Journal of Applied and Natural Science
11(2): 346- 351 (2019)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Non rhizobial endophytic bacteria from chickpea (*Cicer arietinum* L.) tissues and their antagonistic traits

Deepika Chhabra*
Department of Microbiology, Punjab Agricultural University, Ludhiana-114004 (Punjab), India
Poonam Sharma
Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana-114004 (Punjab), India

*Corresponding author. E-mail: chhabra.deepika@gmail.com

Abstract
Bacteria that colonize plant tissues other than rhizobia and are beneficial for plant growth referred to non rhizobial plant growth-promoting endophytic bacteria (PGPEB). This study was designed to assay the biocontrol activity of plant growth promoting endophytic bacterial isolates those found positive for P. solubilization, ACC deaminase, Indole acetic acid and Gibberelic acid production. These bacterial isolates were obtained from chickpea (*Cicer arietinum* L.) tissues (roots and nodules). In a previous study a total of 263 non rhizobial endophytic bacterial isolates were isolated. Out of 263 isolates, 64.5% and 34.5% were Gram positive and negative, respectively. Further for biochemical characterization, catalase, oxidase, citrate utilization, nitrate reduction, methyl red and Voges Proskauer's tests, were performed. On the basis of P solubilization, ACC deaminase, Indole acetic acid and Gibberelic acid production 75 potential isolates were selected and screened for their biocontrol activity *viz*. (production of cell wall degrading enzymes, production of HCN and fluorescent pigment). Out of 75 isolates, only 29 isolates produced cellulase, 64 isolates were able to produce protease and 28 were positive for both cellulose and protease. Of 75 endophytic isolates 12 isolates (7 from root tissue and 5 from nodules tissue, respectively) were positive for HCN production and 16 isolates were found to be fluorescent pigment producer under µv ligh. As chemical fertilizers and pesticides have detrimental effects on the environment. So these bacterial endophytic isolates will be used not only as a biofertilizer because of their plant growth promotional activities but also used as an alternative of synthetic chemicals for control of several plant diseases.

Keywords: Biocontrol, Cellulase, *Cicer arietinum* L., Non rhizobial endophytic bacteria, Protease

Article Info
DOI:10.31018/jans.v11i2.2056
Received: April 5, 2019
Revised: May 4, 2019
Accepted: May 9, 2019

How to Cite
Chhabra, D. and Sharma. P. (2019). Non rhizobial endophytic bacteria from chickpea (*Cicer arietinum* L.) tissues and their antagonistic traits. *Journal of Applied and Natural Science*, 11(2): 346- 351 https://doi.org/10.31018/jans.v11i2.2056

INTRODUCTION

Bacterial endophytes colonize the interior tissue of plant exhibiting no apparent sign of infection or harmful impact (Kusari *et al* 2014). Approxmately 3,00,000 existing plant species are in relation with endophytic inhabitants vary from a small to massive numbers (Dudeja and Giri 2014). Many promising endophytic bacteria like *Azoarcus sp., Glucanoacetobacter diazotrophicus*, *Burkholderia sp., Herbaspirillium sp., Enterobacter* and genus *Serratia* are reported to reinforce yield in various agricultural crops (Vessey 2003). Plant growth promoting endophytic bacteria (PGPEB) are known to effect plant growth by decreasing plant disease and might be useful for sustainable agriculture as an alternative of chemical pesticides for improving the quality and yield of crop (Lugtenberg and Kamilova, 2009). Biological control has been described as eco friendly approach to reduce crop damage due to plant pathogens as compared to the use of chemical control of plant diseases (Wang *et al* 2013).

Endophytic microorganism can enhance plant establishment under stressful conditions in legume and non legume plants and preventing disease via antifungal and outcompeting pathogens for nutrients with siderophore production and better plant general resistance. Some bacterial endophytes show biocontrol activity (antibacterial and antifungal) by producing cell wall degrading enzymes or allelochemicals (antibiotics). Endophytic bacteria can get entry into the root tissues by two ways: actively, by production of hydrolytic enzymes (e.g. endoglucanase, exoglucanase and endopolygalacturonase) and these enzymes in-

volved in cell wall degradation of plant (Compant *et al* 2005) and passively, by penetrating the lateral roots emergence sites or junction of adjacent epidermal cells (Govindasamy *et al* 2008). Production level of these cell wall degrading enzymes differentiated between phytopathogens (deleteriously high levels) and root-colonizing bacterial endophytes (low levels) (Elbeltagy *et al* 2001) and contributed in endophytic bacteria entry into host and their spread inside plant tissue. Endophytic bacteria have the ability to protect their plant host from harmful microorganisms and pests by competition for space and nutrients and antagonism or by initiating the defence mechanisms of plant to respond immediately and efficiently against the pathogens. Antagonism against plant pathogens can be achieved directly by the production of fungal growth inhibitors, antibiotics and antibacterial secondary metabolites.

Most commonly strains of actinobacteria and bacteria viz. *Pseudomonas*, *Bacillus* and *Paenibacillus* spp. are reported as antagonistic for fungal pathogens and have been assayed for control of disease in a wide range of plants, e.g. wheat, potato and black pepper (Aravind *et al* 2009). The *Pseudomonas* spp. (fluorescent) form a long time for their biocontrol activity on plant soil borne pathogens for suppression of diseases. Bacteria with multiple biocontrol mechanisms antibiotics, chitinolytic enzymes, siderophores, HCN are being used widely (Saharan and Nehra 2011).

Associative, endophytic diazotrophic and non rhizobial endophytic bacteria were characterized from different plant species in last couple of years have raised their prospects to be used as biofertilizer (Akhtar and Siddiqui 2009). In recent decades, interest in endophytic microorganisns has been increased, as they have important role in sustainable agriculture. Knowing and understanding the negative impact of artificial fertilizers in agriculture, novel approaches such as the application of endophytic bacteria as biopesticides which are associated with plants, may help to improve plant health and increase productivity.

This study was designed *in vitro* screening of non rhizobial endophytic bacterial isolates for their biocontrol traits. These isolates were previously found positive for their plant growth promotional traits.

MATERIALS AND METHODS

Isolation of endophytic bacteria from chickpea: Healthy plants of chickpea (*Cicer arietinum* L.) were carefully taken out then washed with running tap water to wash off soil from undamaged tissue samples of root and nodules. Soaked in distilled water in a separate beaker and drained. Sample sterilization was done by using $HgCl_3$ (0.1%) for 30 seconds and ethanol (70%) for 3 min for. After sterilization the tissue sample was washed thrice with sterilized water.

Aseptically surface sterilized tissues were homogenized and macerated. After maceration serial dilution of the tissue up to 10^{-6} was prepared and appropriate dilutions (100µl) were used and plates were incubated at 37°C. Further, the isolation of endophytic bacteria was done by streak plate method on Nutrient, Jensen's and *Pseudomonas* agar media. Sterility test was performed by placing the washed tissue on same medium and incubation was done at 28±2°C for 2 -3 days. Carefully bacterial colonies were isolated and streaked over the plate containing their specific medium *viz*. Nutrient agar for *Bacillus* sp., *Klebsiella* sp., Jensen's agar for *Azotobacter* and *Pseudomonas* agar for *Pseudomonas* sp. Further, these isolates were maintained at 4°C on specific medium slants for future use.

Production of HCN: Nutrient agar medium supplemented with glycine (4.4g/litre) was streaked with exponentially grown selected endophytic bacterial isolates with simultaneously keep a filter paper soaked in picric acid (0.5%) in Na_2CO_3 (5%) in the upper lid of Petri dish. Incubation was done at 28±2°C for 2 to 3 days. Change in filter paper colour from yellow to light brown or strong (reddish-brown) represented as positive test for HCN production (Bakker and Schippers 1987).

Cell wall degrading enzyme production

Protease: Skimmed milk agar plates were prepared and spot inoculated with pure culture of test bacteria and incubated at 28±2°C for 2-5 days. Presence of halo clear zone around the growth indicated as positive test for protease production (Chaiharn *et al* 2008).

Cellulase: Cellulase activity of pure cultures was assayed by plating on Carboxy Methyl Cellulose (CMC) agar according to Ariffin *et al* (2006). Spot inoculation with test organism was done and plates were incubated at 28±2ºC for 5 days. Appearance of halo zone around the bacterial growth was considered as positive test for cellulase production. Five days of incubation was done to allow the activity of cellulase on CMC agar plates at 28±2ºC. After incubation, the agar plates were flooded with an aqueous solution of Congo red (1% w/v) for 15 minutes then solution was poured off, and further plates were flooding with 1M NaCl for 15 minutes. Appearance of a clear zone of hydrolysis confirmed cellulose degradation. Isolates with high cellulase activity was selected on the basis of clear zone diameter.

Production of Fluorecent pigment: Non rhizobial endophytic bacterial isolates were spot inoculated on King's B medium and plates were incubated at 28°C for 2 days. Observation of plates was done for yellowish green colour under µv light. Fluorescence ability considered as positive for fluorescent pigment production at 400 nm.

Table 1. Biochemical characterization of non rhizobial endophytic bacteria of chickpea.

Characteristics		Endophytic bacterial isolates (%)	
Biochemical test			
		Positive	**Negative**
Gram's staining		64.5	34.5
Oxidase		87.4	12.6
Catalase		74.4	25.6
Citrate utilization		12.6	87.4
Methyl red (MR)		37.4	62.6
VogesProskauer (VP)		55.7	44.3
Nitrate reduction (NR)		36.6	63.4
Carbohydrate utilization Test	**Different sources of sugar**	**Acid producers**	**Gas producers**
	Dextrose	38.6	6.7
	Fructose	29.6	5.2
	Sucrose	48	13
	Sorbitol	18.7	2.7

RESULTS AND DISCUSSION

Morphological characteristics of non-rhizobial endophytic bacteria and their biochemical characterization: On the basis of morphological studies, out of 263 endophytic bacterial isolates 124 on nutrient agar medium produced large sized, irregular shaped, off-white and rough colonies, whereas 6 isolates showed rhizoid growth and were tentatively identified as *Bacillus* sp. Further, 71 isolates on *Pesudomonas* agar medium produced medium sized, round shaped and raised colonies with smooth margin and light yellow to off white in colour and were provisionally identified *Pseudomonas*. Further, few isolates also produced a fluorescent green pigment on King's B medium. 53 isolates were streaked on nutrient agar medium produced medium, round shaped and raised colonies having entire margin, mucoid and cream in colour were assigned as a *Klebsiella* sp., *Enterobacter* sp. and *Enterococcus* sp. Nine isolates produced yellow colour pigment on nutrient agar with circular, pinhead colonies and were convex with entire margins. On the basis of Gram's reaction, out of 263 non rhizobial endophytic bacterial isolates, 64.5% and 34.5% were Gram positive and negative, respectively. On the basis of biochemical characterization, out of 263 non rhizobial endophytic bacterial isolates 74.4%, 87.4%, 12.6%,36.6%, 37.4% and 55.7% were found to be positive for catalase, oxidase, citrate utilization, nitrate reduction, methyl red and Voges Proskauer's tests, respectively (Table 1). In an another *in vitro* study, further these isolates were screened qualitatively and quantitatively for their plant growth promotional traits *viz.* P solubilisation, IAA and ACC deaminase production. On the basis of plant growth promotion trait only 75 potential isolates were selected for *in vitro* assay of biocontrol traits. Our results were in accordance with Saini *et al* (2015) who isolated 166 endophytic bacteria from root of legumes, chickpea (*Cicer arietinum*), pea (*Pisum sativum*), and lucerne (*Medicago sativa*) and non-legumes wheat (*Triticum aestivum*) and oat (*Avena sativa*) and from nodules of chickpea. Similarly, Zaghloul *et al* (2016) total of 167 endophytic bacterial isolates were isolated from roots, nodules, leaves and stems of faba bean (*Vicia faba*), pea (*Pisum sativum*), fenugreek (*Trigonella foenum-gracum*), lupine (*Lupinus spp*.), common bean (*Phaseolus vulgaris*) and rice (*Oryza sativa*) at flowering stage.

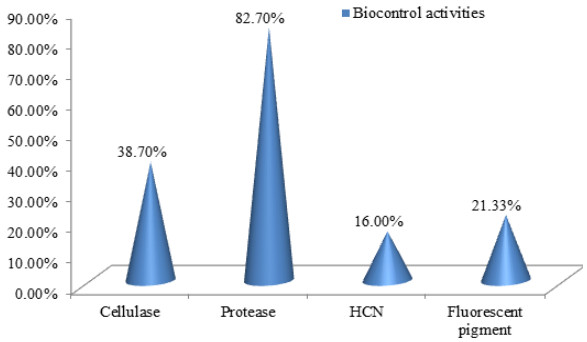

Fig. 1. *Cellulase, protease, HCN and fluorescent pigment production by potential non rhizobial endophytic bacteria of chickpea.*

Biocontrol activity: Out of 75 non rhizobial endophytic bacterial isolates 38.70 %, 82.70%, 16.0% and 21.33% were cellulose, protease, HCN and fluorescent pigment producers, respectively (Fig 1).

Production of cell wall degrading enzymes: All the 75 non rhizobial endophytic bacterial isolates screened for cellulase and protease production (Table 2). Out of 75 isolates, only 29 (38.7%) were cellulose producers and 62 (82.7%) were protease producers. Maximum diameter of zone around bacterial colonies was observed for RBR 34, RBR40 and RBR139 (2.2 cm) on CMC and RBR 155 (2.8) on Skim milk agar media. Our results are well supported by Geetha *et al* (2014) who had also reported from mungbean rhizosphere out of 6 potential bacterial isolates, only 4 (WG-57, TG-60, BG-72and KG-50) were able to produce cellulase whereas 3 showed protease activity. Similarly, Etesami *et al* (2015) procured

Table 2. Cell wall degrading enzyme production by non rhizobial endophytic bacteria of chickpea.

Isolates	Cellulase Zone Dia (cm)	Protease Zone Dia (cm)
RBR11	-	2.2
RBR14	0.6	2.2
RBR17	2.1	2.4
RBR 19	1.1	2.1
RBR20	2.1	2.3
RBR25	-	1.8
RBR34	2.2	2.5
RBR38	-	2.6
RBR40	2.2	2.3
RBR49	0.7	0.9
RBR57	-	2.6
RBR61	-	2.5
RBR75	-	1.4
RBR80	-	2.2
RBR83	-	1.5
RBR89	-	2.5
RBR116	1.4	2.2
RBR119	1.1	2.3
RBR121	-	1.8
RBR127	-	2.2
RBR128	-	2.5
RBR136	1.6	1.6
RBR139	2.2	2.6
RBR144	-	1.9
RBR146	1.4	1.4
RBR155	1.8	2.8
RBR164	1.4	2
RBR165	-	2.5
RBR167	1.3	1.9
RBN2	-	2.6
RBN4	1.4	2
RBN16	-	2.1
RBN17	1.1	1.8
RBN20	-	2.1
RBN25	-	1.2
RBN27	1.3	1.7
RBN28	2.1	-
RBN30	-	2.3
RBN31	1.4	1.9
RBN32	-	2.4
RBN36	-	2.1
RBN38	-	2.3
RBN41	1.6	2.4
RBN44	-	1.7
RBN49	-	1.2
RBN54	-	2.5
RBN59	-	1.5
RBN61	1.1	1.4
RBN63	-	2.3
RBN64	-	2.3
RBN71	-	2.3
RBN75	1	2.1
RBN83	1.6	2.3
RBN86	1.3	1.6
RBN87	1.7	1.5
RBN88	-	2.2
RBN89	0.6	2.1
RBN91	1.4	2
RBN96	-	2.5
LCNE6	-	2.3
LCNE8	1.8	2
LCRE8	-	2
LCRE9	-	2.5
LGR 33	-	2
RB1	-	1.5

(-) No zone

Table 3. HCN and fluorescent pigment production by non rhizobial endophytic bacteria of chickpea.

PGP traits	Isolates
HCN production	RBR89, RBR112, RBR119, RBR127, RBR128, RBR136, RBR139, RBN20, RBN25, RBN30, RBN31, RBN54
Fluorescent pigment production	RBR11, RBR14, RBR17, RBR 19, RBR34, RBR40, RBR75, RBR116, RBN17, RBN20, RBN25, RBN28, RBN54, RBN59, LCNE6, LCRE9

34 isolates of bacteria from two varieties of rice and all isolates were able to produce IAA, 27 isolates were positive for siderophore, 7 were producing HCN, 16 for ACC deaminase, 4 were chitinase, 22 for pectinase and 3 isolates were cellulose producers. Out of 9 isolates from clover plants all produced IAA whereas production of siderophore (6), HCN (2), ACC deanminase (4), chitinase (1), pectinase (6) and cellulose (1) and only 4 were phosphate solubilizers. Egamberdieva *et al* (2016) obtained (40) isolates of bacteria from chickpea root tissues. Isolates EB10 and EB2 were able to produced cellulose, lipase, protease, and chitinase.

HCN production: Data in Table 3 revealed bacterial endophytic isolates (7 and5 from root and nodules, respectively) were positive for HCN production in chickpea. The present findings are well supported by Thirumal *et al* (2017), where out of 15 *Pseudomonas* isolates five *viz*., PVP1, DBP, DMuP, PGuP and RGP detected as moderate (++) whereas ten bacterial cultures (*viz*., DGP1, PSmP, PKP, PRP2, PSP2, DMP2, RGP2, MP1, MP2, SFP1) were scored as weak (+) for HCN production. Similarly, Ahmad *et al* (2008) reported of 72 bacterial isolates 42.85% *Bacillus* and 62.5% *Pseudomonas* spp. were able to produce HCN. Our results are well supported by Geetha *et al* (2014) where out of 6 potential bacterial isolates, only 2 were able to produce HCN. Sharma and Dubey (2017) observed that *Pseudomonas putida* CRN-09 isolated from the rhizosphere of *Vigna radiate* producing hydrogen cyanide (HCN) and also solubilizing phosphate.

Fluorescent pigment production: All the 75 endophytic bacterial isolates grown on King's B medium. Only 16 isolates were putative fluorescent pigment producer under µv light (Table 3). Present investigation is well supported with Boiu-sicuia *et al* (2017) where 20 endophytic bacteria from potato tubers only 6T2 isolate was positive for fluorescent pigment production on King's B agar medium. Similarly, Damodaran *et al* (2013) reported, of 16 rhizobacteria from saline sodic soil only G8, P1 and P2 were positive for fluorescent pigment production.

Conclusion

Two potential isolates RBN20 and RBN25 were found positive for protease, HCN, Fluorescence pigment production. Further, study will be planned to study the biocontrol activity of RBN20 and RBN25 *in vitro* and *in vivo*. These finding will be helpful to design the bacterial biofertilizer with both plant growth promotion and biocontrol acticity and be used as an alternative of chemical based fertilizer and pesticides.

ACKNOWLEDGMENTS

The authors are thankful to Punjab Agricultural University, Ludhiana, India for providing necessary facilities to conduct the present study.

REFERENCES

1. Ahmad, F., Ahmad, I., and Khan, M.S. (2008). Screening of free living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol., Res.* 163: 173-181. DOI:10.1016/ j.micres.2006.04.001
2. Akhtar, M., and Siddiqui Z. (2009). Effects of phosphate solubilizing microorganisms and Rhizobium sp. on the growth, nodulation, yield and root-rot disease complex of chickpea under field condition. *Afr. J. of Biotechnol.,* 8(15): 3489-3496. http:// www.academicjournals.org/AJB
3. Aravind, R., Kumar, A., Eapen, S., and Ramana, K. (2009). Endophytic bacterial flora in root and stem tissues of black pepper (Piper nigrum L.) genotype: isolation, identification and evaluation against Phytophthora capsici. *Lett. in Appl. Microbiol.,* 48: 58-64. DOI: 10.1111/j.1472-765X.2008.02486.x
4. Ariffin, H., Abdullah, N. K., Umi, Y., Shirai, Y., and Hassan, M. A. (2006) Production and characterization by *Bacillus pumilus* EB3. Inter. *J. of Engine. Sci. and Technol.,* 3: 47-53. https:// pdfs.semanticscholar.org/2ddf/2067a5aba34de9ded53aff23439854e0040d.pdf
5. Bakker, A.W., and Schippers, B. (1987). Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas spp mediated plant growth stimulation. *Soil Biol. and Biochem.,* 19: 451-457. https://doi.org/10.1016/0038-0717(87)90037-X
6. Boiu-sicuia, O. A., Constantinescu, F., and Cornea, C. P. (2017). Selection and characterization of new endophytic bacterial strains isolated from potato tuber useful in biocontrol strategies. *Sci. Bulletin,* 21: 23-28. http://biotechnologyjournal.usamv.ro/pdf/2017/Art3.pdf
7. Chaiharn, M., Chunhaleuchanon, S., Kozo, A., and Lumyong, S. (2008). Screening of rhizobacteria for their plant growth promoting activities. *KMITL Sci. and Technol.*, J. 8: 18-23. http:// www.thaiscience.info/journals/Article/ KLST/10424521.pdf
8. Compant, S., Duffy, B., Nowak, J., Clément, C., and Barka, E. A. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. *Appl. and Environ. Microbiol.,* 71: 4951-4959. DOI: 10.1128/AEM.71.9.4951-4959.2005
9. Damodaran, T., Sah, V., Rai, R. B., Sharma, D. K., Mishra, V. K., Jha, S. K., and Kannan, R. (2013). Isolation of salt tolerant endophytic and rhizospheric bacteria by natural selection and screening for promising plant growth-promoting rhizobacteria (PGPR) and growth vigour in tomato under sodic environment. *Afr. J. of Microbiol. Res.*, 7(44) : 5082-5089. DOI: 10.5897/AJMR2013.6003
10. Dudeja, S. S., and Giri, R. (2014). Beneficial properties, colonization, establishment and molecular diversity of endophytic bacteria in legumes and non legumes. Afr. J. of Microbiol. Res. 8(15): 1562-1572. DOI: 10.5897/AJMR2013.654
11. Egamberdieva, D., Li, L., Lindstrom, K., and Rasanen, L. A. (2016). A synergistic interaction between salt-tolerant Pseudomonas and Mesorhizobium strains improves growth and symbiotic performance of liquorice (Glycyrrhiza uralensis Fish.) under salt stress. *Appl. Microbiol. and Biotechnol.*, 100(6): 2829-2841.
12. Elbeltagy, A., Nishioka, K., Sato, T., Suzuki, H., Ye, B., Hamada, T., Isawa, T., Mitsui, H., and Minamisawa, K. (2001). Endophytic colonization and in plant nitrogen fixation by a Herbaspirillum sp isolated from wild rice species. *Appl. Environ. Microbiol.,* 67: 5285-5293. doi: 10.1099/ijs.0.005967-0
13. Etesami H, Alikhani H S and Hosseini H M (2015). Indole-3-acetic acid (IAA) production trait, a useful screening to select endophytic and rhizosphere competent bacteria for rice growth promoting agents. *MethodsX,* 2: 72-78. doi: 10.1016/j.mex.2015.02.008
14. Geetha, K., Venkatesham, E., Hindumathi, A., and Bhadraiah, B. (2014). Isolation, screening and characterization of plant growth promoting bacteria and their effect on Vigna radita (L.) R. Wilczek. *Int. J of Curr Microbiol and Appl Sci.*, 3: 799-809. www.ijcmas.com/vol-3-6/K.Geetha,%20et%20al.pdf
15. Govindasamy, V., kumar, M., Gaikwad, K., and Annapurna, K. (2008). Isolation and characterization of ACC deaminase gene from two plant growth -promoting rhizobacteria. *Curr. Microbiol.,* 57(4): 312-317. DOI https://doi.org/10.1007/978-981-10-8402-7_3
16. Gupta, G., Parihar, S. S., Ahirwar, N. K., Snehi, S. K., and Singh, V. (2015). Plant growth promoting rhizobacteria (PGPR): current and prospects for development of sustainable agriculture. *J. of Microb. and Biochem. Technol.,* 7: 96-102. http:// dx.doi.org/10.4172/1948-5948.1000188
17. Kusari, S., Singh, S., and Jayabaskaran, C., (2014). Biotechnological potential of plant associated endophytic fungi: hope versus hype. *Trends in Biotechnol.,* 32(6): 304-31. http://dx.doi.org/10.1016/ j.tibtech.2014.03.009
18. Lugtenberg, B., and Kamilova, F., (2009). Plant growth promoting rhizobacteria. *Annual Review Microbiology,* 63, 541-556. https://doi.org/10.1146/ annurev.micro.62.081307.162918
19. Saharan, B., and Nehra, V., (2011). Plant growth promoting rhizobacteria: a critical review. *Life Sci. Medical Res.*, 21: 1-30. www.researchgate.net/ publication/284340739_Plant_growth_promoting_rhizobacteria_a_critical_review/citation/download
20. Saini, R., Dudeja, S. S., Giri, R., and Kumar, V., (2015). Isolation, characterization and evaluation of bacterial root and nodule endophytes from chickpea

cultivated in Northern India. *J. of Basic Microbiol.,* 55: 74-81. https://doi.org/10.1002/jobm.201300173

21. Sharma, C., K., and Dubey, R., C., (2017). Plant growth promoting and antagonistic properties of Pseudomonas putida CRN-09 against Macrophomina phaseolina. *Ind. J. of Sci. and Res.*, 13(2): 01-05. http://www.ijsr.in/upload/2064303755chapter%201%20c.pdf
22. Thirumal, G., Reddy, R. S., Triveni, S., Nagaraju, Y., and Prasannakumar, B., (2017). Screening of native rhizobia and pseudomonas strains for plant growth promoting activities. *Int. J. of Current Microbiol. and Appl. Sci.*, 6(7) : 616-662 . DOI: http://dx.doi.org/10.20546/ijcmas.2016.501.075
23. Vessey, J. K., (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil,* 255: 571-586. http://dx.doi.org/10.1023/A:1026037216893
24. Wang, S., Wang, W., Jin, Z., Du, B., Ding, Y., Ni, T., and Jiao, F., (2013). Screening and diversity of plant growth promoting endophytic bacteria from peanut. *Afr. J. of Microbiol. Res.,* 7: 875-884. Zaghloul, R. A., Abou-Aly, H., E., Tewfike, T., A., and Ashry, N., M., (2016). Isolation and characterization of endophytic bacteria isolated from legumes and non-legumes plants in Egypt. *J. of Pure and Appl. Microbiol.,* 10 (1) : 277-290.