cultures than in C-limited continuous cultures or in nonlimited batch cultures. In chemostat cultures of *Anacystis nidulans*, Parrott and Slater (*26*) found that protein content as a percentage of dry weight was greater under $CO_2$ limitation than under light limitation. As mentioned above, increases in RGV have been associated with the onset of growth limitation in laboratory cultures and in bloom-forming populations of blue-green algae. We suggest that only those growth-limiting conditions that favor relative protein synthesis are likely to permit such increases in buoyancy and to promote surface blooms. Unlike N limitation, light and $C_i$ limitation can meet this requirement.

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13 July 1981; revised 16 October 1981

## Fast-Growing Rhizobia Isolated from Root Nodules of Soybean

Abstract. *Fast-growing rhizobia have been isolated from soybean root nodules collected in China. These new isolates are physiologically distinct from slow-growing soybean rhizobia. They formed effective nitrogen-fixing associations with wild soybean and an unbred soybean cultivar from China, but were largely ineffective as nitrogen-fixing symbionts with common commercial cultivars of soybeans.*

*Rhizobium* isolates from nodules of domesticated soybeans and the wild progenitor species of soybeans (*Glycine soja* Sieb. and Zucc.) obtained from three recent expeditions to China (*1*) have been characterized. In all three collections, we found fast-growing *Rhizobium*. They were obtained from the east-central provinces of Shansi, Honan, Shandong, and Shanghai, which are included in the area considered to be the center of origin and diversity for the soybean (*2*). Presumably, the center of origin for its nitrogen-fixing microsymbiont *Rhizobium japonicum* is in the same region. These fast-growing isolates are a previously undescribed group of *Rhizobium* that may be useful in the genetic improvement of *Rhizobium* strains for soybean production.

*Rhizobium* are bacteria capable of forming a nitrogen-fixing symbiosis with leguminous plants. The taxonomic status of *Rhizobium* is controversial because it is based on host infectivity (*3*). For example, strains forming nodules on the soybean plant are given the species rank *R. japonicum*. Within designated species, the bacteria exhibit fairly uniform biochemical characteristics. Two important manifestations of biochemical differences, growth rate and acid production, have been used in differentiating rhizobia with different host affinities (*3, 4*).

Fast-growing species of rhizobia (*R. meliloti, R. leguminosarum, R. trifolii,* and *R. phaseoli*) usually have generation times of 2 to 4 hours, whereas the slow-growing species (*R. japonicum* and *R. lupini*) have generation times of six or more hours (*5*). A fast growth rate is associated with an acid reaction in mannitol medium, whereas slow growth is usually accompanied by an alkaline reaction. A comparison of growth rates and acid production of fast- and slow-growing *R. japonicum* and of other fast-growing species shows that the new soybean rhizobia show more resemblance to other fast-growing species of rhizobia than to typical slow-growing *R. japonicum* (Table 1).

All ten of the soybean cultivars tested formed nodules when inoculated with pure cultures of the fast-growing rhizobia (Table 2). However, only on cultivar Peking [a black-seeded genetically unimproved line from China (*6*)] did the fast-growing *R. japonicum* form effective nitrogen-fixing symbioses. On the commercial soybean cultivars, these bacteria were either completely ineffective or only poorly effective in the fixation of nitrogen. They also formed an effective symbiosis with *G. soja*, the putative wild ancestor-progenitor of the cultivated soybean (*7*). The fast-growing isolates formed ineffective nodules with two *Macroptilium* species and *Sesbania cannabina* Roxb., hosts that associate readily with fast- and slow-growing *Rhizobium*.

Cultures of the fast-growing isolates were verified as *R. japonicum* by repeated soybean (Peking) infection and isolation tests. These cultures were scrutinized for the presence of slow-growing colonies that might be masked by the fast-growing type. None were found.


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The bacterial contents of nodules were also identified by the fluorescent antibody technique (*8*), with rabbit antisera to fast-growing rhizobia.

According to the current classification scheme of *Rhizobium*, which is based on host infectivity, these new isolates are grouped with the slow-growing *R. japonicum*. However, the aspects of their physiology given above, as well as carbohydrate utilization and other biochemical tests (data unreported), indicate these rhizobia are different in many respects from typical *R. japonicum*. Proposals have been made to reclassify *Rhizobium* (*9–11*) and to separate fast- and slow-growing rhizobia into different genera on the basis of their growth rates and biochemical and genetic differences. The new isolates may be an important link between the two distinct groups, since they have the physiological attributes of fast-growing rhizobia, but the symbiotic attributes of slow-growing rhizobia. They should be useful in genetic studies of *R. japonicum*, since fast growers are much easier to manipulate genetically than slow growers. Their contrasting symbiotic reactions on different soybean cultivars also make them potentially useful in studies of the host-determined factors of nitrogen fixation. The ineffective symbioses of the commercial soybean cultivars with the fast-growing *R. japonicum* suggests the presence of host genes similar to those known to control ineffectiveness with slow-growing *R. japonicum* (*12*).

*Rhizobium leguminosarum* collected from the Middle East centers of its host (*Pisum sativum* L.) have also exhibited unusual symbiotic characteristics (*13, 14*). More extensive collection of *Rhizobium* from such centers should broaden the available genetic base of this agriculturally important microorganism.

Table 1. Mean generation times and acid production by fast- and slow-growing rhizobia. Cultures were grown in yeast mannitol (*15*) liquid medium (50 ml) at 28°C on a reciprocating shaker. Samples for determination of mean generation times were taken during the exponential growth phase, and turbidity was measured on a Beckman spectrophotometer (comparison of this method with direct viable counting yield and nearly identical data). Samples for determination of *p*H change were taken after 4 days; the initial *p*H was 6.8. Each result is a mean of three replicates.

| Isolate | Mean generation time (hours) | *p*H |
|---|---|---|
| *Slow-growing* Rhizobium japonicum | | |
| PRC 005 | 9.9 | 6.8 |
| PRC 113-2 | 8.2 | 6.8 |
| PRC 121-6 | 6.9 | 7.2 |
| PRC 2031 | 13.0 | 7.0 |
| PRC B15 | 8.2 | 7.2 |
| USDA 110 | 9.6 | 7.3 |
| USDA 122 | 6.7 | 7.4 |
| Mean | 8.9 | 7.1 |
| *Fast-growing* Rhizobium japonicum | | |
| USDA 191* | 3.7 | 5.2 |
| USDA 192 | 4.1 | 4.7 |
| USDA 193 | 3.8 | 5.9 |
| USDA 194 | 4.8 | 6.5 |
| USDA 201 | 3.5 | 6.7 |
| USDA 205 | 2.9 | 5.2 |
| USDA 206 | 4.1 | 4.7 |
| USDA 208 | 3.5 | 4.8 |
| USDA 214 | 3.5 | 6.1 |
| USDA 217 | 2.9 | 6.5 |
| Mean | 3.7 | 5.6 |
| *Other fast-growing rhizobia*† | | |
| *Rhizobium meliloti* | 2.5 | |
| *Rhizobium trifolii* | 2.9 | |

*This isolate, previously designated number 440, was obtained from the Nitragin Co., Milwaukee, Wisconsin. †Data for *Rhizobium meliloti* and *Rhizobium trifolii* (*16*) are means of six and five strains, respectively.

Table 2. Response of several legumes to inoculation with fast-growing *R. japonicum*. Surface-sterilized seeds were sown in sterilized vermiculite in containers supplied with a nitrogen-free nutrient solution. Approximately $10^7$ cells per strain were added to each seed, and containers were placed either in a growth chamber or greenhouse. Nine strains of the fast-growing *R. japonicum* were tested on each legume, except where noted. The strains are those listed in Table 1, exclusive of USDA 191. Large-seeded legumes were thinned to three plants per container and small-seeded legumes to eight. Each legume-*Rhizobium* treatment was tested in triplicate. After 5 to 6 weeks, plants were scored for color, and roots were examined for the presence of nodules. In addition, one or more of the following measurements were determined: plant top dry weight, nodule fresh weight, nitrogenase activity ($C_2H_2$ reproduction) (*17*), and plant nitrogen content. Tests with the species of *Sesbania*, *Macroptilium*, and *Glycine* included other fully effective strains used as standards.

| |
|---|
| Not nodulated |
| *Leucaena leucocephala* |
| *Medicago sativa* |
| *Trifolium repens* |
| *Trifolium pratense* |
| *Astragalus sinicus* |
| Nodulated: effective symbiosis |
| *Glycine max* cv. Peking |
| *Glycine soja** |
| Nodulated: ineffective symbiosis |
| *Macroptilium atropurpureum* |
| *Macroptilium lathyroides* |
| *Sesbania cannabina* |
| *Glycine max* cv. Lee |
| *Glycine max* cv. Clark† |
| *Glycine max* cv. Williams |
| *Glycine max* cv. Chippewa† |
| *Glycine max* cv. Wilson-6† |
| *Glycine max* cv. Bedford |
| *Glycine max* cv. Hardee |
| *Glycine max* cv. Kent |
| *Glycine max* cv. Mandarin |

*Seed collected in Shansi province, China, in 1980, by T. S. Hu. †Tests were conducted on cultivars Clark, Wilson-6, and Chippewa with seven, six, and four strains of the fast-growing *Rhizobium japonicum*, respectively.

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### References and Notes

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18. This research was supported in part by grants USDA RSSA 4-76 and DSAN-G-0100 (211-d) from the Agency for International Development. We thank S. May for technical assistance and encouragement.

22 October 1981; revised 14 December 1981