Plant and Soil **196:** 1–5, 1997.

N_2-fixation ability of Brazilian soybean cultivars with *Sinorhizobium fredii* and *Sinorhizobium xinjiangensis*

L.M. de O.Chueire and M. Hungria[1]
*EMBRAPA-Soja, Cx. Postal 231, 86001-970, Londrina, PR, Brazil**

Received 6 December 1996. Accepted in revised form 22 May 1997

Key words: *Bradyrhizobium*, *Glycine max*, nodulation, *Rhizobium*, *Sinorhizobium*, symbiosis

Abstract

The main N_2-fixing symbiotic associations with soybean (*Glycine max* (L.) Merrill) plants are realized with bacteria belonging to the species *Bradyrhizobium japonicum* and *B. elkanii.* However, in 1982, fast-growing rhizobia were isolated from soybean root nodules collected in The People's Republic of China and these bacteria are today classified as *Sinorhizobium fredii* and *S. xinjiangensis*. The fast growing strains formed an effective symbiosis with primitive soybean cultivars such as Peking, but not with most North American cultivars, which are the progenitors of almost all Brazilian cultivars. The main purpose of this study was to evaluate the ability of 80 soybean cultivars from the Brazilian germplasm bank to produce effective nodules when inoculated with *S. fredii* or *S. xinjiangensis* strains. Sixty-six percent of the Brazilian genotypes formed effective nodules with both *Sinorhizobium* species. However, when 20 Fix^+ genotypes were inoculated with a mixture of *B. elkanii* and *S. fredii*, at a ratio of 1:1, most or all nodules were occupied by *B. elkanii*. Consequently, there was no relationship between the growth rate *in vitro* and the ability to compete for nodule occupancy. Fast-growing strains have also been isolated from soybean nodules in Brazil, but the ecological importance of these symbiotic associations is still to be determined.

Introduction

Several leguminous and a few non-leguminous plants establish a N_2-fixing symbiosis with bacteria which were described in the past as *Rhizobium* Frank 1889, and the strains able to nodulate soybean (*Glycine max* (L.) Merrill), characterized by the slow growth rate and alkaline reaction in yeast-extract mannitol medium were first classified as *R. japonicum* (Fred et al., 1932; Jordan and Allen, 1974). In the following decades, many differences were shown between *R. japonicum* and other defined rhizobial species resulting, fifty years later, in the reclassification of soybean rhizobia into a new genus and species, *Bradyrhizobium japonicum* (Jordan, 1982). Later, morphological, physiological and genetic differences were reported within the *B. japonicum* species, resulting in a division into two groups, the new species designated *B. elkanii* (Anonymous, 1993; Kuykendall et al., 1992).

Some strains, characterized by the fast growth rate and acid reaction *in vitro*, were isolated in 1982 from soybean nodules collected in the People's Republic of China, within the center of origin and diversity for the soybean rhizobia (Keyser et al., 1982). The fast growing strains were only effective, i.e., with nodules able to fix N_2, with the primitive soybean cultivar Peking (= PI17852.B) and with *G. soja* Sieb. and Zucc., the putative wild ancestor-progenitor of the cultivated soybean. They were either completely ineffective or poorly effective in symbiosis with commercial soybean cultivars grown in the USA (Keyser et al., 1982).

Comparative studies of nutrient requirements, susceptibility to antibiotics, tolerance to NaCl, plasmid profile, localization of nodulation genes, deoxyribonucleic acid (DNA) hybridization, among other characteristics, were reported by Keyser et al. (1982), Sadowsky and Bohlool (1983), Sadowsky et al. (1983), Scholla et al. (1984) and Stowers and Eaglesham (1984) and a new species was proposed, *R. fredii*, with two chemovars, *fredii* and *siensis* (Scholla and Elkan,

* FAX No:+55433716100. E-mail:hungria@cnpso.embrapa.br

1984). Later, other strains were isolated from different areas of China and Vietnam (Buendía-Clavería et al., 1989; Dowdle and Bohlool, 1985; Xu and Ge, 1984) and, as a result of more detailed studies with numerical taxonomy, a new genus was described, with two species *Sinorhizobium fredii* and *S. xinjiangensis* (Chen et al., 1988). Although this nomenclature was not initially endorsed by the taxonomists (Jarvis et al., 1992; Martinez-Romero and Jarvis, 1993), the genus *Sinorhizobium* and the species *S. fredii* were recently revalidated (Lajudie et al., 1994; Lindström et al., 1995), but the taxonomic position of *S. xinjiangensis* remains to be determined (Lajudie et al., 1994).

Soybean is the most important leguminous crop in Brazil and most Brazilian cultivars are derived from North American genotypes. Several studies have shown that American cultivars do not nodulate or form nodules unable to fix N_2 with *S. fredii* (Buendía-Clavería and Ruiz-Saínz, 1985; Devine, 1985; Keyser et al., 1982; Stowers and Eaglesham, 1984). However, recently it was demonstrated that several of these genotypes were able to form N_2-fixing nodules with *S. fredii* strain USDA 257 (Balatti and Pueppke, 1992). The objective of this paper was then to verify the nodulation ability of some cultivars of the Brazilian germplasm bank with *S. fredii* and *S. xinjiangensis* strains. Preliminary studies on nodule occupancy by *S. fredii* and *B. elkanii* were also reported.

Material and methods

Rhizobial strains

- USDA 205 (=ATCC 35423, =LMG6217, =PRC205), type strain of *S. fredii* (received from Dr P van Berkum, USDA, Beltsvile, MD, USA).
- CCBAU 105 (=RX22) - *S. xinjiangensis*, isolated from Xinjiang, Peoples's Republic of China (received from Dr E T Wang, Beijing Agricultural University, Beijing, China).
- CCBAU 114 (=RT15), *S. fredii*, isolated from Tianjin, People's Republic of China (also received from Dr E T Wang).
- 29 w, *B. elkanii*, isolated in Brazil and recommended in commercial inoculants since 1979.

Soybean genotypes

Eighty soybean (*G. max* (L.) Merrill) cultivars were obtained from the Brazilian germplasm bank (Empre-

Table 1. Ability of 80 soybean cultivars to form N_2-fixing nodules with *Sinorhizobium fredii* strains USDA 205, CCBAU 114 and *S. xinjiangensis* strain CCBAU 105

Cultivar	Genealogy	Nodulation
BR-1	Hill × L-356	+[a]
BR-2	Hill × Hood	-
BR-6	Bragg(3) × Santa Rosa	-
BR-8	Bienville × Hampton	+
BR-12	Bienville × Hood	+
BR-13	Bragg(4) × Santa Rosa	-
BR-14	Santa Rosa × Campos Gerais	+
BR-15	Santa Rosa × LoD 76-761	-
BR-16	D 69-B 10-M 58 × Davis	+
BR-24	Paraná × Davis	+
BR-28	Santa Rosa × BR 78-11202	+
BR-35	Selection within Cristalina	+
BR-37	União(2) × Lo 76-1763	-
Bragg	Jackson × D 49-2491	-
Campos Gerais	Arksoy × Ogden	-
CEP 10	IAS 2 × D70-3185	+
CEP 12	Bragg × Hood	+
CEP 16	IAS 2 × Pérola	+
CEP 20	CTS 132 × Forrest	+
Davis	D 49-2573 × N 45-1497	+
Doko	Selection within population RB 72-1	-
Dourados	Selection within Andrews	-
EMBRAPA 9	Lancer × BR 79-251-1	+
EMGOPA 302	Paraná × Mandarin	+
EMGOPA 303	IAC 73-2736-10 × IAC-6	-
EMGOPA 307	GO 79-3090 × Paranagoiana	+
EMGOPA 309	BRB 214	+
FT-2	Selection within IAS-5	-
FT-4	D 65-3076 × D 64-4636	+
FT-5	FT 9510 × Sant'Ana	-
FT-7	FT-8184 (=FT-4) × Davis	+
FT-12	PR 9510 × Prata	-
FT-14	PR 9510 × Sant'Ana	-
FT-16	FT-440 × Campos Gerais	+
FT-17	Selection within FT-2	-
FT-18	PR 9510 × Prata	-
FT-19	Sta. Rosa × (Selection in CajemeX S.Luiz)	-
FT-20	FT 184 (=FT-4) × Davis	+
FT-Bahia	Selection within Cristalina	+
FT-Canarana	Cristalina × FT-1	+
FT-Cristalina	Selection within UFV-1	+
FT-Guaíra	Lancer × União	+
FT-Manacá	FT-907 × Lancer	+
FT-Seriema	M-2 × FT-1	+
GO BR-26	E 77-510-3 × BR 78-11.202	+
IAC-2	La 41-1219 × Yelnanda	+
IAC-4	IAC-2 × Hardee	+
IAC-6	Selection within population RB 72-1	+
IAC-11	Paraná × (Davis × IAC 73-1364)	+
IAC-12	Paraná × IAC 73-231	-
IAC-15	IAC 77-3086 × Paraná	+
IAC -100	IAC 78-2318 × IAC-12	-
Invicta	Lancer × ESSEX	+
IPAGRO 20	(Sta Rosa × Asksoy) × (Majos × Kanro)	+
Ivaí	Majos × Hood	+
Ivorá	(Davis × Shinanomejiro) × (Hogyoku × Amarela Comum)	+
MG BR-22	Bossier × Paraná	+

Table 1. contd.

MS BR-17	Lo 76-732 × Lo D76-66	-
MS BR-21	São Luiz × Davis	+
MS BR-34	D 64-4636 × IAC-7	+
Nova IAC-7	Selection within IAC-7	+
Numbaíra	Davis × IAC 71-1113	+
OCEPAR 3	(Halesoy × Volstate) × (Hood × Rhosa)	+
OCEPAR 4	R 70-733 × Davis	+
OCEPAR 5	Coker 136 × Co 72-260	+
OCEPAR 6	(PI 230.979 × Lee 68) × [(Davis × Bragg) × (Dare × Davis)]	-
OCEPAR 8	Selection in Paraná	+
OCEPAR 11	Davis × Paraná	+
OCEPAR 14	Davis × União	+
Paraná	Hill × D 52-810	+
Paranagoiana	Natural mutation in Paraná	+
Paranaíba	Davis × IAC 72-2211	+
Tiaraju	Industrial × Asomusume	-
Timbira	Selection within population RB 72-1	-
UFV-1	Natural mutation in Viçoja	-
UFV-9	Selection within UFV-1	-
UFV-10	Santa Rosa × UFV-1	-
UFV/ITM-1	Paraná × Viçoja	+
União	D 65-2874 × Hood	+
Viçoja	D 49-2491 (2) × Improved Pelican	-

(+) positive and (-) negative nodulation with the three rhizobial strains, confirmed with three replicates for each strain.

sa Brasileira de Pesquisa Agropecuária, Centro de Pesquisa de Soja, Londrina, PR). Their genealogy is described in Table 1. The material included Brazilian genotypes and two North American cultivars, Davis and Bragg, introduced in Brazil in the earlier sixties.

Nodulation ability with Sinorhizobium

Soybean seeds were surface sterilized, aseptically pre-germinated for three days and transferred to 500-mL glass flasks containing N-free sterile nutrient solution (Andrade and Hamakawa, 1994). Each seedling was then inoculated individually with 1 mL of inoculant (10^9 cells mL^{-1}) of each of the following strains: USDA 205, CCBAU 105 and CCBAU 114. The seedlings were grown under greenhouse conditions in a completely randomized block design with three replicates; plants were harvested 28 days after inoculation. Nodulation was considered positive when all three replicate plants had spherical pink ribbed nodules (average diameter of 2 mm) and green shoots (Fix^+ phenotype). None of the non-inoculated controls nodulated. Nodules of each treatment were also serologically tested, by agglutination, against the anti-sera of each of the inoculated strains (Somasegaran and Hoben, 1985), to confirm the nodulation results.

Nodule occupancy by S. fredii *and* B. japonicum

Twenty cultivars identified as Fix^+ with *Sinorhizobium* strains were inoculated individually with *S. fredii* USDA 205 or *B. elkanii* 29 w or with a mixture of the two strains, in the proportion of 1:1 (v:v), 10^9 cells mL^{-1} of each strain. One mL of the single or mixed inoculum was added to each pre-germinated seedling with the same experimental design and growth conditions as described in the first experiment. Plants were harvested at 28 days after inoculation and nodule occupancy was verified by the serological reaction of all nodules against the anti-sera of USDA 205 and 29 w, as described before.

Results and discussion

Several reports have shown that North American commercial soybean cultivars do not nodulate effectively with *S. fredii* (Buendía-Clavería and Ruiz-Saínz, 1985; Devine, 1985; Keyser et al., 1982), while in primitive or unimproved lines of Asian origin the frequency of Fix^+ symbiosis with type strain USDA 205 ranged from 38 to 85% (Devine, 1985). This suggests some compatibility between soybean and the fast-growing rhizobia has been lost during cultivar development. An inheritance study also showed that the cultivar Peking carries the recessive allele conditioning effective nodulation with USDA 205, in a Mendelian segregation (Devine, 1984).

This study demonstrated that 66% of the 80 Brazilian genotypes tested formed effective nodules with each one of the three *Sinorhizobium* strains tested (Table 1). Nodule number varied from 4 to 20, with an average of 8 nodules per plant (data not shown). Fix^+ genotypes were able to associate effectively with the three strains. The phenotypes observed in the Fix^- cultivars were absence of nodulation, presence of swellings or small irregular nodules without leghemoglobin. Balatti and Pueppke (1992) reported that 17% of 194 North American lines formed N_2-fixing nodules with *S. fredii* strain USDA 257, and the frequency of Fix^+ phenotypes was positively correlated with increasing maturity group. The Brazilian genotypes belong to maturity groups VI or higher, a factor which could have contributed to the very high percentage of Fix^+ phenotypes observed in this study.

Nodulation of cultivar Davis by *S. fredii* USDA 257 has been reported (Balatti and Pueppke, 1992; Dowdle and Bohlool, 1985) and this genotype was a progenitor of 20% of the cultivars identified in this study as positive nodulators. A further 19% of positive nodulators had cultivar Paraná, a Fix$^+$ genotype, as a progenitor. Consequently, the generalized response to *Sinorhizobium* may result from the restricted genetic basis of the germplasm analysed. Indeed, both North American (Delannay et al., 1983) and Brazilian (Hiromoto and Vello, 1986) soybean cultivars come from an extremely narrow range of ancestors.

Some of the claimed advantages of using fast-growing strains for soybean inoculation include the facility of commercial production, easier establishment in soils, displacement of indigenous *B. japonicum* strains and easier manipulation of genes (Buendía-Clavería et al., 1994; Chatterjee et al., 1990; Cregan and Keyser, 1988). However, the poor nodulation with commercial soybean cultivars has discouraged more studies using of *S. fredii*. Furthermore, in controlled environments, *B. japonicum* outcompeted *S. fredii* (Cregan and Keyser, 1988; Dowdle and Bohlool, 1987; McLoughlin et al., 1985). This earlier report was confirmed when 20 cultivars identified as Fix$^+$ in the previous step were co-inoculated with *B. elkanii* and *S. fredii* and most, or all, nodules were occupied by *B. elkanii* (Table 2). Consequentely, growth rate on agar medium was not related with the ability to compete for nodule occupancy. In Spanish alkaline soils devoid of both *B. japonicum and S. fredii*, the fast-growing rhizobia were better competitors (Buendía-Clavería et al., 1994). Almost all Brazilian soils are acid, and this condition appear to favor nodule occupancy by *Bradyrhizobium.* On average, nodule number formed on these cultivars inoculated with USDA 205 (8 nodules plant^{-1}) was significantly lower ($p \leq 0.05$) than when inoculated with 29 w (17.6 nodules) or the mixed inoculum (16.7 nodules). On some cultivars, e.g. Davis, nodule number was lower but not statistically different from the other two inoculation treatments and, in others, e.g., Guaíra, nodule number was similar for all treatments (Table 2).

In Brazilian acid soils, *S. fredii* strains used in this were not suitable for commercial inoculants. It is possible that other fast growing-rhizobia native to Brazilian soils may be able to nodulate soybean cultivars which are Fix$^+$ with *Sinorhizobium.* Indeed, in soil surveys of recent past, between 3 and 5% of the nodules on trap plants of Brazilian cultivars were formed by fast-growing strains. We are now working on the identification of these strains, but the ecological importance of such symbiotic associations is still to be determined.

Table 2. Number of nodules on 20 soybean cultivars inoculated with single or mixed (1:1, v:v) inoculum of *S. fredii* strain USDA 205 and *B. elkanii* strain 29 w at 28 days after inoculation and the proportion of the nodules formed by each strain when the seedlings received the mixed inoculum

Cultivar	Nodule number (n° plant^{-1})			Nodule occupancy (%)	
	USDA 205	29 w	Mixed	29 w	USDA 205
BR-1	4.0 b[a]	20.7 a	22.3 a	100 A[b]	0 B
BR-14	4.0 b	16.7 a	15.7 a	100 A	0 B
BR-16	5.0 c	18.3 a	14.3 b	100 A	0 B
BR-24	9.7 b	16.7 a	21.0 a	100 A	0 B
BR-28	4.0 b	17.3 a	16.3 a	100 A	0 B
CEP 10	6.0 b	22.3 a	26.7 a	50 A	50 A
Davis	11.3 a	16.3 a	19.3 a	75 A	25 B
EMBRAPA 9	8.0 b	18.3 a	16.3 a	50 A	50 A
EMGOPA 307	10.7 a	17.2 a	16.0 a	75 A	25 B
FT-7	8.0 a	14.0 a	13.3 a	100 A	0 B
FT-Cristalina	7.0 a	12.7 a	12.3 a	100 A	0 B
FT-Guaíra	18.0 a	18.0 a	18.0 a	75 A	25 B
IAC-4	4.3 b	17.0 a	16.7 a	75 A	25 B
IAC-6	10.0 a	18.3 a	13.7 a	100 A	0 B
Invicta	18.0 a	27.3 a	18.0 a	100 A	0 B
IPAGRO 20	7.0 b	16.7 a	12.3 ab	100 A	0 B
OCEPAR 3	6.7 b	15.0 a	16.0 a	75 A	25 B
OCEPAR 8	9.0 b	16.0 a	13.3 ab	75 A	25 B
Paraná	9.0 a	15.0 a	10.3 a	100 A	0 B
União	4.0 b	23.0 a	22.0 a	100 A	0 B

[a] Values for nodule number represent the mean of three replicates and, when followed by the same letter within each line did not show statistical difference (Tukey's test, $p \leq 0.05$).
[b] Values followed by the same capital letter within each line did not show statistical difference (Tukey's test, $p \leq 0.05$).

Acknowledgments

The authors acknowledge fellowships from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil). To Rinaldo Benedito Conceição, for technical help. To Dr Leones Alves de Almeida, for the seeds and information about the soybean germplasm, as well as for advice during the experiments. To Dr E T Wang and Dr Peter van Berkum, for sending the strains. To Dr Mercedes C C Panizzi and Robert M Boddey, for suggestions and helpful discussion. To the financial support from FINEP/CNPq/MCT (41.96.0884-00), as a Group of Excellence on Nitrogen Fixation. This paper was approved by the Technical Director of Embrapa-Soja as manuscript 002/96.

References

Andrade D S and Hamakawa P J 1994 Estimativa do número de células viáveis de rizóbio no solo e em inoculantes por infecção em plantas. *In* Manual de Métodos Empregados em Estudos de Microbiologia Agrícola, Brasília. Eds. M Hungria and R S Araujo. pp 63–94. EMBRAPA-SPI, Brasília.

Anonymous 1993 International commitee on systematic bacteriology subcommittee on the taxonomy of *Agrobacterium* and *Rhizobium*. Int. J. Syst. Bacteriol. 43, 622.

Balatti P A and Pueppke S G 1992 Identification of North American soybean lines that form nitrogen-fixing nodules with *Rhizobium fredii* USDA257. Can. J. Plant Sci. 72, 49–55.

Buendía-Clavería A M, Rodriguez-Navarro D N, Santamaría-Linaza C, Ruiz-Saínz J E and Temprano-Vera F 1994 Evaluation of the symbiotic properties of *Rhizobium fredii* in European soils. Syst. Appl. Microbiol 17, 155–160.

Buendía-Clavería A M and Ruiz-Saínz J E 1985 Isolation of mutants of fast-growing soybean strains that are effective on commercial soybean cultivars. Physiol. Plant. 64, 507–512.

Buendía-Clavería A M, Temprano-Vera F, Echevarrieta R O and Ruiz-Saínz J E 1989 *Rhizobium fredii*: Las bacterias de crecimiento rápido que forman nódulos fijadores de nitrógeno en la soja. Dirección General de Investigación y Extensión Agrarias, Centro de Informacion y Documentacion Agraria, Sevilla. 48 p.

Chatterjee A, Balatti P A, Gibbons W and Pueppke S G 1990 Interactions of *Rhizobium fredii* USDA 257 and nodulation mutants derived from it with the agronomically improved soybean cultivar McCall. Planta 180, 303–311.

Chen W X, Yan G H and Li J L 1988 Numerical taxonomic study of fast-growing soybean rhizobia and a proposal that *Rhizobium fredii* be assigned to *Sinorhizobium* gen. nov. Int. J. Syst. Bacteriol. 38, 393–397.

Cregan P B and Keyser H H 1988 Influence of *Glycine* spp. on competitiveness of *Bradyrhizobium japonicum* and *Rhizobium fredii*. Appl. Environ. Microbiol. 54, 803–808.

Delannay X, Rodgers D M and Palmer R G 1983 Relative genetic contributions among ancestral lines of North American soybean cultivars. Crop Sci. 23, 944–949.

Devine T E 1984 Inheritance of soybean nodulation response with a fast-growing strain of *Rhizobium*. J. Hered. 75, 359–361.

Devine T E 1985 Nodulation of soybean plant introduction lines with the fast-growing rhizobial strain USDA 205. Crop Sci. 25, 354–356.

Dowdle S F and Bohlool B B 1985 Predominance of fast-growing *Rhizobium japonicum* in a soybean field in the People's Republic of China. Appl. Environ. Microbiol. 50, 1171–1176.

Dowdle S F and Bohlool B B 1987 Intra- and inter-specific competition in *Rhizobium fredii* and *Bradyrhizobium japonicum* as indigenous and introduced organisms. Can. J. Microbiol. 33, 990–995.

Fred E B, Baldwin I L and McCoy E 1932 Root Nodule Bacteria of Leguminous Plants. The University of Wisconsin Press, Madison.

Hiromoto D M and Vello M A 1986 The genetic base of Brazilian soybean (*Glycine max* (L.) Merrill) cultivars. R. Bras. Genet. 9, 295–306.

Jarvis B D W, Downer H L and Young J P W 1992 Phylogeny of fast-growing soybean-nodulating rhizobia supports synonymy of *Sinorhizobium* and *Rhizobium* and assignment of *Rhizobium fredii*. Int. J. Syst. Bacteriol. 42, 93–96.

Jordan D C 1982 Transfer of *Rhizobium japonicum* Buchanan 1980 to *Bradyrhizobium* gen. nov., a genus of slow growing root-nodule bacteria from leguminous plants. Int. J. Syst. Bacteriol. 32, 136–139.

Jordan D C and Allen N O 1974 Family III. *Rhizobiaceae* Conn 1938. *In* Bergey's Manual of Determinative Bacteriology. pp 235–244. Williams and Wilkins Co., Baltimore.

Keyser H H, Bohlool B B, Hu T S and Weber D F 1982 Fast-growing rhizobia isolated from root nodules of soybeans. Science 215, 1631–1632.

Kuykendall L D, Saxena B, Devine T E and Udell S E 1992. Genetic diversity in *Bradyrhizobium japonicum* Jordan 1982 and a proposal for *Bradyrhizobium elkanii* sp. nov. Can. J. Microbiol. 38, 501–505.

Lajudie P de, Willems A, Pot B, Dewettinck D, Maestrojuan G, Neyra M, Collins M D, Dreyfus B, Kersters K and Gillis M 1994 Polyphasic taxonomy of rhizobia: Emendation of the genus *Sinorhizobium* and description of *Sinorhizobium meliloti* comb. nov., *Sinorhizobium saheli* sp. nov., and *Sinorhizobium teranga* sp. nov. Int. J. Syst. Bacteriol. 44, 715–733.

Lindström K, van Berkum P, Gillis M, Martínez E, Novikova N and Jarvis B 1995 Report from the roundtable on rhizobium taxonomy. *In* Nitrogen Fixation: Fundamentals and Applications. Eds. I A Tikhonovich, N A Provorov, V I Romanov and W E Newton. pp 807–810. Kluwer Academic Publishers, Dordrecht.

Martinez-Romero M E and Jarvis B D W 1993 International committee on systematic bacteriology subcommittee on the taxonomy of *Agrobacterium* and *Rhizobium*. Int. J. Syst. Bacteriol. 43, 622.

McLoughlin T J, Owens P A and Scott A 1985 Competition studies with fast-growing *Rhizobium japonicum* strains. Can. J. Microbiol. 31, 220–223.

Sadowsky M J and Bohlool B B 1983 Possible involvement of a megaplasmid in nodulation of soybean by fast growing rhizobia from China. Appl. Environ. Microbiol. 46, 906–911.

Sadowsky M J, Bohlool B B and Keyser H H 1983 Biochemical characterization of fast and slow-growing rhizobia that nodulate soybeans. Int. J. Syst. Bacteriol. 33, 716–722.

Scholla M H and Elkan G H 1984 *Rhizobium fredii* sp nov, a fast-growing species that effectively nodulates soybeans. Int. J. Syst. Bacteriol. 34, 484–486.

Scholla M H, Moorefield J A and Elkan G H 1984 Deoxyribonucleic acid homology between fast-growing soybean nodulating bacteria and other rhizobia. Int. J. Syst. Bacteriol. 34, 283–286.

Somasegaran P and Hoben H J 1985 Methods in Legume-*Rhizobium* Technology. Niftal, Hawaii. 367 p.

Stowers M D and Eaglesham A R J 1984 Physiological and symbiotic characteristics of fast-growing *Rhizobium japonicum*. Plant Soil 77, 3–14.

Xu L M and Ge C 1984 Physiological-biochemical characteristics and symbiotic responses of the fast-growing *Rhizobium japonicum*. Soybean Sci. 3, 102–109.

Section editor: R O D Dixon