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Hydrogen Uptake (HUP) Activity of *Rhizobium japonicum* from Major U.S. Soybean Production Areas

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Hydrogen Uptake (HUP) Activity of *Rhizobium japonicum* from Major U.S. Soybean Production Areas

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ABSTRACT

The purpose of these experiments was to determine the distribution of the hydrogen uptake (Hup) system among *Rhizobium japonicum* isolated from major soybean production areas of the USA. Soybean nodules were collected from approximately 100 different locations during the 1979 and 1980 growing seasons. The major conclusion is that a majority (>75%) of the *Rhizobium japonicum* strains isolated from major soybean producing areas are missing H_2 uptake activity. The role of the H_2 uptake system in energy efficiency of nitrogen fixation is discussed.

Additional index words: Rhizobium japonicum, Hydrogenase, Nitrogen fixation, *Glycine max* L., Hydrogen uptake.

Root nodules of soybeans (*Glycine max* L.) inoculated with most strains of *Rhizobium japonicum* release considerable amounts of hydrogen; whereas nodules from a minority of strains evolve little if any H_2 (2, 5, 6). The ability to recycle, via H_2 uptake (hup^+) system, the H_2 evolved by nitrogenase can provide energy for nitrogen fixation (3). In greenhouse experiments Hup^- mutants (lacking H_2 uptake) derived from Hup^+ parents fixed significantly less nitrogen compared to the parental Hup^+ strain which synthesized the H_2-oxidizing system (1). However, it should be emphasized that while this is an interesting trend, conclusive field testing will be required to substantiate the agronomic significance of this trait. The capacity to synthesize an active H_2-uptake system may be a desirable characteristic of an energy efficient *Rhizobium japonicum* strain.

At present there is no information on the distribution of the H_2 uptake system among *R. japonicum*-forming nodules in the major soybean production areas. Such information would be useful in the evaluation of strains for use as inoculum. We report here a random sampling of *Rhizobium japonicum* isolates from the major soybean production areas of the USA to determine their hydrogen uptake activity.

MATERIALS AND METHODS

Nodule Collection and Isolation of Bacterial Strains

Random samples of soybean (*Glycine max*) nodules from various locations in different states representing the major soybean production areas in the country were collected during the 1979 and 1980 growing seasons and shipped to Davis in glass vials containing silica gel as dessicant (8). The nodules were rehydrated upon receiving the samples and six representative nodules from three plants in each location were selected at random, surface sterilized with 10% (v/v) sodium hypochlorite for 10 min and washed four times with sterile water. The nodules were crushed and streaked on mannitol-salts-yeast extract (MSY) medium as previously described (5). Twenty single colonies were isolated at random (three to four colonies per nodule) and used as inocula for the assay of hydrogen uptake activity.

Hydrogen Uptake (Hydrogenase) Induction and Assay

Hydrogen uptake (Hup) activity was induced in free-living cultures of *Rhizobium japonicum* as previously described (5) using a glutamate (0.1% w/v) medium with a gas phase consisting of 10% H_2, 5% CO_2 and 1% O_2, and 84% argon. After approximately 6 days incubation at 25 C on a rotary shaker (150 rpm) the cultures were evacuated and filled with argon and assayed for hydrogen uptake activity using the tritium assay (5).

Plant Nodulation Experiments

A selection of the *Rhizobium japonicum* strains isolated (containing both Hup^+ and Hup^- strains) were tested for effectiveness on four different soybean cultivars (var. Evans, Beeson, Dixon, and Harosoy) in order to ensure that the assays of free-living cultures for hydrogen uptake activity reflect the behavior of these strains in the root nodules. Plants were grown under sterile conditions in a mixture of vermiculite-perlite (1:1). The seeds had previously been surface sterilized in 95% ethanol (3 min) then a 0.1% $HgCl_2$/0.06 N HCl solution (3 to 5 min) then rinsed in four changes of sterile water. After germination in a sterile petri dish with adequate moisture content the seedlings were aseptically transferred to a sterile Fleaker (Corning No. 5900) containing the vermiculite-perlite mixture. The plants were inoculated, sterile cotton placed loosely in the mouth of the Fleaker to prevent contamination, and placed in the greenhouse with no supplemental lighting. The plants were harvested 4 to 5 weeks after inoculation and assayed for H_2 evolution, nitrogenase activity (acetylene reduction), and H_2 uptake activity as previously described (5).

RESULTS AND DISCUSSION

Figure 1 shows the results from a survey of more than 2,500 strains of *Rhizobium japonicum* isolated from nodules from nearly 100 different locations in the major soybean production areas during the 1979 and 1980 growing seasons. As far as possible samplings were from commercial fields to avoid bias towards specialized "research" *Rhizobium* strains. It can be seen from the figure that the majority of the states sampled contained mainly Hup^- strains. Strains isolated from nodules obtained from many South-

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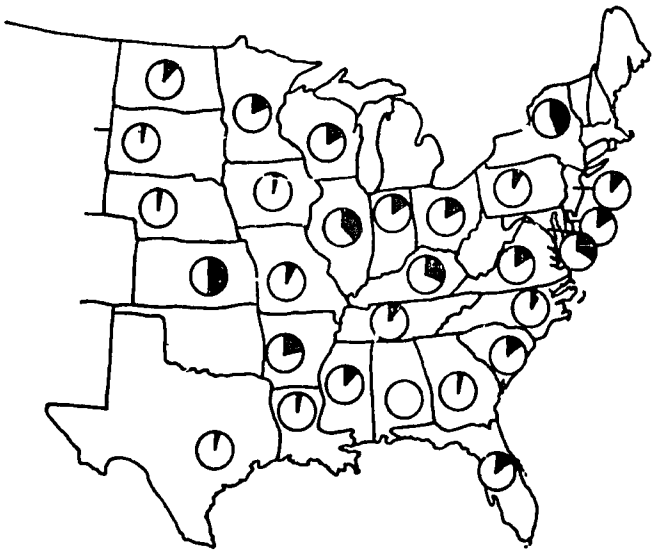

Fig. 1. A summary of the survey of H_2 uptake (Hup) activity in *Rhizobium japonicum* strains from nodules isolated from the major soybean production areas of the USA. One to 10 locations per state were sampled. The size of the dark portion of the circle represents the percentage of Hup⁺ strains.

Table 1. Percentages of Hup⁺ strains of *Rhizobium japonicum* isolated from soybean nodules from Arkansas.

Growth stage	Cultivar	Location		
		Keiser	Marianna	Stuttgart
		% Hup⁺ strains		
Full bloom	Lee	70	0	50
Full bloom	Davis	0	0	40
Full bloom	Tracy	20	0	20
Pod fill	Lee	0	20	80
Pod fill	Davis	0	20	50
Pod fill	Tracy	80	11	0

eastern states, especially Alabama, Florida, Mississippi, North and South Carolina, and Louisiana contained 80% or more Hup⁻ strains. Certain regions, for example locations in New York, Kansas, and Maryland (Beltsville), had high percentage of Hup⁺ strains. The distribution of Hup⁺ strains in different locations within a state could vary greatly. In Illinois, for example, one location in Belleville had 90% Hup⁺ strains, whereas locations in Dekalb and Urbana yielded only Hup⁻ strains. Such results indicate the dominance of different strains in different locations. The period of sampling did not appear to have a significant effect on the percentage of Hup⁺ strains isolated as demonstrated by a more comprehensive survey done in Arkansas, a state which contained a sizable proportion of Hup⁻ strains. Nodules were collected from different sites in three locations with different soil types and soybean cultivars at full bloom and during pod fill (Table 1). Although there is an increase in the percentage of Hup⁺ strains isolated from two sites during pod fill, the overall percentage of Hup⁺ strains from the state sampled during two different periods of growth was similar.

Weber and Miller (9) have shown that soil temperature could have an effect on the distribution of *Rhizobium japonicum* serogroups in soybean nodules. Hence, the effect of annual temperature on the distribution of Hup⁺ strains

Table 2. Analysis of commercial inoculants (*Rhizobium japonicum*) for C_2H_2 reduction, H_2 evolution, and nodule weight. Values were obtained from approximately 5-week-old soybeans (*Glycine max* cv. Evans) grown in the greenhouse.

Inoculants¶	C_2H_4 formed, $\mu mol\ hr^{-1}\ g^{-1}$ nodule wt	H_2 evoluation $\mu mol\ hr^{-1}\ g^{-1}$ nodule wt	Nodule wt, mg $plant^{-1}$
SA	14.3 ± 2.5†	<0.05	168.8
CB	10.5 ± 3.3	5.4 ± 0.6	82.7
XC	19.1 ± 1.4	<0.05 5.1 ± 0.9§	98.5
CD	31.1 ± 4.7	11.9 ± 0.1	109.0
XE	19.9 ± 2.1	<0.05 5.7 ± 0.1§	94.9
XF	23.0 ± 2.1	5.5 ± 0.5	68.2
CG	18.4 ± 7.5	<0.05 5.7 ± 0.2§	112.3
SH	26.7 ± 2.7	<0.05	N.D.‡

† Results are means ± s.e.m.
‡ Not determined.
§ These inoculants contain two distinct kinds of strains—Hup⁺ and Hup⁻.
¶ SA—Nitragin LX848; CB—Nitragin LX872; XC—Felco, Lot #117; CD—Nitragin S299; XE—Research Inoculants, Lot #I 109; XF—Research Inoculants, Lot #I32; CG—Urbana, Lot #I 125; SH—Nitragin LX847.

was investigated. The results (data not shown) showed that although states with significant percentage of Hup⁺ strains (30 to 50%) had annual temperatures of between 10 and 15 C, regression analysis of the data showed that there was little correlation ($r^2 < 0.1$) between possession of H_2 uptake activity and annual temperature. Similar results were obtained when the effect of annual precipitation on distribution of Hup⁺ strains was investigated (data not shown). Other factors were also involved in determining the distribution of Hup⁺ strains. Soybean cultivars grown in past years as well as soil properties could affect the relative abundance and distribution of *Rhizobium japonicum* strains in the field (4, 7, 9).

In addition an analysis of several commercial inoculants commonly used to inoculate soybeans revealed an interesting pattern. Table 2 shows a study of eight different commercial inoculants (consisting of both single and multiple strains) which are used to inoculate *Glycine max* var. Evans, and analyzed for nitrogenase activity (acetylene reduction) and H_2 evolution. A majority of the nodules were found to evolve significant amounts of H_2 — indicating an absence of a H_2 recycling system. At least three of the inoculants (XC, XE, and CG) tested were found to contain both Hup⁺ and Hup⁻ strains since two distinct kinds of nodules were obtained (Table 2).

In summary, the major conclusion from these experimental studies is that a majority of the soybean nodules in major production areas in the USA waste hydrogen due to the absence of an active H_2 uptake system (Hup genes are contributed by *Rhizobium japonicum*.) Several factors such as lack of Hup⁺ strains in commercial inoculants, soil types, and different soybean germplasm (determinate vs. nondeterminate) may contribute to the overall lack of Hup⁺ strains. Experiments on the molecular cloning of Hup genes using recombinant DNA technology are currently in progress with the goal of introduction of Hup plasmids into endemic Hup⁻ strains.

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