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Rhizobium japonicum Serogroup and Hydrogenase Phenotype Distribution in 12 States

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A survey was conducted in 1980 on 972 isolates of *Rhizobium japonicum* obtained from 65 soybean field locations in 12 states. Isolates were examined for the hydrogenase (Hup) phenotype and somatic serogroup identity. Only 20% of the isolates were Hup^+, with a majority of Hup^- isolates occurring in 10 of the 12 states. The most predominant serogroup was 31 (21.5%), followed by 123 (13.6%). Although most serogroups contained a majority of Hup^- isolates, marked differences occurred. None of the isolates in serogroup 135 were Hup^+, but 93% of the isolates in serogroup 122 were Hup^+. The serogroups with relatively high frequencies of Hup^+ isolates (122 and 110) constitute only a small part (<5% each) of the *R. japonicum* field population in the 12 states.

The presence of an active hydrogen uptake (Hup) system in *Rhizobium japonicum* may be a desirable characteristic for an energy-efficient, nitrogen-fixing symbiosis with soybeans (*Glycine max* L. Merr.) (1, 16, 19, 25). In 1979, a large survey of *R. japonicum* isolates from 28 states which make up the major U.S. soybean production area showed that a majority (>75%) were lacking the Hup system (18).

In 1980, a second survey was conducted in those 12 states which in the 1979 survey had the highest percentage of Hup^- isolates. The purpose of the second survey was to confirm the low percentage of Hup^+ isolates in those states, to serologically identify the isolates, and to determine any association between the Hup phenotype and serogroups of *R. japonicum*. The Hup phenotype distribution in the 1979 and 1980 surveys was reported elsewhere (22); this paper presents the results of the 1980 survey regarding serogroup distribution and association between serogroups and the hydrogenase phenotype.

MATERIALS AND METHODS

Nodule collection and isolation of *R. japonicum*. In the 1980 growing season soybean nodules were collected randomly from commerical fields at various locations by cooperators in the following states: Arkansas, Delaware, Florida, Kansas, Louisiana, Minnesota, Mississippi, New Jersey, North Carolina, Pennsylvania, South Carolina, and South Dakota. The procedure used for the isolation of *R. japonicum* from nodules is described elsewhere (22). A total of 972 isolates were obtained from 323 nodules from 65 locations in the 12 states.

Hydrogenase determination. Single-colony isolates were selected, and free-living cultures were tested for hydrogenase activity as described by Lim (17). These cloned isolates were sent to our laboratory in Beltsville, Md., for serological testing.

Serogroup determination. Isolates were grown in yeast-salts-mannitol broth (23) to a density of 10^8 to 10^9 cells per ml and heated at 100°C for 30 min to destroy the heat-labile flagellar antigens. Rabbit antisera prepared against 15 different serotype strains of *R. japonicum* were used in agglutination tests at a 1/100 final concentration. The short agglutination test (23) was performed, and each isolate was tested against all antisera.

RESULTS

The overall frequency of *R. japonicum* serogroups and the percentage of Hup^+ isolates within each serogroup are summarized in Table 1. Serogroups representing less than 1% of the isolates were not listed, whereas the nine serogroups listed in Table 1 accounted for 78.3% of the 972 isolates. The remainder were either unreactive with any antiserum (9.8%) or reactive with more than one antiserum. Only 20.1% of all isolates were Hup^+. Also, 10.8% of the nodules contained different isolates representing more than one serogroup, and 9.3% of the nodules contained isolates within the same serogroup but with different Hup phenotypes. Serogroup 31 was recovered with the highest frequency, containing only 11% Hup^+ isolates. Serogroups 135 and 122 had the most contrasting Hup^+ representation, 0 and 92.7%, respectively

Table 2 summarizes the occurrence by state of *R. japonicum* serogroups and respective Hup phenotypes. Serogroup 31 was predominant in five states (Arkansas, Delaware, Florida, Kansas, and Louisiana) and was well represented in two other states (North Carolina and Mississippi). Serogroups 123, 76, and 46 were also well represented in several states. South Dakota had a high occurrence of isolates in serogroup 135.

DISCUSSION

Of the 12 states surveyed, 10 contained *R. japonicum* isolates which were at least 75% Hup^-. This result is similar to that of the previous survey from 28 states (22). The comparatively low percentage of Hup^- isolates from New Jersey may be a reflection of the low number of locations and nodules sampled (Table 2) or of samples taken from areas of recent soybean plantings that were inoculated with an inoculum containing Hup^+ rhizobia.

Isolates belonging to serogroup 31 occurred with the

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TABLE 1. Serogroup and hydrogenase phenotype frequencies of *R. japonicum* isolates in 1980 survey

Serogroup[a]	No. of isolates	% of total	% Hup$^+$
31[b]	209	21.5	11.0
123	132	13.6	32.6
76	99	10.2	21.2
46[b]	84	8.6	9.5
6[b]	74	7.6	12.2
135	59	6.1	0.0
94	49	5.0	20.4
122	41	4.2	92.7
110	15	1.5	40.0
Unknown	95	9.8	6.3

[a] The number indicates the USDA strain of *R. japonicum* against which antiserum was prepared.

[b] Serotype strains 6, 46, and 31 are also referenced as belonging in the composite serogroups c1, c2, and c3, respectively (5). These serogroups contain many strains with similar serological properties, and in this paper we use the serotype strain designation for the serogroup.

greatest frequency in this survey (Table 1). Previous surveys of *R. japonicum* serogroups in the United States also showed serogroup 31 to be frequent in certain states (3, 5, 12). Serogroup 123 has been reported to be a major component of indigenous *R. japonicum* in Iowa, Illinois, Ohio, and Minnesota (8, 11, 13, 15, 20, 21), and our data show that this serogroup is found in other regions as well. Serogroup 76 was the third most frequent, and its relatively high representation in Mississippi, North Carolina, and Florida confirms an earlier report of its prevalence in southeastern states (3).

Although most serogroups contained a small portion of Hup$^+$ isolates, none of the isolates in serogroup 135 were Hup$^+$, but 92.7% of serogroup 122 isolates were Hup$^+$. All USDA *Rhizobium* Culture Collection strains in serogroup 122 that have been tested (USDA 122, USDA 136, USDA 142, and USDA 143) are also Hup$^+$ (1, 6, 14, 16, 17). Although the serogroup 122 survey isolates are undoubtedly a heterogeneous group for any number of microbiological and symbiotic characteristics, almost all share the Hup$^+$ phenotype.

The existence of different Hup phenotypes in most serogroups is not surprising, as variation for several characteristics within a given serogroup is known (10). Vincent (24) cautions against associating serogroup affinity with any other property, any such relationship being incomplete or no better than chance. However, certain symbiotic characteristics of *R. japonicum* strains do appear to be associated with certain serogroups, such as the ineffective N_2-fixing symbiosis formed by strains in serogroup 122 and most strains in the c1 serogroup with *G. max* cv. Hardee (2) and the ability of strains in serogroups 76 and 94 to nodulate the rj_1rj_1 soybean genotypes (so-called "nonnodulating") (9). The contrasting frequencies of the Hup$^+$ phenotype in serogroups 135 and 122 may indicate an association between these two characteristics, but more extensive research may be needed to confirm this observation.

Information provided by cooperators in each state enabled us to identify 75.2% of the isolates to the cultivar (16 altogether) from which they were obtained. Although the soybean genotype is known to influence serogroup recovery in nodules (4), such a correlation was not possible in this survey due to widely varying representation. However, no particular serogroup-cultivar trend was seen, and *G. max* cv. Williams, which occurs on more soybean acreage than any

TABLE 2. Summary of *R. japonicum* serogroup and hydrogenase phenotype distribution by state

State[a]	No. of isolates of each Hup phenotype in serogroup:																		No. of sample locations	No. of nodules	No. of isolates	% of isolates HUP$^-$	Predominant serogroups
	6		46		31		76		94		110		122		123		135						
	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−					
AK	5		1	31	2	56		9			5		9		10	4			18	66	164	75.0	31, 46
DE		5			4	12	9		5	1		3		3		11			5	28	81	75.3	31, 123
FL					1	21		12					12						3	17	57	77.2	31, 76
KS			3		9	15						4	8		7	7			4	21	67	49.3	31, 123
LA			1	13	1	25	3		3								22		5	26	86	87.2	31, 135
MN		4		3		6							3		17	25			4	20	69	71.0	123
MS	4	5		7	2	21	2	22	1				2			9			6	35	102	90.2	76, 31
NJ		3				3	4						3		9	5			2	9	27	40.7	123
NC		19	1	9	3	17	2	18	1	14						8			6	31	104	89.4	31, 76, 6
SC		14	2	6	1	9	1	7			1					14			5	29	93	86.0	6, 123
PA		13						8		23			3						3	18	60	90.0	94
SD		2		7		1		2								6		37	4	23	62	100	135

[a] AK, Arkansas; DE, Delaware; FL, Florida; KS, Kansas; LA, Louisiana; MN, Minnesota; MS, Mississippi; NJ, New Jersey; NC, North Carolina; SC, South Carolina; PA, Pennsylvania; SD, South Dakota.

other cultivar (7), yielded isolates from 11 different serogroups.

The possession of a hydrogenase uptake enzyme in *R. japonicum* does increase the efficiency of N_2 fixation, with a concomitant increase in plant weight and nitrogen content (1, 16, 25). Most of the *R. japonicum* population in field soils is Hup^-, and the serogroups with a relatively high frequency of Hup^+ isolates (122 and 110) make up only a small part of that population. Recently, Merberg and Maier (19) reported *R. japonicum* mutants, derived from 122DES, a small-colony isolate of USDA 122, with hydrogenase activity higher than that of their parent cultures. If such mutants can be shown at the field level to improve N_2 fixation, yield, or N content in soybeans, they would be valuable as candidates for commercial inocula. Their introduction and competitive establishment would be a worthwhile challenge for microbiologists studying the ecology of *R. japonicum*.

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