Molecular diversity, effectiveness and competitiveness of indigenous rhizobial population infecting mungbean *Vigna radiata* (L. Wilczek) under semi-arid conditions

Suman Kundu · S. S. Dudeja

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Abstract Nodules from mungbean crop raised for the first time at Ram Dhan Singh (RDS) farm of Chaudhary Charan Singh (CCS) Haryana Agricultural University, Hisar were collected from 17 different locations. Twenty-five mungbean rhizobia were isolated and authenticated by plant infection test. DNA of all these rhizobia was extracted purified and amplified using enterobacterial repetitive intergenic consensus (ERIC) primers. All the mungbean rhizobial isolates were clustered into 4 groups at 65% of similarity and were further divided into 17 subclusters at 80% of similarity. All the 4 types of rhizobia were not present at any of the location and group 2 or 4 rhizobia were invariably present. Efficacy of these rhizobia in terms of nodulation, nitrogen uptake and chlorophyll *a* fluorescence was determined under pot culture conditions. Strain MB 307 showed maximum nitrogen uptake of 31.9 mg N plant⁻¹ followed by strain MB 1205, MB 1206(2), MB 308, MB 1524 and strain MB 1521 was found to be the least efficient in terms of N₂ fixation. Nodule occupancy by different rhizobia ranged from 5.5 to 40.3%. Most of the strains belonging to the 2nd group which clustered maximum number of strains were comparatively better competitors and formed 19.5–40.3% of the nodules and were also effective. Isolate MB 307, the most efficient strain, was found to have nodule occupancy of 31.5%. Such type of predominant, efficient and better

competitor strains should be selected for enhancing nodule competitiveness.

Keywords Molecular diversity · Rhizobia · Mungbean · *Vigna radiata* · Competitiveness · N₂ fixation · Symbiosis

Introduction

Biological nitrogen fixation is an important component of sustainable agriculture and rhizobial inoculants have frequently been applied as biofertilizers. Enhanced competitive ability of an inoculant strain is a key requirement for successful colonization of plant roots, nodule formation and subsequent nitrogen fixation. However, traditionally selected efficient strains were found to be poor competitors in the presence of indigenous rhizobia of chickpea, pigeonpea, mungbean and urbean under field conditions of Chaudhary Charan Singh (CCS) Haryana Agricultural University, Hisar. The nodule occupancy by inoculant strains in these crops ranged from 8–21% [1–4].

Native legumes are potential sources of diverse indigenous rhizobial populations. Negligible reports on the molecular diversity of rhizobia infecting legumes of Indian origin and particularly mungbean (*Vigna radiata* (L. Wilczek) are available [5]. Studies on the molecular diversity of root nodule bacteria in rhizosphere, soil or nodules have been conducted to properly classify these rhizobia and also to correlate with promiscuity, symbiotic properties, effectiveness and competitiveness of these bacteria in different legumes but no conclusion has been drawn finally [6–10]. There may be differences between the population densities of different strains in soil and these differences may influence the outcome of nodulation. Dominance of a particular rhizobial

S. Kundu · S. S. Dudeja (✉)
Department of Microbiology,
CCS Haryana Agricultural University,
Hisar - 125 004,
India

Tel: +91 / 1662 / 289292 (O); +91 / 1662 / 243190 (R)
e-mail: ssdudeja@hau.ernet.in, ssdudeja@gmail.com

strain in nodules may result from its higher abundance in soil as reported in *Ensifer meliloti* indigenous for a population [11–13] or may not be correlated [14–16]. Use of three predominant strains as inoculants showed that one strain formed majority of the nodules while the other two were minor occupants in the nodules. It has also been reported that plant preference for a particular strain can be masked by changing the population ratio of the two competing strains. A dominant strain showed 50% nodule occupancy [17], and may have more persistence and its population tended to increase throughout the 4-year experimental period [18]. The possibility of use of molecular diversity of native rhizobia for selection of efficient symbiotic *Medicago* to develop inoculants for management of agro-pastoral systems using local annual medics in Algeria was also explored [19].

In the present study, molecular diversity of rhizobia isolated from mungbean crop grown for the first time at RDS farm, CCS Haryana Agricultural University, as pasture lands were developed for seed production at this farm. Predominant types of rhizobia in the nodules of this crop were identified and then their effectiveness and competitiveness were assessed to develop inoculants with better competitive ability.

Materials and methods

Isolation of mungbean rhizobia

Mungbean plants growing in RDS Farm, CCS Haryana Agricultural University, Hisar were uprooted from 17 sites and their nodules were used for isolation of rhizobia [20] and were maintained on yeast extract mannitol agar (YEMA) slants and stored at 7°C in a refrigerator for further studies. In this way 25 rhizobial isolates were obtained. All the rhizobial isolates were authenticated by plant infection test in test tubes [21]. All the 25 rhizobial isolates were selected for further studies.

Genomic DNA

DNA was obtained from rhizobia grown in 10 ml of yeast extract mannitol (YEM) broth. The log phase cells were harvested and DNA was extracted by boiling method followed by purification with phenol–chloroform [22]. Finally DNA was dissolved in 50–100 µl of TE buffer and quantified.

Amplification of ERIC by polymerase chain reaction

DNA of all the mungbean rhizobia was amplified by enterobacterial repetitive intergenic consensus (ERIC) sequence

using ERIC-1R (5' ATGTAAGCTCCTGGGGATTAC 3') and ERIC-2R (5' AAGTAAGTGACTGGGGTGA GCG 3') primers. Polymerase chain reaction (PCR)-based amplification for each 100 µl of reaction mixture included: PCR buffer (10X) 10 µl, 1.5 mM MgCl₂ (1X), 100–200 ng of pure genomic DNA, 100 pM of each primer, 25 µM each of the deoxynucleotide triphosphates (dNTPs), 3 units of Taq polymerase and the volume was made up with Milli-Q water. DNA amplification was carried out with initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 45°C for 70s and extension at 72°C for 4 min with a final extension at 72°C for 20 min followed with a final cooling to 4°C. Lid temperature was maintained at 105°C. Amplified products were analyzed by horizontal electrophoresis in 3% agarose gel at 100 V for 2 h 30 min and stained with ethidium bromide (0.5 µg ml⁻¹). The gels were visualized under UV in gel documentation system.

Data analysis

The PCR product profiles were converted into two-dimensional binary matrices. The lanes were compared by reading horizontally across the gel. Similarity matrixes were constructed following SimQual coefficient and analyzed by unweighted pair grouping with mathematic average (UPGMA) cluster analysis using biostatistical analysis programme NT-SYS-pc programme 2.1 of Exeter Software USA [23].

Effectiveness of mungbean rhizobia

To assess the N₂ fixing efficiency of mungbean rhizobia, a pot culture experiment was carried out using soils placed in 6–7 kg earthen pots. Analysis of the soil revealed that soil was sandy loam with soil pH 8.0 (H₂O 1:2); organic C 0.39%; electrical conductivity 0.4 dS m⁻¹ (H₂O 1:2); total N 0.052%; phosphorus (Olsen) 10 ppm and 12 ppm as available N. Seeds of mungbean cultivar Asha were surface sterilized with 0.2% mercuric chloride for 2 min followed with alcohol [20] and sown in triplicates. Inoculum of log phase growing cultures of rhizobia (5 ml) containing 10⁷–10⁸ cells per ml was added in each pot along with the seeds. A control was kept without *Rhizobium* inoculation. After germination four plants were maintained in each pot. Pots were irrigated on alternate day or as and when required. After 55 days of growth, plants were uprooted and nodule number, nodule fresh weight, nodule occupancy, shoot and root dry weight and total nitrogen content in shoots was determined. Before uprooting plants, at 55 days of growth, chlorophyll *a* fluorescence was measured. Shoot and root samples were dried

at 80°C. Total nitrogen content of the mungbean plants was estimated by Kjeldahl's steam distillation method [24].

Nodule occupancy of mungbean rhizobia

Nodules were detached from the roots and were surface sterilized with 0.1% mercuric chloride for 2 min followed with alcohol [20]. Nodules were crushed and streaked on YEMA plates with and without 200–400 µg ml⁻¹ of streptomycin. Rhizobia resistant to streptomycin were further assessed for ERIC-PCR banding pattern. Nodule occupancy of only selected eight strains was determined.

Chlorophyll *a* fluorescence measurements

Chlorophyll *a* fluorescence was measured in mungbean plants. In each pot, 3 measurements were recorded. A compact, portable plant efficiency analyzer (PEA – Hansatech, UK) was used to measure chlorophyll *a* fluorescence as detailed earlier [25].

Results

All the mungbean rhizobial isolates were purified and authenticated by plant infection test in test tubes using mungbean seeds of Asha cultivar. The diversity of mung-

bean rhizobial isolates was studied by amplification of ERIC sequence in rhizobial DNA using PCR. There were 7–12 bands of different intensity and molecular weights in different isolates and in total there were 16 bands. All the mungbean rhizobial isolates were clustered into 4 groups at 65% of similarity with other clusters (Fig. 1). Cluster 1 included three mungbean rhizobia MB 308, MB 311 and MB 1524, while cluster 2 included MB 307, MB 706, MB 1202(1), MB 1204, MB 1205, MB 1206(2), MB 1503, MB 1539, MB 1541 (1), MB 1541 (2) and MB 1542(2) (Table 1). In the third cluster MB 1211, MB 1521, MB 1523, MB 1525 and the standard strain S-24 were there and the fourth cluster included MB 306, MB 703, MB 1102, MB 1110(1) and MB 1206(1). However at 80% of similarity these four clusters were further divided into 17 subclusters.

Table 1 Molecular clustering of mungbean rhizobia based on ERIC-PCR amplification

Cluster No.	Rhizobial isolates
1	MB 308, MB 311, MB 1524
2	MB 307, MB 706, MB 1202(1), MB 1204, MB 1205, MB 1206(2), MB 1503, MB 1539, MB 1541(1), MB 1541(2), MB 1542(2)
3	MB 1211, MB 1521, MB 1523, MB 1525, S-24
4	MB 306, MB 703, MB 1102, MB 1110(1), MB 1206(1)

Fig. 1 Unweighted pair grouping with mathematic average (UPGMA) dendrogram of similarity between PCR-amplified ERIC sequences of mungbean rhizobia

Isolates picked from the same nodule do not show 100% similarity as in case of MB 1541(1) and MB 1541(2); and MB 1206(1) and MB 1206(2). However, two isolates from different nodules of the same plant showed 100% similarity and these isolates were: MB 1102 and MB 1110(1); MB 1541(2) and MB 1542(2). Depending upon the site of RDS farm from where rhizobia were isolated, the distribution of different groups of rhizobia showed that 1–3 groups of rhizobia were present at different locations. It was observed that individual groups of rhizobia were present at one site and rhizobia belonging to cluster 2 or 4 were present at all the sites (Table 2).

To determine the effectiveness and competitiveness of mungbean rhizobia in relation to their relative abundance in the root nodules of mungbean, experiment was conducted under pot culture conditions. All mungbean rhizobia along with standard strain S-24 were used as inoculants for Asha cultivar of mungbean. After 55 days of growth, its nodulation, chlorophyll *a* fluorescence, nodule occupancy and nitrogen uptake by mungbean plants was determined. Strain MB 307 formed highest number of 43 nodules per plant as compared to 9 nodules in control (Table 3). Strain MB 1206(2) followed strain MB 307 in nodule number. Although strain MB 308 formed less number of nodules (30), the nodule fresh weight was highest in this strain. Strain MB 308 produced the maximum nodule fresh weight of 163.3 mg plant⁻¹ as compared to 25 mg plant⁻¹ nodule fresh weight in control.

Strain MB 1205 produced significantly higher root dry weight of 241.6 mg plant⁻¹ as compared to 53.7 mg plant⁻¹ in control, followed by strain MB 308 (192.4 mg plant⁻¹), MB 307, MB 1524 and MB 1206(2). Similarly, strain MB 307 produced maximum shoot dry weight 1003.9 mg plant⁻¹ as compared to 113.0 mg plant⁻¹ in control, followed by MB 1205, MB 1206(2), MB 308 and MB 1524 which produced 693.9, 603.2, 527.8 and 493.4 mg plant⁻¹ of shoot dry weight respectively. Whereas, strain MB 1525 produced least shoot dry weight of 148.0 mg plant⁻¹.

Strain MB 307 showed maximum nitrogen uptake of 31.9 mg N plant⁻¹ as compared to 2.0 mg N plant⁻¹ in control and followed by strain MB 1205, MB 1206(2), MB 308,

MB 1524 which showed 17.1, 16.1, 15.6 and 14.6 mg N plant⁻¹. Strain MB 1521 was found to be least efficient in terms of N₂ fixation and showed 3.1 mg N plant⁻¹.

After 55 days of growth, chlorophyll *a* fluorescence was also measured. Fast fluorescence, data indicative of efficiency of photosystem II and N₂ fixation was highest in MB 307 followed by strains MB 1205, MB 1102, MB 308 and MB 1206 (2) and lowest in control (Fig. 2).

Nodule occupancy of only selected mungbean rhizobia, which were resistant to streptomycin of 400 µg ml⁻¹, belonging to different clusters was also determined. Isolate MB 306 showed the lowest nodule occupancy of 5.5% (Table 4). Mungbean rhizobia of cluster 2 showed nodule occupancy ranging from 19.5 to 40.3% by strains MB 1211 and MB 1503 respectively. Isolate MB 307, the most efficient strain was found to have nodule occupancy of 31.5%. Table 5 shows that the relatedness of effectiveness with nodule occupancy and nitrogen uptake by plants was highly correlated with all the parameters.

Discussion

Little is known about the molecular diversity of rhizobia infecting different legumes in India, though the functional diversity of indigenous rhizobia is well known [26]. Therefore, in the present study the molecular diversity of mungbean rhizobia in nodules was assessed and their effectiveness and competitiveness was also determined. The molecular diversity was assessed using amplification of ERIC fragments by PCR. Depending upon the similarity coefficient and UPGMA analysis mungbean rhizobia were grouped into 4 groups at a similarity level of 65% and into 17 groups at a similarity level of 80% indicating an immense molecular diversity of mungbean nodule bacteria as has been shown in other rhizobia using random ERIC and REP fragments [18, 27–32]. Studies on the genetic diversity and phylogeny of slow-growing rhizobia isolated from *Vigna radiata* at main ecotypes of China were reported using 16S rRNA gene PCR-restriction fragment length polymorphism (RFLP),

Table 2 Distribution of ERIC-PCR groups of mungbean rhizobia on the basis of location of sampling

Location No.	Rhizobial isolates	Cluster No.
3	MB 306, MB 307, MB 308, MB 311	1, 4
7	MB 703, MB 706	2, 4
11	MB 1102, MB 1110(1)	4
12	MB 1202(1), MB 1204, MB 1205, MB 1206(1), MB 1206(2), MB 1211	2, 3, 4
15	MB 1503, MB 1521, MB 1523, MB 1524, MB 1525, MB 1539, MB 1541(1), MB 1541(2), MB 1542(2)	1, 2, 3

Table 3 Symbiotic effectiveness of different ERIC-PCR groups of mungbean rhizobia under pot culture conditions

Inoculation with rhizobia	Nodules per plant	Nodule fresh weight (mg per plant)	Dry weight (mg per plant)		Total N uptake by plant (mg per plant)
			Root	Shoot	
Control	9.0	25.0	53.7	113.0	2.0
MB 306	13.0	49.4	108.3	277.8	7.6
MB 307	43.0	147.5	189.3	1003.9	31.9
MB 308	30.0	163.3	192.4	527.8	15.6
MB 311	17.0	72.5	150.9	488.2	14.4
MB 312	18.0	46.5	92.3	224.9	4.8
MB 703	12.0	80.0	122.9	281.2	5.1
MB 706	10.0	92.3	111.7	361.3	11.6
MB 1102	24.0	83.3	126.9	303.7	8.1
MB 1110(1)	24.0	66.7	111.9	334.8	9.5
MB 1202(1)	6.0	50.3	134.1	245.4	6.3
MB 1204	10.0	63.5	140.7	275.6	5.5
MB 1205	24.0	125.5	241.6	693.9	17.1
MB 1206(1)	10.0	31.0	132.8	224.8	7.0
MB 1206(2)	41.0	114.6	174.0	603.2	16.1
MB 1211	11.0	48.1	127.5	199.3	5.1
MB 1503	11.0	53.4	154.5	294.8	7.4
MB 1521	5.0	29.3	107.9	170.5	3.1
MB 1523	9.0	40.6	105.8	204.3	4.7
MB 1524	9.0	84.6	178.9	493.4	14.6
MB 1525	4.0	33.7	93.9	148.0	3.5
MB 1539	10.0	29.8	108.0	275.1	6.3
MB 1541(1)	7.0	67.3	148.4	350.9	8.3
MB 1541(2)	3.0	27.7	88.9	157.4	3.6
MB 1542(2)	4.0	34.2	90.2	160.5	3.9
S-24	7.0	46.6	74.3	211.8	5.3
SE(m)	4.2	29.5	29.1	123.8	4.3
CD at 5%	12.0	NS	82.9	352.3	12.3

Table 4 Nodule occupancy of mungbean rhizobia belonging to different ERIC-PCR clusters

Cluster No.	Strains	Nodule occupancy (%)
1	MB 308	33.5
2	MB 307	31.5
	MB 1202 (1)	39.5
	MB 1211	19.5
	MB 1503	40.3
	MB 1539	26.3
3	S-24	27.8
	MB 1523	30.5
4	MB 306	5.5
	MB 703	24.5

16S rRNA gene sequencing and 16S-23S rRNA IGS PCR-restriction fragment length polymorphism (RFLP) assays [33]. All the strains clustered into three groups at the similarity of 76%. Group I contains 13 slow-growing rhizobia; Group II consists of 21 strains closely related to *B. japonicum* and *B. liaoningense*, and group III isolates were closely related to *B. elkanii*. Diversity in the rhizobia was observed from the isolates made even from same nodules of a plant. Rhizobia isolated from different plants and different sites were dissimilar as reported earlier in other rhizobia [6].

Mungbean rhizobia belonging to 2nd cluster were predominant ones while other groups were less represented. Depending upon the site of RDS farm from where rhizobia were isolated, the distribution of different groups of rhizobia showed that 1–3 groups of rhizobia were present at

Fig. 2 Chlorophyll *a* fluorescence of mungbean plants inoculated with different rhizobia

Table 5 Correlation between effectiveness and nodulation competitiveness of mungbean rhizobia

Pearson correlation	Nodule No.	Nodule fresh weight	Root dry weight	Shoot dry weight	Total N uptake
Nodule fresh weight	0.11				
Root dry weight	0.23	0.91**			
Shoot dry weight	0.49	0.83**	0.90**		
Total N uptake	0.19	0.96**	0.82**	0.79*	
Nodule occupancy	0.18	0.96**	0.81**	0.79*	0.99**

* Significant at $P = 0.01$; ** Significant at $P = 0.05$

different locations. At two locations three groups were present and at these locations native nodulations was comparatively better. It was observed that individual groups of rhizobia were present at one site and rhizobia belonging to cluster 2 or 4 were present at all the sites. In case of *Bradyrhizobium* infecting tree legume, bradyrhizobial strains were clustered into 16 groups with six predominant groups [34]. Further symbiotic characterization of these rhizobia showed that mungbean rhizobia differed in number of nodules formed, nodule fresh weight, root dry weight, shoot dry weight and total N uptake by plant. Such variability in nitrogen fixation efficiency among various native rhizobial isolates has been reported earlier [18, 26]. Within a cluster, rhizobial strains

showed varied effectiveness and the most efficient strain MB 307 belonged to 2nd predominant cluster indicating that all the predominant strains may not be effective, therefore, among these predominant types, the effective strains such as MB 307 should be selected.

Percentage of nodules formed by the inoculant strains as assessed by streptomycin resistance and further confirmed by ERIC-PCR DNA fingerprinting also varied from different rhizobial isolates. An efficient strain may or may not be competitive as indicated by strain MB 307, which was the most efficient strain but had nodule occupancy of 31.5%, as compared to MB 1503 strain which had highest nodule occupancy of 40.3%. Similarly, the most competitive strain

may not be an effective strain as indicated by strain MB 1503 which showed 7.4 mg nitrogen uptake per plant while had a nodule occupancy of 40.3%. Other workers reported that nodule-dominant genotypes from soil populations do not necessarily show superior competitiveness for nodulation compared to minor occupants when evaluated [14, 35]. In case of chickpea, by using such type of molecularly predominant rhizobia under different locations could enhance nodule occupancy by 15% as observed in case of traditionally selected rhizobia [36]. Predominant strains of mungbean rhizobia were relatively more or even most efficient and effective in nodulation and nitrogen fixation and were also better to the strains having highest nodule occupancy.

High level of genetic diversity of mungbean rhizobia at RDS farm also strongly encourages other studies of rhizobial diversity in India. Mungbean isolates from nodules were moderate to good competitor and this may just either reflect their prevalence in soil which may be due to their ability to survive in the soil under prevailing environmental conditions where the ambient temperature ranges from -2 to 47°C or other strong saprophytic competitive ability in the absence of host plant, as mungbean crop was raised for the first time. This could be true otherwise also, i.e. such rhizobia may not necessarily be dominant in bulk soil but they are better competitors. Selection of the symbiotically most efficient mungbean rhizobia among the predominant ones may prove to be a better way of selecting rhizobia with a better competitive ability as has been proven in the case of chickpea rhizobia.

References

- Dudeja SS and Khurana AL (1988) Survival and competitiveness of *Bradyrhizobium* spp. in the rhizosphere of pigeonpea (*Cajanus cajan*). *Biol Fertil Soils* 7:63–67
- Dudeja SS, Khurana AL, Sharma PK, Dogra RC and Garg FC (1995) Symbiotic effectivity of *hup⁺* and *hup⁻* *Rhizobium* strains on mungbean and urdbean under field conditions. *Indian J Microbiol* 35:189–194
- Khurana AL, Sharma PK and Dudeja SS (1991) Influence of host, moisture and native rhizobial population on nodule occupancy in chickpea (*Cicer arietinum*). *Zentralbl Microbiol* 146:137–141
- Sheoran A, Khurana AL and Dudeja SS (1997) Nodulation competitiveness in the *Rhizobium*-chickpea nodulation variants symbiosis. *Microbiol Res* 152:407–412
- McInnes A, Thies JE, Abbott LK and Howieson JG (2004) Structure and diversity among rhizobial strains, populations and communities—a review. *Soil Biol Biochem* 36:1295–1308
- Chen WM, Moulin L, Bontemps C, Vandamme P, Béna G and Boivin-Masson C (2003) Legume symbiotic nitrogen fixation by beta-proteobacteria is widespread in nature. *J Bacteriol* 185:7266–7272
- Laguerre G, Louvrier P, Allard MR and Amarger N (2003) Compatibility of rhizobial genotypes within natural populations of *Rhizobium leguminosarum* biovar *viciae* for nodulation of host legumes. *Appl Environ Microbiol* 69:2276–2283
- Rodriguez-Echeverria S, Perez-Fernandez MA, Vlaar S and Finnan T (2003) Analysis of the legume-rhizobia symbiosis in shrubs from central western Spain. *J Appl Microbiol* 95:1367–1374
- Rosenblueth M and Martinez-Romero E (2004) *Rhizobium etli* maize populations and their competitiveness for root colonization. *Arch Microbiol* 181:337–344
- Safronova VI, Piluzza G, Belimov AA and Bullitta S (2004) Phenotypic and genotypic analysis of rhizobia isolated from pasture legumes native of Sardinia and Asinara Island. *Antonie van Leeuwenhoek* 85:115–127
- Bromfield ESP, Barran LR and Wheatcroft R (1995) Relative genetic structure of a population of *Rhizobium meliloti* isolated directly from soil and from nodules of alfalfa (*Medicago sativa*) and sweet clover (*Melilotus alba*). *Mol Ecol* 4:183–188
- Hartmann A, Giraud JJ and Catroux G (1998) Genotypic diversity of *Sinorhizobium* (formerly *Rhizobium*) *meliloti* strains isolated directly from a soil and from nodules of alfalfa (*Medicago sativa*) grown in the same soil. *FEMS Microbiol Ecol* 25:107–116
- Velasquez E, Mateos PF, Velasco N, Santos F, Burgos PA, Villadas P, Toro N and Martinez-Molina E (1999) Symbiotic characteristics and selection of autochthonous strains of *Sinorhizobium meliloti* populations in different soil. *Soil Biol Biochem* 31:1039–1047
- Leung K, Wanjage FN and Bottomley PJ (1994) Symbiotic characteristics of *Rhizobium leguminosarum* bv. *trifolii* isolates which represent major and minor nodule-occupying chromosomal types of field-grown subclover (*Trifolium subterraneum* L.). *Appl Environ Microbiol* 60:427–433
- Moawad HA, Ellis WR and Schmidt EL (1984) Rhizosphere response as a factor in competition among three serogroups of indigenous *Rhizobium japonicum* for nodulation of field-grown soybeans. *Appl Environ Microbiol* 47:607–612
- Robert FM and Schmidt EL (1985) Response of three indigenous serogroups of *Rhizobium japonicum* to the rhizosphere of pre-emergent seedlings of soybean. *Soil Biol Biochem* 17:579–580
- Bromfield ESP, Barran LR and Prevost D (1989) Is frequency of occurrence of indigenous *Rhizobium meliloti* in nodules of field grown plants related to intrinsic competitiveness? *Soil Biol Biochem* 21:607–609
- Svenning MM, Gudmundsson J, Fagerli IL and Leinonen P (2001) Competition for nodule occupancy between introduced strains of *Rhizobium leguminosarum* bv. *trifolii* and its influence on plant production. *Ann Bot* 88:781–787
- Sebbane N, Sahnoune M, Zakhia F, Willems A, Benallaoua S and de Lajudie P (2006). Phenotypic and genotypic characteristics of root-nodulating bacteria isolated from annual *Medicago* spp. in Soummam Valley (Algeria). *Lett Appl Microbiol* 42:235–241
- Vincent JM (1970) A manual for the practical study of root nodule bacteria. IBP Handbook 15. Blackwell, Oxford

21. Somasegaran P and Hoben HJ (1994) Handbook for rhizobia: Methods in legume-*Rhizobium* Technology, Springer-Verlag, New York
22. Dudeja SS and Chaudhary P (2008) High and low nodulation in relation to molecular diversity of chickpea mesorhizobia in Indian soils. *Arch Agron Soil Sci* 54:109–120
23. Rohlf FJ (1998) NTSYS-pc: numerical taxonomy and multivariate analysis system. 5 Version 2.1. Exeter Software, Setauket, New York, USA
24. Bremner JM (1965) Total nitrogen. In: Black CA et al. (eds) Methods of soil analysis. American Society of Agronomy, Madison, pp 1149–1178
25. Dudeja SS and Chaudhary P (2005) Fast chlorophyll fluorescence transient and nitrogen fixing ability of chickpea nodulation variants. *Photosynthetica* 43:253–259
26. Dudeja SS and Duhan JS (2005). Biological nitrogen fixation research in pulses with special reference to mungbean and urdbean. *Indian J Pulses Research* 18 (2):107–118 (*Forum paper*)
27. de Bruijn FJ (1992) Use of repetitive (repetitive extragenic palindromic and enterobacterial repetitive intergenic consensus) sequences and polymerase chain reaction to fingerprint the genomes of *Rhizobium meliloti* isolates and other soil bacteria *Appl Environ Microbiol* 58:2180–2187
28. Vásquez-Arroyo J, Sessitsch A, Martínez E and Peña-Cabriales JJ (1998) Nitrogen fixation and nodule occupancy by native strains of *Rhizobium* on different cultivars of common bean (*Phaseolus vulgaris* L.). *Plant and Soil* 204:147–154
29. Li J, Xu LM, Fan H, Li L, Ge C and Yang DS (1999) Genetic diversity of Chinese peanut rhizobia by REP-PCR analysis. *Wei Shang Wu Xue Bao* 39:296–304
30. Chen LS, Figueredo A, Pedrosa FO and Hungria M (2000) Genetic characterization of soybean rhizobia in Paraguay. *Appl Environ Microbiol* 66:5099–5103
31. Tajima S, Hirashita T, Yoshihara K, Bhromsiri A and Nomura M (2000) Application of repetitive extragenic palindromic (REP)-PCR and enterobacterial repetitive intergenic consensus (ERIC)-PCR analysis to the identification and classification of Japan and Thai local isolates of *Bradyrhizobium japonicum*, *Sinorhizobium meliloti* and *Rhizobium leguminosarum*. *Soil Sci Plant Nutr* 46:241–247
32. Saldaña G, Martínez-Alcántara V, Vinardell JM, Bellogín R, Ruíz-Sainz JE and Balatti PA (2003) Genetic diversity of fast-growing rhizobia that nodulate soybean (*Glycine max* L. Merr). *Arch Microbiol* 180:45–52
33. Yuan TY, Yang JK, Zhang WT Zhou JC (2006). Studies on genetic diversity and phylogeny of slow-growing rhizobia isolated from *Vigna radiata* at main ecotypes of China. *Wei Sheng Wu Xue Bao* 46(6):869–874
34. Doignon-Bourcier F, Willems A, Coopman R, Laguerre G, Gillis M and de Lajudie P (2000) Genotypic characterization of *Bradyrhizobium* strains nodulating small Senegalese legumes by 16S–23S rRNA intergenic gene spacers and amplified fragment length polymorphism fingerprint analyses. *Appl Environ Microbiol* 66:3987–3997
35. Meade J, Higgins P and O’Gara F (1985) Studies on the inoculation and competitiveness of a *Rhizobium leguminosarum* strain in soils containing indigenous rhizobia. *Appl Environ Microbiol* 49:899–903
36. Dudeja SS, Gupta SC, Majumdar VL and Chaudhary P (2007) Competitiveness of molecularly predominant and host specific chickpea rhizobia under field conditions. In: Vorisek K. et al. (eds) Practical solutions for managing optimum C and N content in agricultural soils IV. International Conference 2007 held at Prague, June 20th–22nd, 2007. Czeck University of Life Sciences and Crop Research Institute, Prague, Czech Republic, pp 38–52