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Screening of Phenotypic and Functional Traits of Soybean Rhizobia

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Nine authenticated strains of rhizobia were collected from different All India co-ordinated research centres (AICR) of soybean in India. *Rhizobium* strains (IND1, IND2, LSBR3, LSER7, LSER8, PANT1, PANT 2) along with reference strains (DS-1, SB-271) were characterized on the basis of morphological, phenotypical and physiological characteristics. Phenotypic characteristics studies included colony morphology, resistance to antibiotics, phosphate solubilising activity, Bromothymol blue (BTB) agar activity and hydrogenase activity. It was observed that the colonies were circular, light pink, convex, rod shaped, aerobic, motile and non spore former. Out of nine strains, 5 strains were identified as slow grower *Bradyrhizobium* strains and 4 strains as fast grower *Ensifer* strains on Yeast extract mannitol agar YEMA (BTB) medium. Significantly high IAA was recorded with LSER8 (25.75μg ml^{-1}) in the presence of L-tryptophan (0.01%). In qualitative screening of hydrogenase activity 2 *Bradyrhizobium* and 3 *Ensifer* strains showed red coloration on YEMA media amended with 0.01% TTC dye. In qualitative screening of phosphate solubilization 80% of isolates produced halo zone on Pikovaskaya's and NBRIP media. These rhizobial strains can be explored as future biofertilizer to promote growth and yield in soybean.

Key words: Biofertilizer, Phenotypic characteristics, *Rhizobium,* Soybean**.**

Rhizobia are gram negative soil bacteria capable of inducing formation of nodules on leguminous plants in which atmospheric nitrogen is reduced to ammonia. This mutualistic symbiotic relationship between rhizobia and legumes is the most important biological mechanism for providing nitrogen to the plants as an alternative to the energy expensive ammonium fertilizers. Inoculation of leguminous seeds with the selected rhizobial strains is being widely practiced to ameliorate the plant yield by enhanced root nodulation and nitrogen uptake of the plant. Due to their paramount environmental and agricultural significance, these legume symbionts are being extensively characterized[1]. Most of these bacterial species are in the Rhizobiacae family as alpha-proteobacteria and classified either in *Rhizobium*, *Mesorhizobium*, *Ensifer*, or *Bradyrhizobium* genera[2]. Legume inoculation with *Rhizobium* is an old aged practice that has been carried out for more than a century in agricultural systems[3]. Soybean is the world foremost provider of protein and oil. It is often called the miracle crop as it contains high protein content (38-45%) as well as high oil content (20%)[4] The N_2 fixation potential of soybean varied from 0-185 kg ha^{-1} atmospheric nitrogen annually with the help of root nodule bacteria[5]. The high N requirement of the crop is mainly fulfilled by

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establishing N_2-fixation symbiosis with rhizobia. Nitrogen fixation by these bacteria can take place only when they grow in association with the host plant; they fail in fixing nitrogen when living free of the host. Each kind of leguminous crops requires specific kinds of *Rhizobium* to cause nodulation. It has been proven that plant productivity increases when the rhizobia are present in rhizosphere. It provides the major biological source of fixed nitrogen in agricultural soils[7].

A well established practice for maintaining soil fertility has been the cultivation of leguminous plants which replenish atmosphere nitrogen through symbiosis with rhizobia in rotation with non leguminous plants. Thus objective of this study was to characterize the potential strains of *Bradyrhizobium/ Ensifer* by several approaches, including the evaluation of phenotypic and physiological properties.

MATERIALS AND METHODS

Procurement of cultures

Nine authenticed strains of *Rhizobium* were procured from Indian Agricultural Research Institute (IARI), New Delhi and Department of Microbiology, Punjab Agricultural University, Ludhiana, respectively (Table 1).

Purification of *Bradyrhizobium* and *Ensifer* strains of soybean

Growth on Congo red Medium

Rhizobium colonies appeared white, translucent, gummy, glistening, elevated and comparatively small with entire margins were selected in contrast to stained colonies of *Agrobacterium* on congo red medium which were red in color.

Gram staining

Gram staining was done to ensure purity and freedom from gram positive bacteria. Gram staining reaction was carried out by using a loopful of pure culture grown on YEM (yeast extract mannitol) broth and stained as per the standard Gram's procedure[8].

Ketolactose test

The principle of this test is based on the ability of *Agrobacterium*, a common contaminant of *Rhizobium* to produce ketolactase enzyme which converts lactose to ketolactose. *Rhizobium* cultures were streaked on lactose medium in the centre. After incubation for 4 days at 28±2°C, 5 ml of Benedict's reagent was poured in each Petriplate to detect ketolactose and kept at room temperature for 1-1½ hours. *Agrobacterium* growth was surrounded by yellowish zone of Cu_2O whereas no such yellow zone was observed around the growth of rhizobia[9].

Morphological studies

The thick bacterial smear of all the isolates was gram stained and morphological characterization was done on the basis of colony morphology including shape, color and surface margin.

Differentiation between *Bradyrhizobium* and *Ensifer* strains of soybean

Bromothymol Blue (BTB) agar medium was used for differentiating *Bradyrhizobium* from *Ensifer* strains. The cultures were streaked on BTB agar plates. BTB agar was made by adding 5 ml of (0.5% BTB in ethanol) to 1 litre of YEMA medium. The plates were incubated at 28±2ºC for 2-10 days. The change in color of medium was observed. The isolates were classified as slow growers (medium turns blue) or fast growers (medium turns yellow) on their reaction on YEMA supplemented with BTB[8]. Purified colonies were then transferred to YEMA medium slants. These slants were incubated at 28±2°C for 2-10 days and stored in refrigerator at 4°C for further studies.

Screening for functionality traits of *Bradyrhizobium* and *Ensifer* strains of soybean

Indole acetic acid production

IAA production was detected according to Gordon and Weber[10] and Patten and Glick[11]by inoculating the rhizobial cultures in YEMB medium supplemented with 0.01% tryptophan separately and incubated for 3 days at 28±2ºC. Exponential phase culture was centrifuged at 10, 000 rpm for 20 min at 4ºC to collect the supernatant. Two drops of orthophosphoric acid was added to 2 ml of supernatant. Appearance of pink color confirmed the production of IAA. The amount of IAA (µg/ml) was determined quantitatively by adding 4 ml of Salkowski's reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) to 2 ml of culture supernatant. Absorbance was measured at 535 nm after 20 min. Uninoculated broth with Salkowski's reagent was utilized as reference. The values were ascertained with the help of standard curve.

Screening of phosphate solubilizer activity of *Bradyrhizobium and Ensifer*

Petri plates containing Pikovaskaya's[12] and NBRIP (National Botanical Research Institute's Phosphate)[13] media were inoculated with different *Bradyrhizobium and Ensifer* strains. Petriplates were incubated at 28±2ºC for 4-5 days. Formation of halo zone and yellow zone around the bacterial growth on Pikovaskaya's and NBRIP media respectively indicated the qualitative phosphate solubilization activity of the organism.

Microbial solubilization of insoluble phosphates in liquid medium

One hundred ml of Pikovaskaya's broth was dispensed in 250 ml conical flasks. One hundred mg P_2O_5 as tricalcium phosphate (TCP) was added separately to each flask and the contents were sterilized at 15 lb for 15 minutes. The flasks were inoculated with 1 ml suspension of overnight grown culture. The inoculated flasks were incubated at 28±2ºC.

P- estimation

To 1 ml of culture supernatant, 2 ml of 2 N HNO_3 and 5 ml distilled water was added. One ml of AM: AV reagent was added to each tube[14]. The yellow color intensity of the solution was measured at 420 nm after 25minutes. The values were ascertained with the help of standard curve made of 5 ppm KH_2PO_4.

Screening of *Bradyrhizobium/Ensifer* strains for Hydrogenase uptake system

For evaluation of hydrogenase uptake system bacterial culture was grown in defined Hup medium of Maier *et al*[15] and YEMA medium for the expression of hydrogenase in free living rhizobia with Triphenyl Tetrazolium Chloride (TTC) dye (0.01% W/V). The plates were incubated at 28±2 ºC for 2 days. Production of TTC dye was examined up to 10 days. *Rhizobium* cultures possessing Hup^+ system showed red coloration; whereas Hup^- strains were unable to reduce TTC showed no red coloration and remained as colorless.

RESULTS AND DISCUSSION

Nine authentic strains were procured from different All India co-ordinated research (AICR) centres of soybean in India. Purified strains of soybean rhizobia were gram negative motile rods, creamy white translucent slime colonies with regular/entire margin which is parallel to the results[16]. Similarly Somasegaran and Hoben[17]suggested that typical rhizobial colonies should show little or no congo red absorption in dark. The rhizobial isolates in the current study were further tested on YEMA plates containing BTB indicated that 4 fast growing isolates were found to produce yellow colonies due to acid production on the medium with high amount of mucus after 2 days of incubation. Whereas, remaining 5 isolates along with reference strains DS 1 and SB 271 produced blue color colonies, which indicated the presence of alkali producers, considered as slow growing rhizobia. YMA-BTB medium used for categorizing indigenous soybean root nodulating as fast and slow growing rhizobia (Table 2) based on acid/alkali production[18]. This was also reported[19] in Vietnam and[20,16] in India , On BTB agar plates categorized fast and slow growing rhizobia. Authenticated isolates were classified as

Table 1. Procurement of cultures from different research centres

Reference Cultures	Procurement
IND 1& IND 2 (*Bradyrhizobium* sp. & *Ensifer* sp.)	Directorate of Soybean Research, Indore
LSBR3,LSER7&LSER8 (*Bradyrhizobium* sp., *Ensifer* sp. & *Ensifer* sp.)	Pulses Microbiology Laboratory Punjab Agricultural University (PAU), Ludhiana
PANT1 & PANT2 (*Bradyrhizobium* sp. *& Ensifer* sp.)	Department of soil Science, G B Pant University of Agricultural & Technology , Pantnagar
DS1 (*Bradyrhizobium japonicum*)	Division of Microbiology ,Indian Agricultural Research (IARI) New Dehli
SB271 (*Bradyrhizobium* sp.)	Department of Microbiology, Punjab Agricultural University (PAU), Ludhiana

Table 2. Differentiation of fast and slow growing strains of soybean rhizobia on YEMA (BTB) medium

Isolates	Color produced on BTB agar	Fast/Slow grower
IND1	Blue	Slow
IND 2	Yellow	Fast
LSBR3	Blue	Slow
LSER 7	Yellow	Fast
LSER 8	Yellow	Fast
PANT1	Blue	Slow
PANT2	Yellow	Fast
SB 271	Blue	Slow
DS 1	Blue	Slow

Table 3. Quantitative measurement of Indole acetic acid (IAA) production by different *Bradyrhizobium* and *Ensifer* strains in presence and absence of L-tryptophan

Bradyrhizobium and *Ensifer* strains	IAA production (µg/ml)	
	L-TRP (-)	L-TRP (+)
Control	0.27	0.35
IND 1	2.25	2.65
IND 2	1.50	2.00
LSBR3	10.5	18.35
LSER7	13.55	23.70
LSER8	12.25	25.75
PANT 1	2.60	2.80
PANT 2	4.25	4.30
SB 271	12.10	14.70
DS 1	10.50	15.80
CD5%	0.25	0.78

Table 4. Measurement of phosphate solubilization by *Bradyrhizobium* and *Ensifer* strains of soybean on Pikovaskaya's medium as a function of time

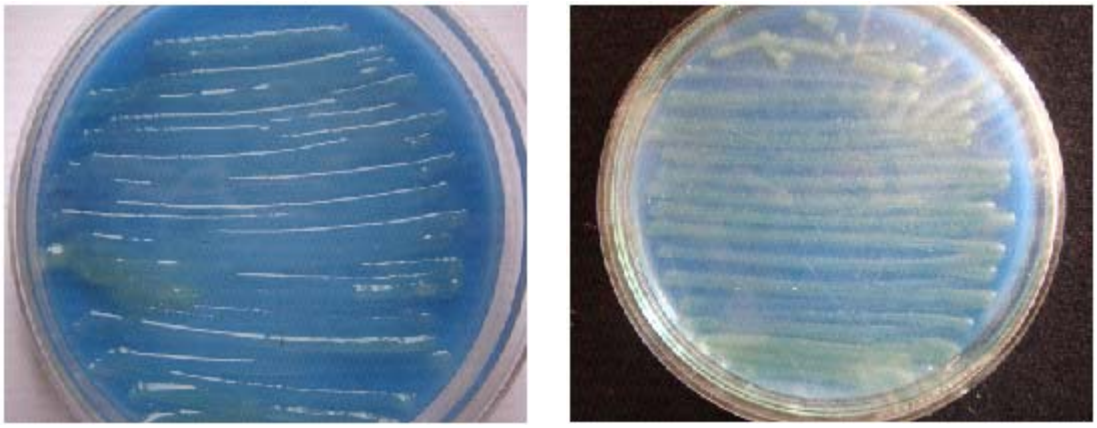
Cultures	P-solubilization (mg/100ml) Incubation period (days)				
	3rd	6th	9th	12th	15th
IND 1	0.39	1.89	1.98	3.58	0.95
IND 2	0.63	2.41	5.63	5.80	2.54
LSBR3	0.27	0.63	3.36	3.62	2.25
LSER7	0.45	0.73	0.95	1.76	0.72
LSER8	0.61	1.00	2.09	3.47	2.70
PANT 1	0.25	0.45	2.27	2.48	2.34
PANT 2	0.37	1.22	1.51	1.76	0.41
SB 271	0.27	0.90	1.42	2.18	0.34
DS 1	0.34	3.04	3.53	3.71	2.79
CD5%	NS	1.03	0.28	0.13	0.24

fast (turn medium yellow) and slow growing (turn medium blue) rhizobia on YEMA supplemented with BTB (Figure 1).Out of 4 fast growers, two *Ensifer* strains LSER 7, LSER 8 and *Bradyrhizobium* strains LSBR 3 produced gum. Our results indicated that 50% *Ensifer* strains produced gum and our findings are in close agreement with other reporters[21,22.]

All isolates were assessed for their ability to produce IAA both in the presence and absence of precursor L- tryptophan (Table 3). Significant difference was found among isolates in their ability to produce IAA. A low amount of IAA was produced by all isolates in the absence of L-tryptophan, which ranged from (1.50-13.55 µg/ml). In the presence of L- tryptophan the amount of IAA produced by all isolates was found to be increased from 2.00-25.75µg/ml. Maximum IAA was produced by LSER 8 (25.75 µg/ml) followed by LSER 7 (23.70 µg/ml) and LSBR 3 (18.35 µg/ml). The lowest amount of IAA was observed in IND 2 (2.00 µg/ml of IAA). Variation in IAA production by different isolates could be due to variation in utilization of L-tryphtophan (Figure 2).

Our results are in accordance with earlier findings[23]where all 50 isolates of rhizobia produce variable amount of IAA. Sridevi and Mallaiah[24]also revealed variation in IAA production by different rhizobia in YEM broth supplemented with L-tryptophan. Production of IAA is wide spread among plant associated bacteria and results are also in close confirmation of Boddey and Hungria[25], where *B. japonicum* reference strain accumulated between 4.88 to 7.08 µm of IAA, while with *B. elkanii* strain concentration reached 44.36 µm of IAA. Appunu *et al*[26]also observed that in the presence of L-tryptophan nearly 9% of slow-growers and 10% of fast growers synthesized IAA. Maximum production of IAA in the presence of L-tryptophan by *Ensifer* strain LSER 8 and LSER 7, also supported with the earlier findings of Annapurana[27], Zahir[28]and Kumar and Ram[29]where auxin biosynthesis by rhizobia increased many folds with suitable precursor (L-tryptophan). *Rhizobium* sp. isolated from the root nodules of *Desmodium gangeticum* and *Clitoria ternatea* L. produced a high amount of IAA from L-tryptophan in culture[30].

Eighty percent of total isolates showed P-solubilizing potential on basis of halo zone on



(a) (b)

Fig. 1. Differentiation between (a) *Bradyrhizobium* and (b) *Ensifer strains*

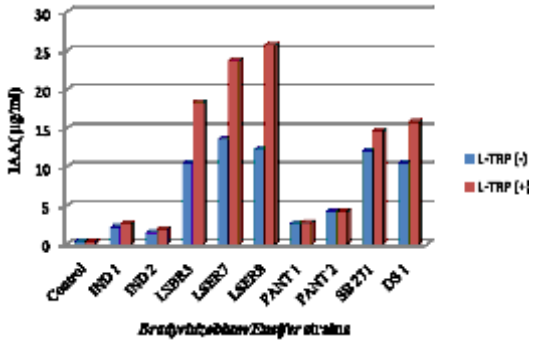
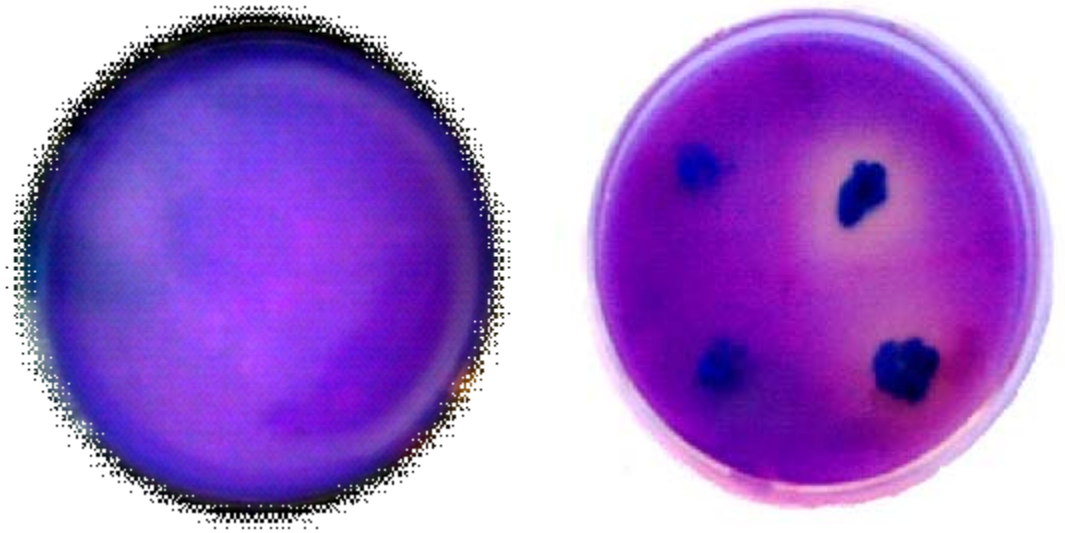


Fig. 2. Quantitative measurement of Indole acetoc acid (IAA) prodiction by different *Bradyrhizobium* and *Ensifer* strains in presence and absence of L-tryptophan

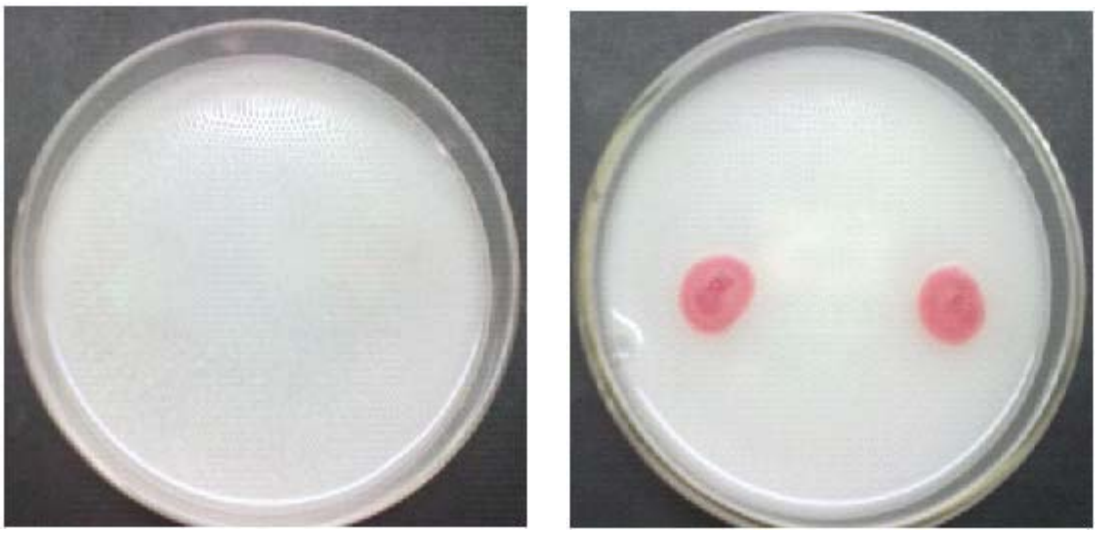
Pikovaskaya's and NBRIP (Figure 3) media were selected, however, the zones varied in size. Approximately 98% of phosphorus in soil is present as insoluble phosphates and can severely limit plant growth and productivity in legumes. To circumvent the problem of P deficiency use of rhizobia with phosphate solubilizing activity can help in mobilizing unavailable phosphorus to plants.

The relative efficiency of the nine isolates of *Ensifer* and *Bradyrhizobium* strains was studied for solubilizing TCP at different intervals of time (3, 6, 9, 12 and 15 days) (Table 4). It was seen that increasing amount of P was released by different isolates with increasing period of incubation till



Uninoculated Inoculated

Fig. 3. Phosphate solubilization on NBRIP medium



Uninoculated Inoculated

Fig. 4. Hydrogenase uptake system in *Bradyrhizobium/Ensifer* strains

12th day. The phosphate solubilizing activity was observed upto 15th day. Significant variation was observed among isolates in their ability to solubilize phosphate. The maximum phosphate solubilization was observed on 12th day, which ranged from (1.76 to 5.80 mg 100 ml^{-1}). Maximum phosphate was solubilized by IND 2 (5.80 mg 100 ml^{-1}) and LSBR 3 (3.62 mg 100 ml^{-1}). Whereas very low phosphate solubilizing activity was recorded in local reference culture SB 271 (solubilized 0.34 mg 100 ml^{-1}) at 15thday.

A drop in phosphate solubilizing activity at 12th day might be due to deficiency of nutrients in the culture medium. Similar decline in phosphate solubilizing activity after 12 days was in close agreement with earlier findings[31]. Out of 9 strains of *Bradyrhizobium* LSBR 3 *and Ensifer* IND 2 strains were able to mobilize P from TCP in liquid medium. This observation was in close association with the earlier findings[32]where out of 57 strains of *B. japonicum* only 13 strains were able to solubilize P from TCP.

The presence of Hup system in rhizobia is desirable trait for an energy efficient process of N_2 fixation with nitrogen fixing symbiosis in legumes. Three each of bradyrhizobial (LSBR 3, DS1 and SB271) and *Ensifer* strains (LSER7, LSER 8 and PANT 2) showed red coloration on Maier's *et al* and YEMA media amended with 0.01% TTC dye (Figure 4).

The present research aimed to investigate potential strains of *Bradyrhizobium/ Ensifer* and ability to adapt in prevailing environmental conditions for improving BNF and yield in soybean. It was concluded that LSBR 3, PANT1 and LSER 8 emerged as effective strains for biological nitrogen fixation. So selection of strains with improved BNF can be exploited for converting potential *Bradyrhizobium/Ensifer* strains into commercial inoculants of soybean.

REFERENCES

1. Rai,R., Dash,K.P., Mohapatra,T., Singh, A. Phenotypic and molecular characterization of indigenous rhizobia nodulating chickpea in India. *J. Exp. Biol*., 2012; **50**: 340-50.
2. Weir,B.S. The current taxonomy of rhizobia. NZ Rhizobia website. 2012; http**:**// www.rhizobia.co.nz/taxonomy/rhizobia
3. Qureshi, M.A., Ahmad, M.J., Naveed, M., Iqbal, A., Akhtar, N., Niazi, K.H. Co-inoculation with and for improving growth, nodulation and yield of chickpea (L.). *Soil. Environ*., 2009; **2**: 124-29.
4. Hamayun, M., Khan, S. A., Shinwari, Z. K., Khan, A. L., Ahmad, N., Lee, I. J. Effect of polyethylene glycol induced drought stress on physio-hormonal attributes of soybean. *Pak. J. Bot*., 2010; **42**: 977-86.
5. Abbasi, M.K., Manzoor, M., Tahir, M.M. Efficiency of *Rhizobium* inoculation and P

fertilization in enhancing nodulation, seed yield and phosphorous use efficiency by field grown soybean under hilly region of Rawalakot Azad Jammu and Kashmir, Pakistan. *J. Pl. Nut*., 2010; **33**: 1080-1102.

6. Jain, R.K., Srivastav, A., Sharma, D.K. Isolation of crop specific indigenous strains and study their effects on seed germination, Indian. *J. L. Sci.*, 2012; **2**: 41-45.
7. Shahzad, F., Shafee, M., Abbas, F., Babar, S., Tariq, M.M., Ahmad, Z. Isolation and biochemical characterization of *Rhizobium Meliloti* from root nodules of Alfalfa (*Medico Sativa*). *J. Anima & Pl. sci.*, 2012; **22**: 522-24.
8. Somasegaran, P., Hoben, H.J. Handbook for Rhizobia**:** *Methods in legume-Rhizobium Technology.* Springer-Verlag, New York. 1994; pp 1-450.
9. Bernaerts, M.J., Deley, J.A biochemical test for crown gall bacteria. *Nature*., 1963; **197**: 406-07.
10. Gorden, S.A.,Weber, R. P. Calorimetric estimation of IAA. *Pl. Physoil.*, 1951; **26**: 192-95.
11. Patten,C.L., Glick,B.R. Bacterial biosynthesis of indole-3-acetic acid. *Can. J. Microbiol.*, 42 (1996) 207–20.
12. Pikovaskaya, R.I. Mobilization of phosphate dissolving bacteria in rhizosphere of some cultivated legumes. *Pl. Soil*., 1948; **35**: 127-32.
13. Nautiyal, C.S. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms, *FEMS. Microbiol. Lett*., 1999; **170**: 265-70.
14. Jackson, M.L. Estimation of phosphorus content. Soil chemical analysis, Printer Hall, New Delhi (India); 1973.
15. Maier, R.J., Campbell, N.E.R., Hanus, J.F., Simpson, F.B., Russel, S.A., Evans, H.J. Expression of hydrogenase activity in free living *Rhizobium japonicum*. *Proc. Natt. Acad. Sci. USA.*, 1978; **75**: 3258-262.
16. Meghvansi, M.K., Prasad, K., Mahna. Symbiotic potential, competitiveness and compatibility of indigenous *Bradyrhizobium japonicum* isolates to three soybean genotypes of two distinct agro-climatic regions of Rajasthan, India. *Saudi. J. Biol. Sci*., 2010; **17**: 303–10.
17. Somasegaran, P., Hoben, H.J. Methods in Legume-*Rhizobium* Technology. NifTAL project and MIRCEN. Department of Agronomy, 2nd Soil Science Hawaii Institute Tropical Agriculture Human research, Univ Hawaii Manoa, 1985; pp 1-52.
18. Hameed, S., Yasmin, S., Malik, K.A., Zafar, Y., Hafeez, F. *Rhizobium, Bradyrhizobium* and *Agrobacterium* strains isolated from cultivated legumes. *Biol. Fertil. Soils.*, 2004; **39**: 179–85
19. Saeki, Y., Kaneko, A., Hara, T., Suzuki, K., Yamakawa, T., Nguyen, M.T., Nagatomo, Y., Akao, S. Phylogenetic analysis of soybean-nodulating rhizobia isolated from alkaline soils in Vietnam. *Soil. Sci. Pl. Nutr*.-, 2005; **51**: 1043–52.
20. Sharma, M.P., Srivastava, K., Sharma, S.K. Biochemical characterization and metabolic diversity of soybean rhizobia isolated from Malwa region of Central India. *Pl. Soil. Environ.*, 2010; **56**: 375–83.
21. Baoling, H., ChengQun, L., Bo, W., LiQin, F. A rhizobia strain isolated from root nodule of gymnosperm *Podocarpusm acrophyllus*. *Sci. Chin. Ser. C-Life. Sci*., 2007; **50**: 1-6.
22. Hungria, M., Campo, R.J., Chueire, L.M.O., Grange, L., Megias, M. Symbiotic effectiveness of fast-growing rhizobial strains isolated from soybean nodules in Brazil. *Biol. Fertil. Soils*., 2001; **33**: 387–94.
23. Leelahawonge, C., Pongslep, N. Factors influencing indole-3-acetic acid biosynthesis of root-nodule bacteria isolated from various leguminous plants. Thammasat. *Int. J. Sc. Tech*., 2009; **14**: 2-4.
24. Sridevi, M., Mallaiah, K.V. Bioproduction of indole acetic acid by *Rhizobium* strains isolated from root nodules of green manure crop, *Sesbania sesban* (L.) Merr. short communication, Iranian. *J. Biotech*., 2007; **5**: 178-82.
25. Boddey, L.H., Hungria, M. Phenotypic grouping of Brazilian *Bradyrhizobium* strains which nodulate soybean. *Biol. Fertil. Soils*., 1997; **25**: 407-15.
26. Appunu, C., Sasirekha, N., Ramalingam, V., Prabavathy., Nair, S. A significant proportion of indigenous rhizobia from India associated with soybean (*Glycine max* L.) distinctly belong to *Bradyrhizobium* and *Ensifer* genera. *Biol. Fertil. Soils*., 2009; **47**: 56-63.
27. Annapurna, K., Balakrishnan, N., Vital, L. Verification and rapid identification of soybean rhizobia in Indian soils. *Curr. Microbiol*., 2007; **54**: 287-91.
28. Zahir, Z.A, Shah, M.K., Naveed, M., Akhtar, M.J. Substrate dependent auxin production by *Rhizobium phaseoli* improve the growth and yield of *Vigna radiate* L. under salt stress conditions. *J. Microbiol. Biotech*., 2010; **20**: 1288-94.
29. Kumar, P.R., Ram, M.R. Production of indole acetic acid by *Rhizobium* isolates from *vigna trilobata* (L) Verdc. *African. J. Microbial. Res*., 2012; **6**: 5536-41.
30. Roy, M., Basu, P.S. Studies on root nodules of

leguminous plants bioproduction of indole acetic acid by a *Rhizobium* sp. from a twiner *Clitoria ternatea* L. Acta. *Biotech*., 2004; **12**: 453-60.

31. Kang, S.S., Hat, C.G., Lee, T.G., Maheshwari, D.K. Solubilization of insoluble inorganic phosphates by a soil-inhabiting fungus *Fomitopsis* sp. PS 102. *Curr. Sci*., 2002; **82**: 439-42.

32. Alikhani, H.A., Saleh, R.N., Antoun, H. Phosphate solubilization activity of rhizobia native to Iranian soils. *Pl. Soil*., 2006; **287**: 35-41.