

Numerical Taxonomic Study of Fast-Growing Soybean Rhizobia and a Proposal that *Rhizobium fredii* Be Assigned to *Sinorhizobium* gen. nov.

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A total of 33 strains of fast-growing soybean rhizobia isolated from soil and soybean nodules collected in China and 25 strains belonging to the genera *Rhizobium*, *Bradyrhizobium*, and *Agrobacterium* were compared by numerical taxonomic techniques, using 240 different characters. Our results indicated that all of the strains of fast-growing soybean rhizobia which we examined are closely related (guanine-plus-cytosine content, 59.9 to 63.8 mol%) and are separated from *Rhizobium* and *Bradyrhizobium* at the generic level. Based on numerical taxonomy, deoxyribonucleic acid (DNA) base ratio determinations, DNA-DNA hybridization data, serological analysis data, the composition of extracellular gum, bacteriophage typing data, and soluble protein patterns, we propose that the fast-growing soybean rhizobia represent members of a new genus rather than a species of *Rhizobium* (*Rhizobium fredii*); we propose *Sinorhizobium* gen. nov. as an appropriate generic name. The type species of the new genus is *Sinorhizobium fredii* comb. nov. (basonym, *Rhizobium fredii* Scholla and Elkan 1984), and the type strain is strain ATCC 35423 (= USDA 205). For the other species we propose the name *Sinorhizobium xinjiangensis* sp. nov.; the type strain of this species is strain CCBAU 110, which has been deposited in the Beijing Agricultural University Culture Collection, Beijing, People's Republic of China.

Root nodule bacteria of leguminous plants are presently classified into two genera, *Rhizobium* and *Bradyrhizobium* (4). *Rhizobium* includes three species, *Rhizobium meliloti*, *Rhizobium loti*, and *Rhizobium leguminosarum*. *Bradyrhizobium* has only one species, *Bradyrhizobium japonicum*, and includes strains that are capable of nodulating lupines, soybeans, and certain other legumes (4).

Recently, a new group of fast-growing soybean rhizobia was identified from soil and soybean nodules collected in the People's Republic of China by Chinese and American scientists (6, 25). The taxonomic status of these bacteria was uncertain, but they may represent a transitional group falling between the genera *Rhizobium* and *Bradyrhizobium* (4).

Scholla and Elkan (13) proposed a new species name for this group, *Rhizobium fredii*, based mainly on deoxyribonucleic acid (DNA) hybridization comparisons of five strains of these bacteria with representatives of the genera *Rhizobium* and *Bradyrhizobium* and on a review of some of the characteristics of 11 strains of fast-growing soybean rhizobia.

To extend the current information on the fast-growing soybean bacteria, we performed a numerical taxonomic study of a large number of strains. We propose that they be assembled in a new genus that includes two species.

MATERIALS AND METHODS

Organisms. A total of 58 strains were used in this study (Table 1); 33 strains were fast-growing soybean rhizobia isolated from soil and soybean nodules collected in the People's Republic of China, and the other 25 strains were representatives of *Rhizobium*, *Bradyrhizobium*, and *Agrobacterium*.

Features. Each of the 58 strains was characterized by determining 240 different coding features. The following features were considered: (i) utilization as sole carbon sources (0.1%) of cellobiose, D-mannose, salicin, ammonium tartrate, casein hydrolysate, fructose, sodium acetate, sorbose, raffinose, D-melezitose monohydrate, hexose 6-phos-

phate, L-rhamnose, D-ribose, sodium hippurate, lactose, dulcitol, creatinine, sodium D-gluconate, urea, sodium succinate, meso-erythritol, glucose, D-erythrose, D-turanose, D-galactose, D-(-)-tagatose, D-lyxose, D-amygdalin, malate, xylose, D-glutamic acid, tris(hydroxymethyl)aminomethane, 2-methionine, sodium benzoate, magnesium citrate, D-malic acid, ammonium oxalate, pyruvate, L-cysteine, DL-asparagine, vanillic acid, glycine, D-fucose, methyl-DL-glucoside, D-sorbitol, sucrose, calcium malonate, sodium alginate, and inositol; (ii) utilization as sole nitrogen sources (0.1%) of L-phenylalanine, D-glutamic acid, DL-glutamine, L-valine, DL-serine, L-lysine, DL-cysteine hydrochloride, DL-lysine hydrochloride, D-tryptophan, L-arginine, DL-valine, L-histidine, DL-aspartic acid, glycyl-DL-valine, DL-cystine, L-tryptophan, D-arginine, D-serine, L-serine, L-isoleucine, DL-proline, DL-alanine, DL-asparagine, DL-arginine hydrochloride, DL-glutamic acid, L-cystine hydrochloride, glycine, D-cystine, L-arginine hydrochloride, D-histidine monohydrochloride, isocytosine, and pyrimidine; (iii) requirement for folic acid, thioctic acid, vitamin B₁, calcium D-pantothenate, riboflavin, ascorbic acid, nicotinic acid, nicotinamide, vitamin B₁₂, D-biotin, and pyridoxine hydrochloride; (iv) tolerance to dyes (0.1%, wt/vol), such as sudan 1, erythrosin A, picrocarmine, safranin T, orcein, rosanilin, brilliant cresyl blue, gentian violet, bromthymol blue, bromophenol blue, methyl red, bromocresol purple, neutral red, auramine O.B.S, light green SF, methyl green, iodine green, bismarck brown, fuchsin basic, and nigrosine; (v) tolerance to the antibiotics doxycycline (1, 5, and 25 µg/ml), tetracycline (1, 5, and 25 µg/ml), aureomycin (1, 5, and 25 µg/ml), vancomycin (5, 25, and 125 µg/ml), chloramphenicol (5, 25, and 125 µg/ml), terramycin (1, 5, and 25 µg/ml), erythromycin (25 and 125 µg/ml), penicillin (5 and 25 µg/ml), kanamycin (1, 5, and 25 µg/ml), gentamicin (5 and 25 µg/ml), streptomycin (1, 5, and 125 µg/ml), medemycin (125 µg/ml), jiemycin (125 µg/ml), and amphotericin B (125 µg/ml); (vi) tolerance to the pesticides 50% malathion (2,000× and 1,000×), 72.0% chlordimeform (2,000×, 1,000×, and 500×), 38.08% dimethoate (2,000×, 1,000×, 500×, and 250×), 50%

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TABLE 1. Origins and hosts of the strains of bacteria examined

Strain	Host	Origin	Source ^a
102F-28	<i>Medicago sativa</i>	?	UCD
H ₁	<i>Melilotus albus</i>	Heilongjiang	CCBAU
1002 ^T (= ATCC 9930 ^T) (<i>R. meliloti</i>) ^b	?	?	USDA
002	<i>Medicago sativa</i>	Xinjiang	CCBAU
162X68 (<i>R. leguminosarum</i> biovar <i>trifolii</i>)	?	?	USDA
2370 ^T (= ATCC 10004 ^T)	?	?	USDA
(<i>R. leguminosarum</i> biovar <i>viceae</i>)			
127K17 (<i>R. leguminosarum</i> biovar <i>phaseoli</i>)	?	?	USDA
1-3	<i>Vicia sepium</i>	Beijing	CCBAU
38	<i>Astragalus sinicus</i>	Nanjing	NAU
7034	<i>Astragalus sinicus</i>	Nanjing	NAU
103	<i>Astragalus sinicus</i>	Nanjing	NAU
Bs12	<i>Astragalus sinicus</i>	Hubei	HAU
CCBAU 101 (= 6-1)	<i>Vigna savi</i>	Beijing	CCBAU
CCBAU 102 (= 131)	<i>Glycine soja</i>	Heilongjiang	CCBAU
2054	<i>Glycine soja</i>	Liaoning	SFRI (CAAS)
2053	<i>Glycine soja</i>	Liaoning	SFRI (CAAS)
2048	<i>Glycine soja</i>	Liaoning	SFRI (CAAS)
2047	<i>Glycine soja</i>	Liaoning	SFRI (CAAS)
CCBAU 103 (= RX11)	<i>Glycine max</i>	Xinjiang	CCBAU (S. S. Yang)
CCBAU 104 (= RX12)	<i>Glycine max</i>	Xinjiang	CCBAU (S. S. Yang)
CCBAU 105 (= RX22)	<i>Glycine max</i>	Xinjiang	CCBAU (S. S. Yang)
CCBAU 106 (= RX23)	<i>Glycine max</i>	Xinjiang	CCBAU (S. S. Yang)
CCBAU 107 (= RX31)	<i>Glycine max</i>	Xinjiang	CCBAU (S. S. Yang)
CCBAU 108 (= RX33)	<i>Glycine max</i>	Xinjiang	CCBAU (S. S. Yang)
CCBAU 109 (= RX41)	<i>Glycine max</i>	Xinjiang	CCBAU (S. S. Yang)
CCBAU 110 ^T (= RX42 ^T)	<i>Glycine max</i>	Xinjiang	CCBAU (S. S. Yang)
CCBAU 111 (= RT7)	<i>Glycine max</i>	Tianjin	CCBAU (S. S. Yang)
CCBAU 112 (= RT10)	<i>Glycine max</i>	Tianjin	CCBAU (S. S. Yang)
CCBAU 113 (= RT13)	<i>Glycine max</i>	Tianjin	CCBAU (S. S. Yang)
CCBAU 114 (= RT15)	<i>Glycine max</i>	Tianjin	CCBAU (S. S. Yang)
CCBAU 115 (= RT18)	<i>Glycine max</i>	Tianjin	CCBAU (S. S. Yang)
CCBAU 116 (= RT20)	<i>Glycine max</i>	Tianjin	CCBAU (S. S. Yang)
CCBAU 117 (= RT22)	<i>Glycine max</i>	Tianjin	CCBAU (S. S. Yang)
CCBAU 118 (= RT41)	<i>Glycine max</i>	Tianjin	CCBAU (S. S. Yang)
USDA 191	From soil	Shanghai	USDA
USDA 192	<i>Glycine soja</i>	Shandong	USDA
USDA 193	<i>Glycine soja</i>	Shanxi	USDA
USDA 194	<i>Glycine soja</i>	Henan	USDA
USDA 201	<i>Glycine soja</i>	Henan	USDA
USDA 205 ^T	<i>Glycine soja</i>	Henan	USDA
USDA 206	<i>Glycine soja</i>	Henan	USDA
USDA 208	<i>Glycine soja</i>	Henan	USDA
USDA 214	<i>Glycine soja</i>	Henan	USDA
USDA 217	<i>Glycine soja</i>	Henan	USDA
USDA 257	<i>Glycine soja</i>	Henan	USDA
122-3	<i>Glycine max</i>	Hubei	IOC (CAAS)
305	<i>Glycine max</i>	Hubei	IOC (CAAS)
B15	<i>Glycine max</i>	Liaoning	IOC (CAAS)
2110	<i>Glycine max</i>	United States	
Y11	<i>Glycine soja</i>	Hubei	IOC (CAAS)
USDA 6 ^T (= ATCC 10324 ^T) (<i>B. japonicum</i>)	<i>Glycine max</i>	?	USDA
110	<i>Glycine max</i>	?	USDA
SM	<i>Glycine max</i>	?	UW
Ry61	<i>Glycine max</i>	?	CCBAU
113-2	<i>Glycine max</i>	?	IOC (CAAS)
B6S5 (<i>Agrobacterium tumefaciens</i>)			IBAC
C ₅₈ (<i>Agrobacterium tumefaciens</i>)			IBAC
1.150 (<i>Agrobacterium radiobacter</i>)			IMAC

^a USDA, United States Department of Agriculture, Beltsville, Md.; CCBAU, Beijing Agricultural University Culture Collection, Beijing, People's Republic of China; SFRI (CAAS), Soil and Fertilizer Research Institute, Chinese Academy of Agricultural Sciences, Beijing, People's Republic of China; IOC (CAAS), Institute of Oil Crops, Chinese Academy of Agricultural Sciences, Beijing, People's Republic of China; UCD, University of California, Davis; NAU, Nanjing Agricultural University, Nanjing, People's Republic of China; HAU, Huazhong Agricultural University, Wuhan, People's Republic of China; UW, University of Wisconsin, Madison; IBAC, Institute of Botany, Academia Sinica, Beijing, People's Republic of China; IMAC, Institute of Microbiology, Academia Sinica, Beijing, People's Republic of China.

^b T = type strain.

phoxin (2,000 $\times$, 1,000 $\times$, 500 $\times$, and 250 $\times$), 20% carbendazol (125 $\times$), and 40% omethoate (100 $\times$, 500 $\times$, and 250 $\times$); (vii) tolerance to potassium tellurite (0.01, 0.025 and 0.05%), silver nitrate (0.05%), sodium deoxycholate (0.05, 0.1, and 0.2%), sodium hypochlorite (2%), and sodium nitrite (0.05 and 0.1%); (viii) growth at pH 4.5, 5.0, 5.5, 6.0, 7.0, 8.5, 9.0, 9.5, 10.0, and 10.5; (ix) growth on yeast mannitol agar (YMA) (21) supplemented with NaCl at concentrations of 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, and 4.5%; (x) reaction in litmus milk; (xi) growth in peptone broth; (xii) reduction of methyl blue; (xiii) growth at 10, 35, and 40°C; (xiv) production of catalase, urease, L-phenylalaninase, peroxidase, and cytochrome oxidase; (xv) nitrate reduction; (xvi) Nile blue reduction; (xvii) acid or alkali production on YMA; (xviii) colonies low and convex, high and convex, butyrous, with entire margins, and translucent or opaque; (xix) turbidity, pellicle, viscous mass, or ring of growth in broth; and (xx) flagella peritrichous, polar, lateral, single, or more than three or six.

Methods. Most of the biochemical tests were conducted on plates inoculated with a multipoint inoculator (5); other tests were conducted in broth. Most cultures were incubated at 28°C; the exceptions were those cultures examined for growth at 10, 35, and 40°C. Growth was observed after incubation for 3 to 7 days for fast growers and for 7 to 10 days for slow growers.

Carbohydrate and organic acid utilization was determined on the medium of White (24) containing the microelements of medium C₅7 (10). Sole nitrogen source utilization was determined on the same medium, except that NaNO₃ was replaced by various nitrogen sources and mannitol was used as the carbon source. Requirements for vitamins and growth factors were studied on Zhou medium (26). Tolerance to antibiotics, pesticides, dyes, other chemicals, and various concentrations of NaCl was tested on YMA. The pH range for growth was examined on YMA by using the method of Thompson and Skerman (20). Reaction in litmus milk was determined by the usual method, and results were observed after 1 to 8 weeks of incubation. Acid or alkali production was determined on YMA supplemented with bromthymol blue indicator, and nitrate reduction was examined by using the modified method of Pohlman (11). Methyl blue reduction was observed by adding 1 drop of 1% methyl blue to a test tube of inoculated milk after 7 days of incubation at 28°C and then incubating the preparation at 37°C for 1 h; disappearance of the blue color was regarded as a positive reaction. Nile blue reduction was detected by using the method of Skinner et al. (16). Urease was detected by the methods of Smibert and Krieg (17), whereas the catalase, phenylalaninase, oxidase, peroxidase, and cytochrome oxidase tests were those described by Skerman (15).

Numerical analysis. Coding of traits, calculating similarity by using the S_j formula, determining the center strain of the group, and clustering by average linkage were all performed by using the methods of Sneath and Sokal (18). Guanine-plus-cytosine (G+C) contents of the DNAs were determined by the method of Marmur and Doty (8).

RESULTS

The results of the computer analysis are presented as a dendrogram in Fig. 1. All of the strains examined were linked at the 38% similarity level, while all of the strains of the fast-growing soybean rhizobia which we examined were grouped together at the 58.5% similarity level and members of the genus *Rhizobium* and of *B. japonicum* were grouped

together at the 62.3 and 70.0% similarity levels, respectively. The three representative strains of the known species of *Agrobacterium* were closely grouped at the 91.8% similarity level and were included in the group *Rhizobium*.

Representative strains of *R. leguminosarum* and *R. meliloti*, and those strains isolated from *Astragalus sinicus* were grouped together at the 83.0, 80, and 80.5% similarity levels, respectively.

Figure 1 shows that the fast-growing soybean rhizobium group exhibits little similarity to the *Rhizobium* group (50%) or *Bradyrhizobium* (38%), and it is obviously separated from these taxa at the generic level. Within the group of fast-growing soybean rhizobia there are two distinct phena. Phenon I includes 25 strains (4 strains isolated from Liaoning, 11 strains isolated from Xinjiang, Heilongjiang, and the suburbs of Beijing and Tianjin, and all of the strains obtained from the United States Department of Agriculture, except strain USDA 194, which is distinct from both phenon I and phenon II). Phenon II includes only six of the strains which we examined, all of which were isolated from the region of Xinjiang.

The differential characteristics of the fast-growing soybean rhizobia and members of *Rhizobium* and *Bradyrhizobium* are shown in Table 2. The differential characteristics of phenon I and II of the fast-growing soybean rhizobium group are shown in Table 3.

The G+C contents of the DNAs of five strains of fast-growing soybean rhizobia range from 59.9 to 63.8 mol% (Table 4).

DISCUSSION

Recently, Scholla and Elkan (13) proposed a new species name for the fast-growing soybean rhizobia (*R. fredii*), but this proposal does not appear to correspond to the DNA homology results of these authors. The mean level of DNA homology between the five strains of fast-growing soybean rhizobia which they used and the type strains of the other species of *Rhizobium* was only 19%, and the mean level of homology between their strains and *Bradyrhizobium* was 21% (14). Our data indicate that the fast-growing soybean rhizobia have a very low level of similarity to either *Rhizobium* or *Bradyrhizobium*. Therefore, it seems incorrect to place the fast-growing rhizobia in the genus *Rhizobium*. They probably should be placed outside both *Rhizobium* and *Bradyrhizobium*.

Patterns of soluble proteins resulting from polyacrylamide gel electrophoresis and ultraviolet scanning show a clear distinction between the fast-growing soybean rhizobia and members of *Rhizobium* and *Bradyrhizobium* (22). Lim and Tan (7) showed that the compositions of extracellular polysaccharides and lipopolysaccharides from fast-growing soybean rhizobia differed not only from those of strains of *Rhizobium* but also from those of strains of *Bradyrhizobium*.

More recently, the relationships between *R. fredii* and the recognized species of *Rhizobium* and *Bradyrhizobium* were investigated by using DNA-DNA hybridization, legume nodulation tests, and phage typing (23). The results provided evidence that the *R. fredii* strains form a distinct DNA homology group which can be divided into subgroups. At 65°C the mean levels of relative homology of 11 strains of *R. fredii* with *R. fredii* reference strains USDA 208 and USDA 191 were 86 and 80%, respectively. These values were significantly higher than the mean levels of relative homology with DNAs from other groups of rhizobia. Based on the results of cross-inoculation, phage-typing, and DNA-DNA

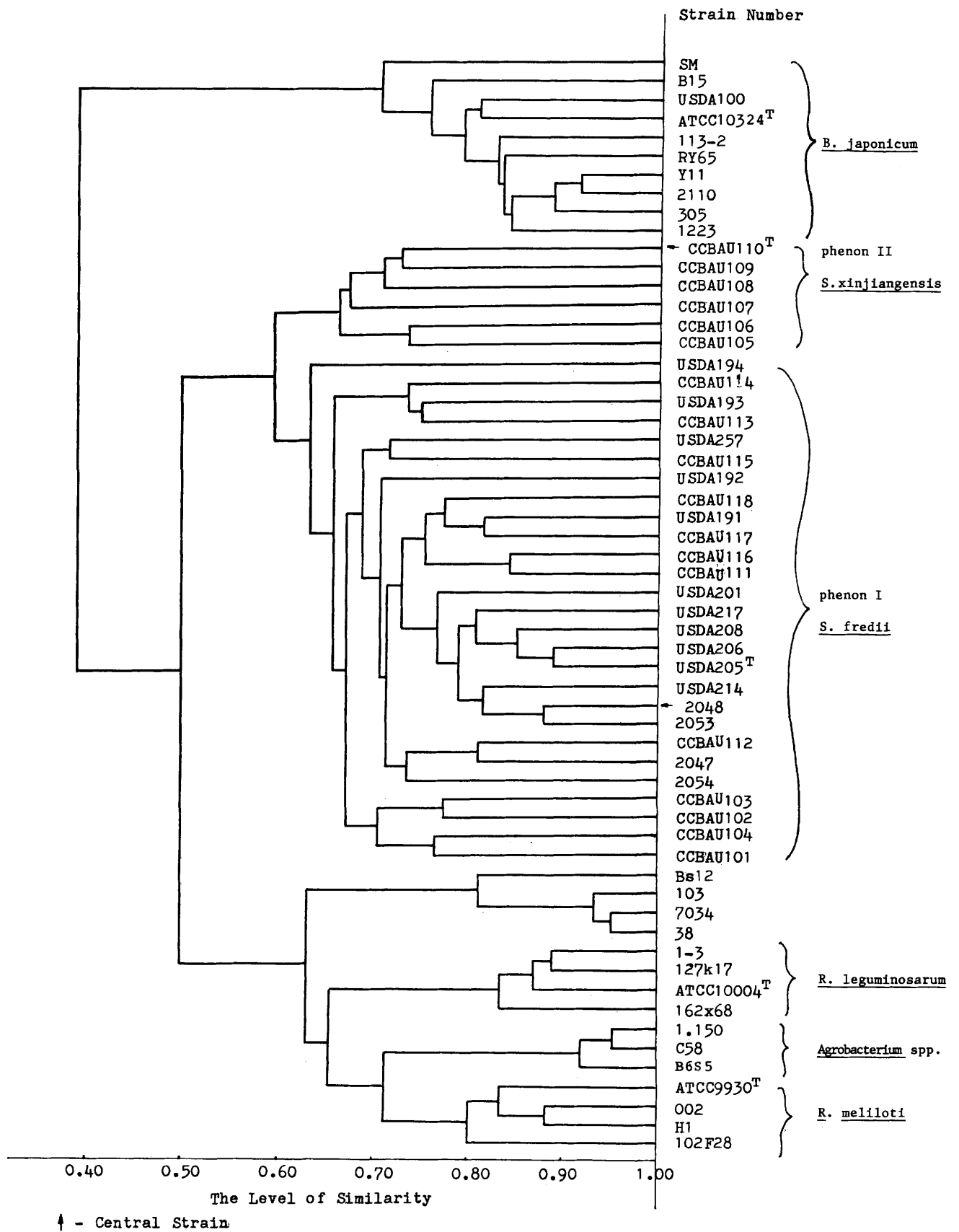


FIG. 1. Dendrogram (average linking clustering mode).

TABLE 2. Differential characteristics of fast-growing soybean rhizobia and members of *Rhizobium* and *Bradyrhizobium*

Characteristic	Fast-growing soybean rhizobia	<i>Rhizobium</i>	<i>Bradyrhizobium</i>
Flagellar arrangement			
Monotrichous	d ^a	—	+
Peritrichous	d	+	—
Growth at pH 5.5	d	d	+
Growth at 35°C	d	+	—
Acid production on YMA	+	+	—
Enzyme production			
Cytochrome oxidase	+	d	d
L-Phenylalaninase	+	—	—
Carbon source utilization			
Cellobiose	+	+	—
D-Mannose	+	+	—
Dulcitol	—	+	—
D-(–)-Tagatose	—	d	—
Salicin	+	d	—
Casein hydrolysate	d	+	+
Erythrose	d	+	—
D-Sorbitol	d	+	+
Sucrose	d	+	—
Inositol	d	+	—
Raffinose	d	+	—
Amonium tartrate	—	d	+
Nitrogen source utilization			
D-Glutamic acid	—	d	+
L-Lysine	d	+	+
L-Histidine	—	+	+
L-Arginine	d	+	+
L-Serine	d	+	+
DL-Asparagine	—	+	—
L-Tryptophan	d	+	+
External requirement for:			
Calcium D-pantothenate	+	—	d
Ascorbic acid	d	+	—
Folic acid	d	—	—
Biotin	d	—	+
Tolerance to:			
Chloramphenicol (5 µg/ml)	+	d	+
Erythromycin (25 µg/ml)	+	d	+
Streptomycin (5 µg/ml)	d	+	+
Vancomycin (5 µg/ml)	+	d	+
Kanamycin (1 µg/ml)	+	d	+
Terramycin (5 µg/ml)	—	d	+
Dentamycin (5 µg/ml)	d	+	+
Sodium nitrite (0.1%)	d	—	d
Brilliant cresyl blue	—	+	+
Nile blue reduction	d	—	—

^a +, At least 95% of the strains are positive; —, more than 95% of the strains are negative; d, less than 95% of the strains are positive.

hybridization experiments, it was concluded that *R. fredii* is taxonomically distinct from other known species in the genera *Rhizobium* and *Bradyrhizobium* (23).

In addition, according to DNA homology data (23) and serological relatedness data (3, 12), strains USDA 194, USDA 201, and USDA 257 were distinguished from other strains of fast-growing soybean rhizobia. However, based on our results, only strain USDA 194 can be separated from phenon I and II.

Based on our numerical taxonomic study and on the data mentioned above, we propose that the fast-growing soybean rhizobia represent a new genus, for which we propose the name *Sinorhizobium* gen. nov. (Si.no.rhi.zóbi.um. L. n. *sinae*, China; Gr. n. *rhiza*, root; Gr. n. *bios*, life; M. L. neut. n. *Sinorhizobium*, rhizobia isolated from roots in China).

Within this genus there are at least two species (phenon I

TABLE 3. Differential characteristics of *Sinorhizobium* species

Characteristic	<i>S. fredii</i> (phenon I)	<i>S. tianjinensis</i> (phenon II)
Carbon source utilization		
Sodium acetate	+ ^a	d
Raffinose	+	d
Rhamnose	+	d
D-Amygdalin	d	—
Malate	+	d
DL-Asparagine	d	—
Nitrogen source utilization		
L-Valine	d	—
Lysine	d	—
L-Arginine	+	d
monohydrochloride		
Tolerance to:		
Vancomycin (25 µg/ml)	d	—
Chloramphenicol (125 µg/ml)	d	—
Penicillin (25 µg/ml)	d	—
Gentamicin (25 µg/ml)	d	—
Streptomycin (5 µg/ml)	d	—
72.0% Chloridimeform (2,000×)	d	—
Growth at pH 5.5	d	—
Growth at pH 8.5	d	—
Litmus milk acid production	d	+
Growth at 10°C	d	—
Nitrate reduction	d	—

^a +, At least 95% of the strains are positive; —, more than 95% of the strains are negative; d, less than 95% of the strains are positive.

and II). Because the type strain of *R. fredii* cv. *fredii* (strain USDA 205) and the type strain of *R. Fredii* cv. *sinensis* (strain USDA 201) fall into phenon I, the type species of this genus should be *Sinorhizobium fredii* comb. nov. For the other species we propose the name *Sinorhizobium xinjiangensis* sp. nov. (xin.ji.ang.eńsis. M. L. adj. *xinjiangensis*, from Xinjiang, referring to rhizobia isolated from the suburbs of Xinjiang, People's Republic of China).

The characteristics of this new genus are described below. Rods 0.5 to 0.9 by 1.2 to 3.0 µm, usually containing granules of poly-β-hydroxybutyrate. Nonsporeforming. Gram negative. Motile by means of one polar flagellum or one to more than three peritrichous flagella. Aerobic, possessing a respiratory type of metabolism with oxygen as the terminal electron acceptor. Colonies are circular, convex, semitranslucent, raised, and mucilaginous, usually 2 to 4 mm in diameter within 3 to 5 days of YMA. Pronounced turbidity develops after 2 to 3 days in agitated broth. The optimum temperature is 25 to 30°C, but most strains grow at 35°C and some strains grow at 10°C. Optimum pH, 6 to 8. Some

TABLE 4. G+C contents of DNAs of the fast-growing soybean rhizobia^a

Source of DNA (strain)	Melting temp (°C)	G+C content (mol%)
2048	94.22 ^b	60.94
CCBAU 103	79.60	61.33
CCBAU 110 ^T	79.60	61.38
CCBAU 111	80.60	63.82
CCBAU 113	79.00	59.90

^a The melting temperatures and G+C values are the means of two replicate determinations.

^b Determined in 1.0× SSC (0.15 M NaCl plus 0.015 M sodium citrate). The other values were all determined in 0.1× SSC.

strains grow at pH 5.0, and other strains grow at pH 10.5. Most strains grow in the presence of 1.0% NaCl, but not 1.5% NaCl, although a few strains grow well on YMA containing 4.5% NaCl.

D-Arabinose, cellobiose, fructose, D-galactose, glucose, L-glutamine, lactose, D-mannose, mannitol, D-ribose, sodium succinate, xylose, and D-turanose are utilized as sole carbon sources, but ammonium tartrate, ammonium oxalate, cellulose, dulcitol, fucose, glycine, sorbose, sodium alginate, and vanillic acid are not. All strains produce acid on YMA.

Ammonium chloride and nitrates rather than amino acids are the preferred sole nitrogen sources, but some strains utilize certain amino acids. Peptone is poorly utilized. All strains require pantothenate and nicotinic acid, but their reactions to other vitamins are variable. All strains are susceptible to doxycycline (1 µg/ml), tetracycline (1 µg/ml), aureomycin (1 µg/ml), terramycin (1 µg/ml), and streptomycin (125 µg/ml), but resistant to vancomycin (5 µg/ml), chloromphenicol (5 µg/ml), erythromycin (25 µg/ml), penicillin (5 µg/ml), medemycin (125 µg/ml), amphotericin B (125 µg/ml), and kanamycin (1 µg/ml). Reactions to pesticides are variable, but all strains are susceptible to 38.08% dimetheate (250×), 50% phoxin (250×), and 40% omthoate (500×), as well as to 0.01% potassium tellurite and calcium hypochlorite (2%). All strains produce cytochrome oxidase and catalase.

The members of this genus do not exhibit a wide host range, but effectively nodulate *Glycine soja*, *Glycine max* cv. TGm119, *G. max* cv. TGm120, *G. max* cv. TGm344, *G. max* cv. Kai-Yu No. 8, *Vigna unguiculata* cv. VITA-3, and *Cajanus cajan* cv. CITA-1. An ineffective symbiosis is formed on *Vigna radiata*, *Macroptilium atropurpureum*, *Macroptilium lathyroides*, and *Sesbania cannabina* (2, 19, 25).

Large plasmids have been demonstrated in most strains, and the structural *nif* genes are contained on large plasmids (the only known exception is strain USDA 194) (9). *nif* genes and *nod* genes are located on a single plasmid (1).

The G+C content of the DNA ranges from 59.9 to 63.8 mol%.

For *Sinorhizobium xinjiangensis* sp. nov., the characteristics are those described above for the genus and in Tables 2 and 3. The cells have one polar flagellum or peritrichous flagella.

The type strain of *S. xinjiangensis* (strain CCBAU 110) is the center strain of the species subgroup in numerical analysis.

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LITERATURE CITED

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