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Predominance of Fast-Growing *Rhizobium japonicum* in a Soybean Field in the People's Republic of China'

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Soybean rhizobia were isolated from two soils with different cropping histories from Hubei province in central China. The first, from Honghu county, has been under soybean cultivation for decades. All of the isolates obtained from nodules on soybeans growing in this soil were fast-growing, acid-producing rhizobia. However, slow-growing, alkali-producing isolates were obtained at higher dilutions of the same soil. The second soil, from Wuchang county, has been under rice cultivation with no record of previous soybean cultivation. All of the soybean rhizobia recovered from this soil, and at higher dilutions of the soil, were typical slow-growing, alkali-producing isolates. The isolates from both soils were grouped by using intrinsic antibiotic resistance, gel immunodiffusion, and fluorescent-antibody procedures. Representative isolates were tested for symbiotic effectiveness with four soybean cultivars (Peking, Davis, Williams, and Ai Jiao Zao) in a pot experiment. There were significant cultivar-rhizobial interactions. Moreover, on each cultivar, there was at least one fast-growing isolate among these new rhizobia that was as effective as the highly effective slow-growing reference strain USDA 110.

Bacteria of the genus *Rhizobium* nodulate and fix nitrogen in symbiosis with many legumes. The various species in this genus make up two broad groups of fast- and slow-growing strains based on growth rate and effect on the pH of yeast extract-mannitol (YEM) medium (12, 26). On the basis of these and other fundamental differences, the slow-growing strains were transferred to a newly named genus *(Bradyrhizobium* gen. nov.), while the fast growers were retained in the genus *Rhizobium (11).* This new taxonomy, however, does not readily accommodate fast-growing soybean rhizobia.

Soybeans are considered to be commonly nodulated by slow-growing rhizobia only. Recently, Keyser et al. (14) reported fast-growing strains of rhizobia isolated from soybean root nodules collected in the People's Republic of China. Studies have shown these fast-growing soybean rhizobia to be distinct in their microbiological and symbiotic properties from the "typical" slow-growing type (15, 19, 22).

Initial studies on the symbiotic effectiveness of the fast growers set forth the notion that they are effective only with certain soybean genotypes from Asia but are generally ineffective with several North American-adapted soybeans (14). Subsequent studies revealed greater diversity in the symbiotic response between fast growers and soybean cultivars (8, 22, 23), with fast growers forming effective symbioses with several commercial soybean cultivars.

Since China is the center of origin of soybeans (10) and presumably of their rhizobia, studies in China of the composition of indigenous populations of soybean rhizobia are particularly important. Since one of us (S.F.D.) was in China for 16 months we had the opportunity to compare the indigenous populations in two soils with different cropping histories. In this study we show that in an uninoculated soybean field although effective slow-growing soybean rhizobia were present in relatively high numbers the majority of nodules were formed by fast-growing rhizobia.

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MATERIALS AND METHODS

Soils. The two soils, both located in Hubei province in central China, have markedly different cropping histories. The first, Honghu soil from Honghu county, has been under soybean cultivation without inoculation for as long as people could recall. The second, Wuhan soil from Wuchang county, has been under continuous rice cultivation with no record of prior soybean cultivation. Fifty pounds (22.7 kg) of each soil was collected, transported to Hawaii, and stored at 4°C until use (approximately 3 months). Soil analyses were kindly done by Ada Chu of the Benchmark Soils Project, University of Hawaii. The following procedures (21) were used: carbon, section 6Ala; total N, section 6B1; Fe, section 6C1; P, Olsen; cations, section 5A1; pH, H_2O and KCl, 1:1 suspension and 1-h equilibration. The chemical properties of the soils were similar except for soil pH (Table 1).

Soybean cultivars. Five soybean *(Glycine max* L. Merr.) cultivars were used in this study. Ou Huang no. 3 and Ai Jiao Zao are improved, yellow-seeded cultivars released by the Oils and Root Crops Institute in Wuhan for use in Hubei Province. Ai Jiao Zao was the cultivar planted in Honghu where the Honghu soil was collected. Peking is an unimproved black-seeded cultivar. Davis and Williams are common commercial cultivars grown in North America.

Isolation of rhizobia. Cultivar On Huang no. 3 was used as the trap host. The methodology for isolation was devised to obtain a heterogeneous population of indigenous rhizobia. Since the soils had been in cold storage, the rhizobial population was stimulated by growing soybeans. After 10 days the seedlings were removed. Rhizosphere soil was collected by carefully removing the seedlings, gently shaking the intact root system to remove soil loosely adhering to the roots, and placing the root system with the remaining soil adhering to the roots in 100 ml of YEM (25) salts. The rhizosphere soil suspensions were shaken for 15 min on a wrist-action shaker. Five 10-fold serial dilutions of the suspensions were made, and 1 ml of each dilution was added to soybeans planted in sterile vermiculite. In addition, soybeans were planted directly in the enriched soil. Eight nodules were collected from each dilution and from the

TABLE 1. Chemical analysis of a soybean soil from Honghu county and a rice soil from Wuchang county

Soil	Organic C (%)	Total N (%)	C/N	Extractable iron (%)		Extractable P (ppm)	Extractable bases (meq/100 g of soil)				CEC[a]	pH		
				Fe	Fe_2O_3		Ca	Mg	Na	K		H_2O	KCl	Difference
Honghu	1.1	0.12	10	4.5	6.4	7.33	11.5	1.6	0.04	1.1	12.6	7.1	6.0	−1.1
Wuchang	0.8	0.08	11	4.9	6.9	8.72	5.3	2.3	0.07	1.1	16.6	5.3	4.1	−1.2

[a] CEC, Cation exchange capacity (meq/100 g of soil).

undiluted soil treatment. One rhizobial isolate was obtained from each nodule. Nodules were rinsed extensively in tap water, immersed in 95% ethanol for 20 s, and immersed in 4% H_2O_2 for 4 min. Nodules were crushed in 2 ml of YEM salts. Serial 10-fold dilutions of the nodule crushate were made, and 0.1 ml of the appropriate dilutions was spread on YEM agar plates containing 0.25 mg of bromthymol blue per liter. All plates were incubated at 28°C for 7 to 10 days before isolations were made.

Purification, authentication, and cataloging. For each nodule isolate a single colony was selected and re-streaked on YEM agar plates and checked for purity: Once pure cultures were confirmed, each isolate was streaked on YEM agar plates containing Congo red (25). Each isolate was confirmed to be soybean rhizobia by inoculating *Gycine soja* seedlings growing in test tubes with Hoagland nitrogen-free plant nutrient agar (9). All isolates were maintained on YEM agar slants; the agar slants used for the maintenance of fast growing isolates contained 0.05% $CaC0_3$. Isolates were cataloged as follows: isolates with the prefix HH were isolated from the Honghu soil; isolates with the prefix WU were isolated from the Wuhan soil; isolates with the initial number 0 were isolated from plants grown directly in the soil; isolates with the initial number 1 were isolated from the 10^{-1} dilution of the rhizosphere soil; isolates with the initial number 2 were isolated from the 10^{-2} dilution of the rhizosphere soil; and so on. Thus HH504 is an isolate from the 10^{-5} dilution of the Honghu rhizosphere soil.

Generation times. Growth and pH responses were determined in YEM and Bishop (2) media for three fast-growing isolates (USDA 205, HH103, and HH303) and three slowgrowing isolates (USDA 110, WU002, and WU006). USDA 205 is one of the fast-growing soybean rhizobia isolated previously (14); HH103 and HH303 are fast-growing soybean rhizobia isolated from the Honghu soil. USDA 110 is a slow-growing strain from the USDA Culture Collection, Beltsville, Md.; WU002 and WU006 are slow-growing soybean rhizobia isolated from the Wuhan soil. Both media were adjusted to pH 6.9 before inoculation. Fast-growing isolates were pregrown in each medium for 3 days, while slow-growing isolates were pregrown for 5 days. Inocula were added to an initial density of 106 cells per ml into 50 ml of the medium in 125-ml sidearm Erlenmeyer flasks. Flasks were agitated at 25°C in a water bath shaker. Cell growth was monitored with a Klett-Summerson photoelectric colorimeter (equipped with a no. 66 red filter), and the pH was determined after 4 days with an Orion Research (model 501) pH meter and a glass combination electrode.

Intrinsic antibiotic resistance (IAR). Resistance to low levels of antibiotics was determined by the method of Josey et al. (13). Fresh solutions of antibiotics (obtained from Sigma Chemical Co., St. Louis, Mo.) were filtered sterilized (0.4-ìm pore-size Nuclepore filter) and added to cooled (48°C) YEM agar medium to give the following concentrations (ìg/ml): chloramphenicol, 12 and 25; kanamycin sulfate, 10; nalidixic acid, 10; neomycin sulfate, 2.5; polymyxin B sulfate, 20; rifampin, 1 and 6; streptomycin sulfate, 2.5 and 10; tetracycline hydrochloride, 1; and vancomycin, 1.5 and 5. Antibiotic stock solutions were prepared in sterile distilled water at a concentration of 10 mg/ml, except chloramphenicol (10 mg/ml in 95% ethanol), nalidixic acid (10 mg/ml of 1 N NaOH), and rifampin (10 mg/ml in methanol). The use of a multiple inoculator allowed for simultaneous inoculation of up to 28 cultures per petri plate. Each culture was replicated four times per antibiotic concentration used. Controls consisted of YEM agar plates without antibiotics. Duplicate plates of each antibiotic concentration were incubated in the dark for 7 days, and isolates showing growth were scored as positive.

Immunofiuorescence. Fluorescent antibodies (FAs) were

TABLE 2. Composition of indigenous rhizobia from two soil samples from China[a]

Soil	Soybean-soil rhizobia		Rice-soil rhizobia					
	Fast growing (%)	Slow growing (%)	Fast growing (%)	Slow growing (%)	Serogroup			
					USDA 110	CB1809	USDA 31	Unidentified[b]
Enriched soil	100	0	0	100	+	+	+	+
Dilutions of rhizosphere soil								
10^{-1}	100	0	0	100	+	+	+	−
10^{-2}	75	25	0	100	+	+	−	−
10^{-3}	100	0	0	100	+	+	−	−
10^{-4}	75	25	0	100	+	+	−	−
10^{-5}	100	0	—[c]	—	—	—	—	—

[a] The soybean soil used in this study was from an uninoculated soybean field in Honghu county; the rice soil was from a rice paddy in Wuchang county with no known history of soybean cultivation. Values in the table are percentage of total nodules; +, presence; −, absence.

[b] In addition to the three FAs listed, unidentified isolates were reacted with FAs prepared against the following slow-growing strains: USDA 123, USDA 138, USDA 76, USDA 46, USDA 94, USDA 6, USDA 135, HU005, HU121–6, and HU2031.

[c] —, No nodules.

TABLE 3. Intrinsic resistance of fast- and slow-growing rhizobia to antibiotics

Antibiotic (wg/ml)	% of resistant rhizobia	
	Fast growing (*n* = 43)	Slow growing (*n* = 47)
Chloramphenicol (12)	88	100
Chloramphenicol (25)	22	100
Kanamycin (10)	34	100
Naladixic acid (10)	100	100
Neomycin (2.5)	100	100
Polymyxin (20)	5	100
Rifampin (1)	5	100
Rifampin (6)	0	81
Streptomycin (2.5)	5	100
Streptomycin (10)	5	92
Tetracycline (1)	0	100
Vancomycin (1.5)	100	100
Vancomycin (5)	83	100

prepared from sera against the somatic components of soybean rhizobia strains by the procedures of Schmidt et al. (20). Gelatin-rhodamine isothiocyanate (3) was used to suppress nonspecific adsorption. The microscopy techniques have been described elsewhere (18).

Immunodiffusion. Immunodiffusion procedures have been described elsewhere (16). Antigens for immunodiffusion analyses were prepared from cells grown on the surface of B5 (6) medium. Cells were harvested from agar flats after 3 days of growth, suspended in 0.85% saline containing thimerosal (1:10,000, final concentration), and stored at 4°C until use. Gels were incubated for 6 days in a moist, dark chamber at room temperature, rinsed exhaustively for several days with frequent changes of 0.85% saline solution, stained with amido black (0.1% amido black, 4.25 x 10^{-1} M acetic acid, 4.25 x 10^{-1} M sodium acetate, 15% glycerol), and destained with 2.0% acetic acid for several days until the background was clear. The precipitin bands were recorded photographically.

Host range and symbiotic efficiency. Seeds were surface sterilized as follows: *Vigna unguiculata*, 4% calcium hypochlorite, 20 min; *Sesbania cannabina* PI180050, concentrated H_2S0_4, 20 min; *Macroptilium atropurpureum*, concentrated HCI, 3 min; and planted in sterile vermiculite moistened with 1/4-strength Hoagland nitrogen-free solution in Leonard jars. Five-day-old seedlings were inoculated with the desired strains, and the top of the vermiculite was covered with sterile perlite and a layer of paraffin-coated sand. There were three replicates per treatment. Plants were harvested 5 weeks after inoculation.

A glasshouse pot study was designed to evaluate the symbiotic efficiency of selected isolates on four soybean cultivars (Ai Jiao Zao, Davis, Peking, and Williams). A mixture of sand, perlite, and vermiculite (1:1:1 by vol) was placed in 3-gal (11.3-liter) pots (25-cm diameter) lined with plastic bags. The pots were divided into four sections with two 20-cm sheets of fiber glass extending to the bottom of the pots. A 20-cm (10-mm diameter) polyvinyl chloride pipe in the center of the pots facilitated watering with 1/4-strength Hoagland nitrogen-free solution.

Seeds were surface sterilized for 20 min in 4% calcium hypochlorite and washed extensively in sterile water, and four seeds of each cultivar were planted in each pot. Three day old seedlings were thinned to leave one seedling of uniform size of each cultivar per pot. The four seedlings in each pot were inoculated with the same isolate of rhizobia by adding 1 ml of the turbid culture to each seedling. After inoculation the top of the sand-perlite-vermiculite mixture was covered with a 3-cm layer of fine gravel. There were three replicates for each treatment. Plants were harvested 4 weeks after inoculation. Plant tops were dried at 70°C and weighed, and nitrogen content was determined by Kjeldahl analysis.

RESULTS

The method used to enrich and isolate rhizobia from the two soils yielded a heterogeneous population of indigenous rhizobia (Table 2). In the Honghu soil, which has a long history of soybean cultivation, fast-growing isolates were predominant. Moreover, different isolates (i.e., belonging to different IAR groups and having different gel immunodiffusion patterns) were obtained at the various dilutions of the rhizosphere soil. Slow-growing isolates were also present in this soil in high numbers as indicated by their recovery at the higher dilutions of the rhizosphere soil. In the Wuhan rice soil, with no prior record of soybean cultivation, only slow-growing isolates were recovered. The slow-growing isolates were tested for serological affinity with strainspecific FAs. Nearly 100% of the isolates from the Wuhan soil could be identified with three FAs: FA USDA 110, FA CB1809, and FA USDA 31. The slow-growing isolates from the Honghu soil did not react with any of the 13 FAs tested.

The fast-growing isolates were grouped according to their intrinsic resistance to low levels of antibiotics (Table 3). There were eight patterns of antibiotic resistance among the fast growers (Table 4). The differences detected among the slow-growing isolates were primarily due to the slow growing isolates from the Honghu soil.

The results of immunodiffusion cross-reactions of two fast-growing isolates from each IAR pattern with somatic

TABLE 4. Summary of IAR patterns for some fast-growing soybean rhizobia

IAR pattern no.	No. of isolates	Chl[a]		Kan	Nal	Neo	Pol	Rif		Str		Tet	Van	
		12	25	(10)	(10)	(2.5)	(20)	1	6	2.5	10	(1)	1.5	5
1	17	+	−	−	+	+	−	−	−	−	−	−	+	+
2	3	−	−	−	+	+	−	−	−	−	−	−	+	−
3	6	+	−	+	+	+	−	−	−	−	−	−	+	+
4	2	−	−	−	+	+	−	−	−	−	−	−	+	+
5	4	+	−	−	+	+	−	−	−	−	−	−	+	−
6	2	+	+	+	+	+	+	+	−	+	+	−	+	+
7	6	+	+	+	+	+	−	−	−	−	−	−	+	+
8	1	+	+	−	+	+	−	−	−	−	−	−	+	+

[a] Numbers are antibiotic concentration in micrograms per milliliter. +, Growth indicating resistance; −, no growth indicating sensitivity. Chl, Chloramphenicol; Kan, kanamycin; Nal, nalidixic acid; Neo, neomycin; Pol, polymyxin; Rif, rifampin; Str, streptomycin; Tet, tetracycline; Van, vancomycin.

TABLE 5. Immunodiffusion analysis of fast-growing soybean rhizobia

Group	Isolates	Antiserum prepared against somatic antigen of[a]:		
		USDA 205	USDA 192	USDA 194
I	USDA 205, HH107, HH205, HH504	2	0	0
II	HH002, HH003, HH203, HH208	1	0	0
III	USDA 194, HH402, HH502	0	0	1
IV	USDA 192	0	2	1
V	HH102, HH103, HH106, HH303, HH307, HH505	0	0	0

[a] Numbers represent the number of precipitin bands formed.

cell antisera produced against fast-growing soybean rhizobia strains USDA 192, 194, and 205 are summarized in Table 5. The reactions with somatic antisera indicated serological relatedness between some of the Honghu isolates and the USDA fast-growing strains reported previously. Of the 15 isolates tested, 7 formed at least one precipitin band with antisera produced against USDA 205, two isolates formed one band with USDA 194, while the remaining isolates formed no bands. The reactions with whole-cell antisera (data not shown) could not be used to separate the isolates into serogroups, as all of the isolates shared several heatlabile antigens.

Two slow-growing isolates, WU002 and WU006, which cross-react with FA CB1809 and FA USDA 110, respectively, and two fast-growing isolates, HH103 and HH303, were selected, and mean generation times were determined in two media (Table 6). One known slow-growing strain, USDA 110, and one known fast-growing strain, USDA 205, were included as reference strains. In complex medium (YEM) the fast-growing isolates had mean generation times three to four times lower than the slow-growing isolates, while in a defined medium (Bishop) they were four to five times lower. The fast growers acidified both media while the slow growers made both media more alkaline.

The three fast growers tested were able to nodulate V. *unguiculata, M. atropurpureum,* and *S. cannabina,* but formed effective nodules only with *Vigna* sp. and *Macroptilium* sp. (Table 7).

The responses of four soybean cultivars to inoculation with fast- and slow-growing isolates are presented in Tables 8 and 9. There were significant cultivar-strain interactions. Among the slow growers from the rice soil, one of the predominant isolates, WU002, formed an ineffective symbiosis with cultivar Ai Jiao Zao. One slow-growing isolate, HH401, from the soybean soil formed effective nodules on the cultivar Peking but induced chlorosis on the leaves which resulted in reduced plant weight. The fast growers were highly effective on the two cultivars from China, Peking and Ai Jiao Zao, whereas they were generally less effective on the two North American cultivars, Williams and Davis. It is important to note, however, that on each cultivar tested there was at least one fast-growing isolate that was as effective as the highly effective slow-growing reference strain USDA 110.

DISCUSSION

The methodology to isolate rhizobia from soil was similar to a method used by Belser and Schmidt (1) to isolate ammonia-oxidizing nitrifiers. They obtained different genera of nitrifiers at different dilutions of the soil. In our study, slow-growing rhizobia were recovered in the soybean soil at higher dilutions presumably because the soil factors contributing to competitiveness were less emphatic. The advantage of this methodology for analyzing the composition of an indigenous population is the possibility that bacteria might be recovered independent of their competitive ability. Initial sampling of nodules from soybeans growing in the Honghu field revealed that 100% of the nodules contained fastgrowing rhizobia (data not shown), indicating that the recovery of fast-growing rhizobia in this study was not peculiar to our methodology.

The fast-growing soybean rhizobia reported previously (14) were isolated from soybean root nodules collected in four east-central provinces. In this study fast growers were isolated from a soil in Hubei province in central China where agriculture is primarily paddy rice with only scattered acreage of soybeans. The implication is that fast-growing soybean rhizobia may be a common component of the natural microflora in China. In contrast to the soybean soil, the indigenous soybean rhizobia in the rice soil consisted only of the slow-growing group. Despite the fact that paddy rice, but never soybeans, had been cultivated in this soil, soybean

TABLE 6. Mean generation time (MGT) and final pH of the medium of several fast- and slow-growing soybean rhizobia

Isolate	Medium			
	YEM[a] (pH 6.9)		Defined[b] (pH 6.9)	
	MGT (h)	Final pH	MGT (h)	Final pH
Slow growing				
USDA 110	11.1	7.08	16.9	7.83
WU002	8.2	7.08	16.9	7.98
WU006	14.1	7.10	26.0	7.39
$\bar{x}$	11.1	7.09	19.9	7.73
Fast growing				
USDA 205	3.6	6.58	3.6	6.96
HH103	3.4	5.50	4.1	5.51
HH303	2.6	6.44	5.9	6.72
$\bar{x}$	3.2	6.17	4.5	6.40

[a] Schmidt et al. (20).
[b] Bishop et al. (2).

TABLE 7. Response of two legumes, *V. unguiculata* and *M. atropurpureum*, to inoculation with fast- and slow-growing soybean rhizobia

Inoculum strain	*V. unguiculata*		*M. atropurpureum*	
	Top dry wt[a]	Nodule dry wt[b]	Top dry wt[a]	Nodule dry wt[b]
Uninoculated control	0.34		0.04	
USDA 123	1.13	128	0.10	13.1
HH003	2.59	212	0.29	40.9
HH103	2.26	526	0.12	9.6
HH303	1.91	155	0.05	5.8
LSD[c]	1.0	118	0.07	16.8

[a] Values are grams per plant and are the means of three replicates.
[b] Values are milligrams per plant and are the means of three replicates.
[c] LSD, Least-significant difference ($P = 0.05$).

TABLE 8. Response of four soybean cultivars to inoculation with fast- and slow-growing soybean rhizobia. I. Percent nitrogen in tops[a]

Peking		Davis		Williams		Ai Jiao Zao	
Isolate	%N	Isolate	%N	Isolate	%N	Isolate	%N
Uninoculated	0.80	Uninoculated	0.82	Uninoculated	0.84	Uninoculated	0.82
WU002	1.98	*HH103*	1.42	*USDA 205*	0.87	WU002	1.01
WU108	2.02	*HH504*	1.45	*HH205*	1.40	HH401	2.23
WU006	2.11	*USDA 205*	1.82	*HH507*	1.79	*HH504*	2.28
USDA 110	2.23	*HH502*	1.85	*HH504*	1.81	*HH303*	2.39
WU104	2.26	*HH205*	1.86	*HH502*	1.84	USDA 110	2.44
WU003	2.39	*HH208*	1.88	*HH208*	2.14	*HH103*	2.46
HH303	2.43	*HH507*	1.93	HH401	2.21	*HH507*	2.47
HH504	2.46	*HH003*	2.29	WU003	2.26	WU006	2.48
HH201	2.49	USDA 110	2.38	*HH303*	2.33	*HH205*	2.49
HH103	2.63	*HH303*	2.42	WU006	2.37	*HH003*	2.51
HH507	2.63	WU006	2.45	*HH003*	2.38	WU104	2.52
USDA 205	2.64	HH401	2.45	*HH103*	2.41	*HH502*	2.56
HH205	2.72	WU002	2.56	USDA 110	2.41	WU003	2.56
HH003	2.75	HH201	2.59	WU002	2.49	*USDA 205*	2.63
HH208	2.76	WU108	2.63	WU108	2.51	WU108	2.63
HH502	2.83	WU003	2.72	HH201	2.62	HH201	2.72
HH401	3.04	WU104	2.77	WU104	2.71	*HH208*	2.77
LSD[b]	0.51	LSD	0.42	LSD	0.39	LSD	0.35

[a] Italics denote fast-growing isolates. Values are the means of three replicates.
[b] LSD, Least-significant difference ($P = 0.05$).

rhizobia were present, albeit in low numbers (data not shown). In China, it is common for soils to contain soybean rhizobia irrespective of their cropping histories, presenting a particular challenge when introducing highly effective inoculum strains on soybeans. In the United States, in soils where soybeans have been grown previously, establishment of selected inoculum strains of rhizobia has been largely unsuccessful due to competition from indigenous rhizobia (7, 17, 24).

It is interesting that the bulk of the slow growers in the rice soil cross-reacted with FAs prepared against strains USDA 110 and CB1809, two highly effective and widely used inoculum strains (H. H. Keyser, personal communication).

In addition, two of the more extreme rhizobium-cultivar interactions reported in the literature were encountered in our limited sampling: the bacterium-induced chlorosis (5) by the slow-growing isolate HH401 on the cultivar Peking and the ineffective response between Ai Jiao Zao and WU002 which is similar to the ineffective response between strains in the 122 serogroup (e.g., CB1809) and the cultivar Hardee (4). As noted above WU002 also falls within the 122 serogroup.

The fast-growing soybean rhizobia previously reported could be separated into at least three distinct serogroups based on immunodiffusion reactions with the somatic antisera produced against USDA 192, 194, and 205 (M. J.

TABLE 9. Response of four soybean cultivars to inoculation with fast- and slow-growing soybean rhizobia. II. Top dry weight [a]

Peking		Davis		Williams		Ai Jiao Zao	
Isolate	Dry wt	Isolate	Dry wt	Isolate	Dry wt	Isolate	Dry wt
Uninoculated	0.49	Uninoculated	0.63	*USDA 205*	0.65	Uninoculated	0.78
HH401	0.58	*HH504*	0.83	*HH205*	0.86	WU002	0.86
WU108	0.71	*HH205*	0.85	Uninoculated	0.98	*HH205*	1.64
WU006	0.75	*HH103*	1.04	*HH504*	1.08	WU104	1.68
WU104	0.76	*USDA 205*	1.08	*HH502*	1.13	*HH504*	1.68
WU002	0.84	*HH502*	1.10	*HH208*	1.13	WU003	1.69
USDA 110	0.84	*HH208*	1.17	*HH507*	1.19	*HH208*	1.76
HH201	0.98	*HH507*	1.23	*HH003*	1.24	*HH003*	1.80
WU003	1.01	*HH003*	1.32	*HH303*	1.25	WU108	1.81
HH303	1.04	*HH303*	1.35	HH201	1.39	HH201	1.84
HH208	1.10	WU104	1.47	*HH103*	1.58	*USDA 205*	1.88
HH507	1.14	WU006	1.49	WU104	1.58	USDA 110	1.88
HH003	1.17	WU003	1.61	HH401	1.64	HH401	1.89
HH205	1.19	USDA 110	1.69	USDA 110	1.68	*HH303*	1.89
HH504	1.21	WU002	1.83	WU108	1.78	WU006	1.91
HH502	1.21	WU108	1.87	WU006	1.82	*HH507*	1.91
HH103	1.31	HH401	1.89	WU003	1.85	*HH502*	1.91
USDA 205	1.35	HH201	1.95	WU002	2.06	*HH103*	2.68
LSD[b]	0.39	LSD	0.42	LSD	0.54	LSD	0.54

[a] Italics denote fast-growing isolates. Values are grams per plant and are the means of three replicates.
[b] LSD, Least-significant difference ($P = 0.05$).

Sadowsky, Ph.D. thesis, University *of* Hawaii, 1983). Several *of* the fast-growing isolates in this study are distinct from the previously reported fast growers and did not fall into any one *of* the three serogroups. In addition the host range for effective nodulation *of* these isolates was different from that reported earlier. Keyser et al. (14) reported that the fast growers nodulated *M. atropurpureum, S. cannabina,* and G. *max cv.* Williams ineffectively, whereas some *of* the isolates in this study formed an effective symbiosis on these hosts. The highly significant cultivar-strain interactions, not found with the slow growers to the same extent, deserve further investigation which could lead to identification *of* the genes responsible for host-strain specificity.

So far as we are aware, this is the first report illustrating the predominance *of* fast-growing soybean rhizobia under natural conditions. This belies the conclusion that fast growing soybean rhizobia represent an anomalous situation *of* little practical significance. Since the results presented in this study emanate from samples taken from one soybean field in China, we must exercise restraint in making generalizations. More collections from similar fields in China are required to establish a better understanding *of* indigenous soybean rhizobia populations. However, we did find: (i) in a soil that has been under soybean cultivation for decades fast-growing rhizobia were predominant; (ii) this population had diverse microbiological and symbiotic characteristics; (iii) there were highly significant cultivar-strain interactions; and (iv) in a rice soil that had no prior history *of* soybean cultivation the predominant soybean rhizobia were effective slow-growing strains.

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