12th days and few at 15th day and varied from 0.8 to 21.0 mg 100ml$^{-1.}$ All 38 isolates (non rhizobial endophytic bacteria) positive for P- solubilization were further screened for quantitative IAA, gibberelic acid and ACC deaminase. These 38 endophytic bacterial isolates were able to produce GA in nutrient broth and ranged from 87.78 µgml^{-1} to 112.15 µgml^{-1}. All 38 endophytic bacterial isolates were subjected for their ACC deaminase production on Dworkin and Foster's minimal medium. The ability of isolates to utilize ACC as a source of N was assessed on the basis of bacterial growth on plates containing substrate ACC and $(NH_4)_2SO_4$. Sixteen isolates were found to show growth but considerable variation was observed with respect to growth, when compared with control. Based on preliminary qualitative test for screening of endophytic bacterial isolates positive for ACC deaminase, 16 isolates were further assessed on the basis of bacterial growth in liquid medium selected for their ability to utilize ACC as a sole source of N in terms of optical density (OD at 600). The study of non rhizobial endophytic bacteria will be a good strategy to understand soil-plant-microbe interactions and to the development of efficient inoculants for sustainable agriculture.
Keywords: ACC deaminase, Endophytes, Gibberelic acid, Non rhizobial, P solubilization,

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INTRODUCTION

In recent decades, interest in endophytic microorganisms has been increased, as they have important role in sustainable agriculture. Endophytic bacteria are defined as interior colonizers of the plant *viz*. root, seed, stem and leaf without showing any harmful impact on host plant. *Enterobacter* sp., *Pseudomonas* sp. and *Bacillus* sp. are most abundant non rhizobial endophytic bacteria in legumes and other plants [18]. Plant Growth Promoting endophytic bacteria (PGPEB) also colonize plant root tissues and increase plant growth by different mechanisms [19]. Endophytic bacteria were better protected from biotic (viruses, parasites, weeds, beneficial and harmful insects) and abiotic (drought, heat, salinity, high temperature) stress than the rhizospheric bacteria [17]. Endophytic microbes promote growth of plant by acquiring nutrients *via* nitrogen fixation, P and Zn solubilisation, iron chelation, production of HCN, siderophore, antibacterial or antifungal agents, competition for nutrients and by inducing systemic resistance of plant [9]. Phosphate solubilization by endophytic bacteria is an important trait to enhance the soil fertility, crop production, N_2 fixation in legumes, crop quality and resistance to plant diseases [12]. Phosphate solubilizing endophytic bacteria (PSEBs) may be helpful to reverse the process of phosphate fixation [16]. The endophytic bacterial isolates representing the genera *Erwinia*, *Pseudomonas, Bacillus* and

Flavobacterium are effective IAA producers. Further, the endophytic bacterial communities were considered more efficient IAA producers (as measured in cultures) than the rhizoplanic ones. Gibberelic acid speed up germination by stimulating hydrolytic enzymes which degrade the cells surrounding of the radicle and thus enhancing seedling elongation in cereal seeds [11]. Endophytic isolates of *B. cereus* (CLB2), *Bacillus weihenstephanensis* (TSB4), *B. subtilis* (TSB5), *B. licheniformis* (TSB4) and *B. cereus* (TSB4D) were capable of producing plant growth hormones viz., indole acetic acid and gibberlic acid [22]. ACC and ethylene synthesis is induced by many biotic and abiotic stress *viz.* pathogen attack, flooding or drought and wounding. Furthermore, synthesis of ethylene is triggered by auxins, particularly IAA and inhibited by abscisic acid (ABA). Production of excess ethylene in stress response inhibits root and growth elongation. Ethylene has shown direct effect on transport and signalling of auxin [26]. Some of plant growth promoting bacteria have the ability to degrade the ethylene precursor ACC by ACC deaminase enzyme (bacterially-encoded) and utilize the end products (carbon and nitrogen sources) hence, forms an efficient sink for ACC. Concomitantly, these endophytic bacteria decrease the ethylene levels in its host plant tissue and increase plant growth under stressful conditions [7]. Limited studies are available on non rhizobial endophytic bacteria for improving growth, symbiosis and productivity in chickpea. Knowing and understanding the negative impact of artificial fertilizers in agriculture, novel approaches such as the application of non rhizobial endophytic bacteria as biofertilizer which are associated with plants, may help to increase productivity and improve plant health.

MATERIAL AND METHODS

Plant Growth Promotional (PGP) traits for isolates of endophytic bacteria

Determination of Phosphate (P) solubilization

Phosphate solubilization index (PSI)

"Qualitative assay for P solubilization of plant associated endophytic bacteria was done by streaking of pure culture on NBRIP medium containg plates (National Botanic al Research Institute's Phosphate growth) [1]. " Appearence of clear halo zone around bacterial colony after 5-7 days incubation period at 28±2^{o}C was indicated positive for P solubilization [20]. Following formula was used to calculate Phosphate solubilization index (PSI):

PSI Index= A/B

A= Total diameter (colony + halo zone)

B= Diameter of colony.

Further quantitative phosphate solubilization was performed with promising P solubilizers with presence of clear halo zone around bacterial growth in plate assay method.

Quantitative estimation

Pikovaskaya's broth (100 ml) and 0.1g P_2O_5 as tri-calcium phosphate (TCP) was added in 250 ml conical flask as an inorganic phosphate substrate and the flasks containing broth were autoclaved at 121° C for 15 min. The broth was inoculated with 1 ml of overnight grown pure culture suspension and incubation was done at 28±2°C for 15 days. Equal ratio of ammonium molybdate and ammonium vandate was added to culture supernatant and incubated for 25 minutes, development of yellow colour indicated phosphate solubilizing activity. Intensity of the yellow colour of solution was measured spectroscopically (Elico UV-VIS spectrophotometer) at 420 nm for quantitative estimation [10].

Qualitative and quantitative analysis for Indole acetic acid (IAA) production:

IAA production in different isolates of endophytes were detected [8] by inoculating pure bacterial culture in 10 ml Luria Bertanni broth with or without tryptophan (0.01% L-Trp) and incubation was done at 28-30°C for 3-6 days. Presence of pink colour showed production of IAA which was indicative of positive test. Quantative estimation was done for IAA (μgml^{-1}) by addition of 2 ml of Salkowski's reagent (1 ml of 0.5m $FeCl_3$ in 50 ml of 35% $HClO_4$) into culture supernatant (1 ml) alongwith uninoculated broth with Salkowski's reagent as a reference. After 20 min, absorbance of pink colour was measured spectroscopically (Elico UV-VIS spectrophotometer) at 535 nm and quantification of IAA was done by using standard curve.

Quantative measurement of Gibberellic acid production

Quantative measurement of gibberellic acid by endophytic bacteria was estimated as per method of Borrow *et al* [2].

Reagents

(a) Zinc acetate solution: Zinc acetate (21.9g) was added into 80ml of distilled water and 1ml of glacial acetic acid to make the volume upto 100ml with distilled water.

(b)Potassium ferrocyanide solution: Potassium ferrocyanide (10.6g) was mixed in 100ml distilled water.

Cultures inoculated in their relevant broth containing tubes and incubation was done at 37^0C for seven days. After end of incubation period, cultures were centrifuged for 10 min at 8000 rpm. After centrifugation two ml of zinc acetate solution was added into fifteen ml of the culture supernatant. After two minutes, 2 ml of potassium ferrocyanide solution was added and again centrifuged for 10 min at 8000 rpm. Equal volume of supernatant (5 ml) was added to 30 % hydrochloric acid (5ml) and the test tube was incubated at 27°C for 1 hr 15min. HCL (5%) was used as a blank. UV-VIS spectrophotometer was used to measure the absorbance at 254nm. Gibberellic acid solution of known strength was used to prepare standard curve to quantify the gibberellic acid produced by the cultures and expressed as μgml^{-1} broth.

Determination of ACC deaminase production

Qualitative assay was done as per standard method. Plates containing Dworkin's and Foster (DF) minimal medium with ACC as a sole nitrogen source were streaked with pure culture of endophytic isolates and bacterial growth was observed. [4]. Incubation was done for 3-4 days at 28±1°C.

Quantitative estimation

Liquid DF minimal medium with $(NH_4)_2SO_4$, with ACC (sigma, Ltd) and without ACC was used to culture endophytic isolates individually. Growth of bacterial isolates in different media was measured at 600nm by using UV-VIS Spectrophotometer [24].

RESULT AND DISCUSSION

For qualitative P- solubilization, all the 96 isolates from nodules were tested on NBRIP medium amended with 0.5% tri calcium phosphate (TCP) as inorganic source of phosphorus. Out of 96 bacterial endophytes, 36 bacterial isolates shown clear halo yellow zone on NBRIP medium indicated the positive test for P-solubilization. The 2 isolates (LCNE6 and LCNE9) procured from Pulses Microbiology Laboratory, Department of Plant Breeding and Genetics and were also found positive for P- solubilization. Therefore, total 39.58% (38) isolates from nodules were positive for P- solubilization on NBRIP medium. Phosphate solubilization index (PSI) of nodule endophytic bacterial isolates was ranged from 1.27 to 2.25. Highest P solubilization index was recorded in RBN17 (2.25) followed by LCNE (2.08). Out of 38 nodule non rhizobial endophytic isolates 55.2%, 28.90% and 15.60 % were shown high, medium and low PSI, respectively. Similarly, Maghraoui *et al.,* [14] observed that of 4 rhizobial strains RHOF147 (1.37 cm) and RHOF174 (1.2 cm) strains formed clear halos around colonies after 15 days. Similarly, Ghosh *et al.,* [6] documented PSI values ranged from 1.2 to > 2.7 in 6 rhizospheric bacterial isolates of seagrass. Mahalakshmi and Reetha [15] studied that 83.3% of *Pseudomonas* isolates were P-solubilizers in tomato crop. Sherathia *et al.,* [25] observed of total 154 rhizobacteria from groundnut rhizosphere, 11 were positive for phosphate solubilization on Pikovskaya's agar plates containing tri-calcium phosphate (TCP) with holozone diameter ranges from 10.6- 19.9 mm. From qualitative estimation test on NBRIP medium, out of 96, 38 nodule non rhizobial endophytic bacterial isolates showed positive results for P-solubilization, and were further subjected for quantitative estimation of P-solubilization in 100ml of Pikovaskaya's broth with TCP (0.1%) as inorganic substrates with initial pH 7.0. P-solubilization activity of different isolates was measured at different time intervals (3, 6, 9 12 and 15 days) as given in Table 2. The phosphate solubilizing activity was observed up to the 15th day. It was observed that P solubilization by different isolates was increased with increased in time interval up to 15^{th} days. PSB isolates showed maximum amount of P-solubilization at 12^{th} days and few at 15^{th} day and varied from 0.8 to 21.0 mg $100ml^{-1.}$ Nodule's non rhizobial endophytic bacterial isolate maximum P-solubilization was noticed with RBN17 (21.0 $mg100ml^{-1}$) followed by RBN61 (17.3 $mg100ml^{-1}$) of chickpea. Similarly, variation in P solubilization was also noticed with *Pseudomonas putida, P. fluorescens chao and P. fluorescens tabriz* released 51, 29 and 62 % P, respectively; with highest value of 0.74 mg P / 50 mL from Fe_2O_3 [5] whereas, *Pseudomonas fluorescens* solubilized 100 mg P/ L containing $Ca_3(PO_4)_2$ or 92 and 51 mg P/ L containing $AlPO_4$ and $FePO_4$, respectively in the broth medium. Selvi *et al.,* [23] also studied phosphate solubilization ability of PSM with variation from 11.85 mg to 61.96 mg P_2O_5 in liquid PVK medium in bacterial isolates recovered from rhizoplane, rhizosphere and non rhizosphere of different leguminous plants. In another study 5 PSB isolates selected from soil samples of five different district of Odisha revealed P solubilization efficiency in NBRIP broths with Ca, Al, and Fe-complexed phosphates. Isolate CTC12 and KHD08 transformed more amount of soluble P from Ca-P (CTC12 393.30 mg/L; KHD08 465.25 mg/L), Al-P (CTC12 40.00 mg/L; KHD08 34.50 mg/L), Fe(III)-P (CTC12 175.50 mg/L; KHD08 168.75 mg/L), and Fe(II)-P (CTC12 47.40 mg/L; KHD08 42.00 mg/L) after 8 days of incubation and were exploited as biofertilizer in peanut [21]. Production of phytohormone (IAA) is an important mechanism of plant growth promotion by endophytic bacteria. This hormone promotes the growth of roots. All 38 isolates (non rhizobial endophytic bacteria) positive for P- solubilization were further

screened for quantitative IAA production and showed red colour reaction with Salkowaski's reagent indicated their ability to produce IAA. Of 38 nodule endophytic bacterial isolates 21.05%, 60.52% and 18.42% were found to be low, medium and high producer of IAA in the presence of tryptophan (Fig 2) . IAA production in non rhizobial endophytic bacterial isolates ranged from 4.12- 18.24 μgml^{-1} (absence of tryptophan) and 21.6-39.44 μgml^{-1}(presence of tryptophan). In the presence o f tryptophan, the isolate LCNE 8 produced the maximum amount of IAA (39.44 μgml^{-1}) whereas in the absence of tryptophan the isolate RBN17 produced the maximum amount of IAA (18.24 μgml^{-1}) as given in Table 3. Dhole *et al.,* [3] also isolated *Chryseobacterium indologenes* AM2 non rhizobial endophytic bacterial isolate from *vigna radiata* with production 87.04 µg ml^{-1} IAA in the presence of tryptophan. Endophytic bacteria have many beneficial effects on their host plant growth by producing phytohormones similar to that of PGPR. Gibberellic acid (GA) is an important plant growth promoter associated with several plant growth and development processes, such as seed germination, stem elongation, flowering and fruit development. All the 38 endophytic bacterial isolates were able to produce GA in nutrient broth and ranged from 87.78 μgml^{-1} to 112.15 μgml^{-1} (Table 4). Of 38 isolates, from nodule 23.68%, 57.89%, 18.42% were found to be low, medium and high producer of GA, respectively (Fig 3). Similarly, Umamaheshwari *et al.,* [27] reported 25 bacterial endophytes from green gram, red gram, cowpea, clover and chickpea plants for gibberellic acid production with maximum amount in isolate GR 19 (2.83 µg ml-1) and minimum with BR13 (0.75 µg ml^{-1}) from green gram and black gram. The results are well in agreement with Lenin and Jayanthi (2012) where of 20 isolates, 5 each of *Azospirillum lipoferum* (6.45 to 7.10 $\mu g25ml^{-1}$), *Azotobacter chroococcum* (6.21 to 6.80 $\mu g25ml^{-1}$), *Pseudomonas fluorescens* (6.14 to 6.64 µg 25 ml^{-1}) and *Bacillus megaterium* (4.04 to 4.50 µg 25 ml^{-1}) were producing gibberellic acid. All 38 endophytic bacterial isolates were subjected for their ACC deaminase production on Dworkin and Foster's minimal medium. The ability of isolates to utilize ACC as a source of N was assessed on the basis of bacterial growth on plates containing substrate ACC and $(NH_4)_2SO_4$. Sixteen isolates were found to show growth but considerable variation was observed with respect to growth, when compared with control (Table 6). Based on preliminary qualitative test for screening of endophytic bacterial isolates positive for ACC deaminase, 16 isolates were further assessed on the basis of bacterial growth in liquid medium selected for their ability to utilize ACC as a sole source of N in terms of optical density (OD at 600). Higher growth of endophytic bacterial isolates was observed in DF broth supplemented with ACC (OD ranged 0.364 to 1.04) as compared to DF broth supplemented with $(NH4)_2$ SO_4 (OD ranged 0.1620 to 0.5471) (Table 6). Data indicated the ability of isolates to use ACC as N source due to the presence of ACC deaminase activity. Little or no growth in DF medium without ACC or $(NH4)_2$ SO_4 was observed due to the absence of N source. RBN16 isolate showed highest growth in DF medium with ACC (1.04) followed by RBN63 (0.8210) (Table 6). All the 16 isolates utilized ACC as N source (i.e. positive for ACC-deaminase enzyme activity) but with different degree of efficacy. Similarly, Shahzad *et al.,* [24] reported 8 rhizobacterial isolates from chickpea rhizosphere with highest growth (OD 0.75) by utilizing ACC as N source. Of the 9 isolates from clover plants, all isolates produced IAA, 6, 2, 4, 1, 6 and one isolate produced siderophore, HCN, ACC deaminase, chitinase, pectinase and cellulase respectively, whereas 4 isolates solubilized phosphorous.

Table: 1 Qualitative measurement of P-solubilization by nodule non rhizobial endophytic bacteria of chickpea on NBRIP medium

RBN2	0.9	1.3	1.44
RBN4	0.8	1.1	1.38
RBN13	0.9	1.6	1.78
RBN16	1.3	1.7	1.31
RBN17	1.2	2.7	2.25
RBN20	1.2	1.9	1.58
RBN25	1.1	1.4	1.27
RBN27	1.1	1.6	1.45
RBN28	0.8	1.5	1.88
RBN29	0.9	1.5	1.67
RBN30	0.9	1.6	1.78
RBN31	1.2	2	1.67
RBN32	1.1	1.9	1.73
RBN36	0.9	1.5	1.67
RBN38	0.8	1.3	1.63

RBN41	1	1.6	1.60
RBN44	1.1	1.9	1.73
RBN49	1	1.7	1.70
RBN54	0.9	1.6	1.78
RBN59	1	1.6	1.60
RBN61	1.1	1.9	1.73
RBN63	1.1	1.9	1.73
RBN64	1.2	2.1	1.75
RBN65	1.3	2.1	1.62
RBN69	0.9	1.6	1.78
RBN71	0.8	1.4	1.75
RBN75	1.1	2	1.82
RBN76	1.1	1.5	1.36
RBN83	1.3	2.3	1.77
RBN84	1.2	2.1	1.75
RBN86	1	1.9	1.90
RBN87	1	1.6	1.60
RBN88	1.2	2.2	1.83
RBN89	1.2	1.9	1.58
RBN91	1	1.7	1.70
RBN96	1.1	1.9	1.73
LCNE6	1.2	2.5	2.08
LCNE8	1	1.9	1.90
LGR33	1.2	1.9	1.58
RB1	0.7	1.3	1.86

Table 2: Quantitative measurement of P-solubilization by nodule non rhizobial endophytic bacteria in Pikovaskaya's broth at different intervals of time

Isolates	**P-solubilization ($mg100ml^{-1}$)**				
	Incubation period (days)				
	3rd	**6th**	**9th**	**12th**	**15th**
RBN2	2.4	4.5	4.7	9.8	12.5
RBN4	0.8	3.0	9.2	7.4	6.4
RBN13	0.8	2.3	4.8	4.9	8.0
RBN16	3.2	2.6	3.1	5.0	6.8
RBN17	3.1	13.5	18.7	21.0	5.7
RBN20	2.4	3.2	5.20	5.7	3.7
RBN25	1.3	1.8	4.1	4.2	6.4
RBN27	5.2	5.9	6.0	7.1	7.2
RBN28	1.2	2.7	9.6	14.6	8.4
RBN29	0.2	2.1	4.0	8.4	5.7
RBN30	2.2	5.0	8.8	8.9	5.0
RBN31	6.2	8.8	10.4	10.8	11.1
RBN32	4.4	5.9	6.1	7.6	5.7
RBN36	0.6	1.7	1.8	5.9	5.4
RBN38	1.9	2.7	6.1	7.4	7.1
RBN41	5.1	5.4	5.8	6.1	4.0
RBN44	4.4	5.1	5.4	5.8	4.8
RBN49	1.1	4.1	5.2	6.2	4.4
RBN54	3.5	5.4	6.2	7.1	6.8
RBN59	3.2	4.8	4.9	11.2	10.8
RBN61	2.0	6.9	10.9	17.3	7.2
RBN63	0.4	2.6	5.4	6.1	1.8
RBN64	2.4	2.3	2.6	5.0	5.8
RBN65	5.3	6.9	7.4	9.5	6.5
RBN69	5.2	5.6	9.3	10.0	3.1
RBN71	2.4	4.5	5.3	9.9	3.0
RBN75	2.8	7.6	8.2	10.6	6.9

Isolates	P-solubilization (mg100ml^{-1}) Incubation period (days)				
	3rd	6th	9th	12th	15th
RBN76	4.8	7.6	10.1	10.2	5.8
RBN83	1.6	2.0	2.6	5.9	3.4
RBN84	5.5	7.7	8.6	9.3	10.5
RBN86	2.9	6.4	12.2	13.7	3.8
RBN87	0.7	4.3	4.4	5.2	4.2
RBN88	2.9	6.1	14.4	15.5	11.1
RBN89	0.6	4.5	10.1	10.7	8.4
RBN91	4.8	5.0	7.9	9.9	6.3
RBN96	2.5	4.4	6.2	6.9	4.8
LCNE6	7.35	8.5	12.06	12.45	7.11
LCNE8	8.20	9.97	10.26	12.06	6.83
LGR33	2.6	4.1	4.9	8.7	3.4
RB1	4.5	7.32	7.31	10.19	7.75
CD @ 5%	0.35	0.39	0.15	0.39	0.45

Table 3: Quantitative measurement of IAA production by nodule non rhizobial endophytic bacteria of chickpea

Isolates	IAA (µg/ml) at 6th day	
	(-)Tryptophan	(+) Tryptophan
RBN2	4.49	24.45
RBN4	4.12	22.77
RBN13	4.23	21.60
RBN16	4.14	27.60
RBN17	18.24	39.24
RBN20	9.37	27.52
RBN25	8.84	24.92
RBN27	4.81	27.80
RBN28	14.06	33.39
RBN29	7.82	25.42
RBN30	7.32	24.80
RBN31	8.11	29.60
RBN32	7.16	29.10
RBN36	8.05	22.74
RBN38	8.36	28.07
RBN41	9.25	25.20
RBN44	6.03	29.95
RBN49	9.21	29.16
RBN54	9.40	25.46
RBN59	5.90	28.92
RBN61	11.37	36.45
RBN63	7.12	28.80
RBN64	9.06	29.57
RBN65	7.89	28.96
RBN69	7.15	29.86
RBN71	9.36	28.35
RBN75	7.54	29.22
RBN76	9.37	26.29
RBN83	8.99	26.3
RBN84	9.76	23.76
RBN86	14.04	38.12
RBN87	9.39	29.37
RBN88	13.24	38.64
RBN89	6.13	27.43
RBN91	9.07	26.17
RBN96	8.83	28.10
LCNE6	14.4	38.29
LCNE8	15.95	39.44
LGR33	15.28	32.45
RB1	14.23	35.72
CD @ 5%	0.02	0.08

Table 4:Quantitative estimation of gibberellic acid by nodule non rhizobial endophytic bacterial isolates of chickpea

Isolates	GA_3 (µg ml^{-1})
RBN2	91.91
RBN4	92.15
RBN13	92.15
RBN16	96.06
RBN17	110.60
RBN20	91.34
RBN25	91.21
RBN27	92.02
RBN28	101.44
RBN29	89.20
RBN30	90.13
RBN31	98.68
RBN32	93.82
RBN36	90.59
RBN38	92.15
RBN41	90.45
RBN44	92.15
RBN49	92.36
RBN54	98.59
RBN59	88.89
RBN61	102.15
RBN63	91.97
RBN64	93.24
RBN65	94.29
RBN69	94.75
RBN71	92.15
RBN75	94.32
RBN76	91.91
RBN83	92.64
RBN84	92.10
RBN86	109.30
RBN87	94.87
RBN88	101.94
RBN89	87.78
RBN91	94.86
RBN96	92.15
LCNE6	112.15
LCNE8	108.29
RB1	80.65
LGR33	111.91
CD @ 5%	0.41

Table 5: Qualitative ACC deaminase production by non rhizobial endophytic bacteria from chickpea nodules

Broth	ACC deaminase positive non rhizobial endophytic bacterial isolates
DF	RBN28, RBN 54 & RBN 64
DF + $(NH_4)SO_4$	RBN27, RBN28, RBN30, RBN31, RBN41, RBN44, RBN54, RBN61, RBN63, RBN64, RBN91 & LCRE8
DF+ACC	RBN2, RBN4, RBN13, RBN16, RBN17, RBN20, RBN28, RBN30, RBN31, RBN44, RBN54, RBN61, RBN71, RBN88, RBN91 & LCRE8

Table 6: Quantative ACC deaminase production by non rhizobial endophytic bacteria from nodules of chickpea in DF minimal medium

Isolates	DF+ACC	DF+$(NH_4)SO_4$
RBN2	0.5678	0.3579
RBN4	0.7259	0.2386
RBN13	0.6737	0.4211
RBN16	1.04	0.3740
RBN17	0.8795	0.5471
RBN20	0.4839	0.3321
RBN30	0.3664	0.2096
RBN31	0.7472	0.3620
RBN41	0.5496	0.2281
RBN44	0.7912	0.4300
RBN61	0.5202	0.1620
RBN63	0.8210	0.3444
RBN71	0.8091	0.2590
RBN88	0.9603	0.3277
RBN91	0.5442	0.5210
LCRE8	0.7821	0.2843
RB1	0.9225	0.5932

Fig 1: Phosphate solubilization Index (% PSI) of nodule non rhizobial endophytic bacteria of chickpea

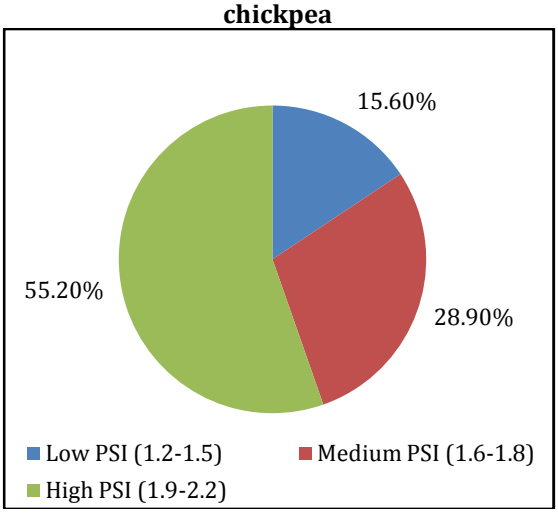


Fig 2: Indole acetic acid production (+ tryptophan) in nodule non rhizobial endophytic bacteria in Luria broth.

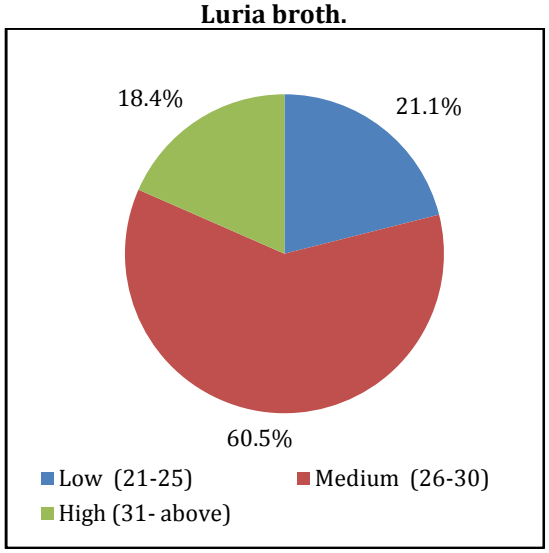
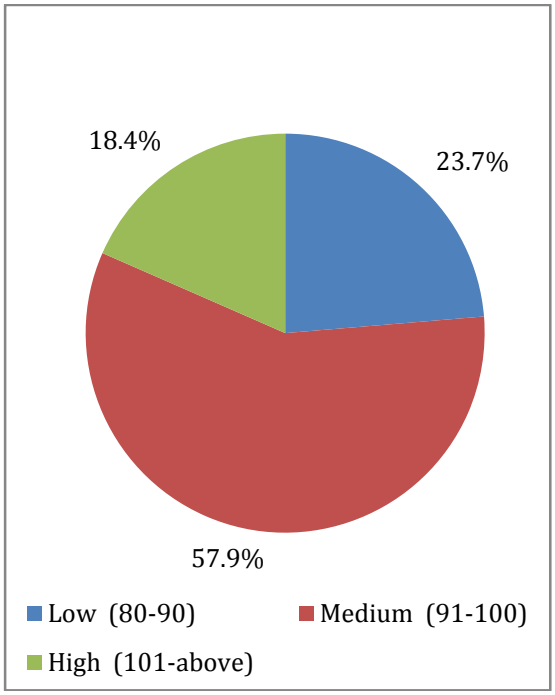


Fig 3: Gibberellic acid production in nodule non rhizobial endophytic bacteria of chickpea in nutrient broth



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