Deoxyribonucleic Acid Homologies Among Acid-Producing Strains of *Rhizobium*

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Plant specificities and deoxyribonucleic acid homologies were studied among 122 strains of *Rhizobium*. Some strains were assigned to species on the basis of their source of isolation and present nodulation capabilities, but many did not fit into one of the six currently recognized species of the genus *Rhizobium*. Among those strains assigned to species were many which also nodulated plants outside their species-specific, cross-inoculation group. Conversely, isolates from a wide variety of plants could be designated *Rhizobium phaseoli* since they were capable of nodulating *Phaseolus vulgaris*. Acid production and growth rate on yeast-mannitol agar were tested for all strains. Some strains grew rapidly but did not produce an acid reaction; these were grouped with the fast growing acid producers. Deoxyribonucleic acid homology was used to identify four genetic groups of fast-growing, acid-producing rhizobia. Group 1 included strains of *Rhizobium trifolii* (except strains obtained from *Trifolium lupinaster*), *Rhizobium leguminosarum*, *Rhizobium phaseoli* (obtained from *Phaseolus vulgaris*), and two strains obtained from *Neptunia gracilis*. Group 2 comprised six American strains obtained from crown vetch (*Coronilla varia*), sainfoin (*Onobrychis vicifolia*), and *Sophora* spp. Species status for this group should remain tentative until further strains have been studied. Group 3 corresponded with *Rhizobium meliloti* as presently defined. Group 4 included fast-growing *Lotus* rhizobia, two strains obtained from *T. lupinaster*, and a wide variety of previously unclassified strains. Nine fast-growing strains could not be included in any of these groups. The nine slow-growing, non-acid producing strains included in this study showed <10% homology with DNAs from seven fast-growing reference strains. The relationships between subgroups in group 1 are discussed, and the genetic diversity of strains obtained from *Phaseolus vulgaris* is examined. It is proposed that fast-growing rhizobia comprise at least four species corresponding with the four genetic groups described.

The classification of legume root-nodule bacteria presented in the 8th edition of *Bergey's Manual of Determinative Bacteriology* (18) places them in one genus, that of *Rhizobium*. This genus is divided into two groups distinguished by differences in growth rate, carbohydrate utilization, and flagellation. The six species of rhizobia described are distinguished by the leguminous plants they nodulate. The unsatisfactory aspects of this species classification have been reviewed previously (6, 15).

Deoxyribonucleic acid (DNA) reassociation has been used to study the relationships between fast-growing, acid-producing rhizobia and agrobacteria (7, 12) and between root-nodule bacteria from legumes indigenous to New Zealand and strains designated as *Rhizobium lupini* (16). Recently, Jarvis et al. (15) used this technique to determine DNA homologies among 27 strains of *Rhizobium trifolii*, 4 strains of *Rhizobium*

leguminosarum, and 4 strains of *Rhizobium phaseoli*. It was proposed that *Rhizobium trifolii* and *Rhizobium leguminosarum* should be combined under the name which has priority: *Rhizobium leguminosarum* Frank. Two *Rhizobium* strains obtained from African *Trifolium* species and two obtained from a northern Asiatic species were less closely related. The average relatedness of *Rhizobium* strains taken from *Phaseolus vulgaris* to those obtained from clover was 46%, but the authors suggested that *Rhizobium phaseoli* should be retained at present as a separate species and examined in more detail.

This paper describes the DNA homologies among 113 strains of fast-growing, acid-producing rhizobia and seven reference DNAs from *Rhizobium* spp. Nine slow-growing, non-acid-producing strains have been included for comparative purposes.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains included in this investigation are listed in Table 1 together with their hosts of isolation and countries of origin. The strains are arranged in groups which correspond with the DNA homology groups in Table 2. The fast-growing rhizobia are divided into five groups and the slow-growing rhizobia form a sixth group. Within each group, all strains are alphabetically ordered according to plant host. In addition, the group 1 *Rhizobium* strains from clovers are organized according to geographic origin.

Cultural methods. The media and methods of culture maintenance we used have been described in a previous communication (15). Acid production was determined on yeast extract-mannitol agar by the method of Norris (22). Growth rate was measured by following the increase in turbidity during log-phase growth of cultures in yeast extract-glucose media (19). Strains with generation times of <5 h were designated fast-growing and those with generation times >5 h, as slow-growing.

Nodulation and effectiveness. The method described by Jarvis et al. (15) was used to determine nodulation and effectiveness of nodulation.

Genome size measurement. The spectrophotometric method of De Ley et al. (4) was used to determine genome size. A UNICAM SP 1800 ultraviolet spectrophotometer with SP876 series 2 temperature-program controller (Pye Unicam Ltd., Cambridge, England) set at 76°C and fitted with a Perkin-Elmer model 56 recorder (Perkin-Elmer, Norwalk, Conn.) was used. The genome size was calculated by using the equation of Gillis et al. (8) and reference *Escherichia coli* DNA (3.28×10^9 daltons; De Ley [3]).

DNA preparation and reassociation experiments. The preparation of unlabeled DNA and DNA labeled with ^{32}P , the DNA reassociation technique with the batch hydroxyapatite procedure to separate unreassociated from reassociated DNA, and the regeneration of hydroxyapatite were as described by Jarvis et al. (15) except for two modifications. In the preparation of labeled DNA, the step after sonication for 56 s, in which the labeled DNA was denatured and then eluted from an hydroxyapatite column, was omitted. The purity and hybridization values obtained with labeled DNA prepared in this way were independent of this step. Secondly, samples of reassociated DNA were frozen at -20°C for up to 48 h before being thawed and analyzed by batchwise elution from hydroxyapatite. The hybridization values were unaffected by storage under these conditions.

RESULTS

Acid production and growth rate. Most strains were classified as fast-growing acid producers on the basis of colony size and marked acid production on yeast extract-mannitol agar. The following strains (with the plant from which they were obtained within parentheses) showed little or no acid production, but on the basis of colony size, generation time, and host range,

they were classified as fast growers: strains NZP5469 (*Lathyrus sativus*), 63 (*Pisum arvense*), CB1170 (*Medicago sativa*), CC1005 (*Hosackia* sp.), 461 (*Lotus* sp.), NZP5302 (*Robinia pseudoacacia*), CB81 (*Leucaena leucocephala*), J1070 (*Mimosa invisa*), and X18b (*Mimosa caesalpiniaefolia*).

Only the following strains (with the plants from which they were obtained within parentheses) were classified as slow-growing, nonacid producers: ES1 (*Erythrina speciosa*), NZP2192 and NZP2257 (*Lotus corniculatus*), NZP2076 (*Lotus suaveoleus*), NZP5087 (*Lupinus angustifolius*), WU425 (*Ornithopus compressus*), NZP5113 (*Ornithopus perpusillus*), 94 (*Phaseolus acutifolius*), and 100 (*Phaseolus aureus*).

Homology and plant specificity. The plant specificities of the various *Rhizobium* strains that we used in this investigation are listed in Table 1. Selected strains of *Rhizobium trifolii*, *Rhizobium leguminosarum*, *Rhizobium phaseoli*, and *Rhizobium meliloti* were tested on a wide variety of hosts, and the results indicate that strains which fit into the classification scheme of *Bergey's Manual of Determinate Bacteriology* on the basis of plant origin or nodulating capacity may also nodulate a variety of other hosts which are not formally included in their species-specific, cross-inoculation groups; many of the remaining strains did not fall into any of the six currently recognized species in the genus *Rhizobium* (18).

Rhizobia obtained from *Trifolium polymorphum* (NU125), *Trifolium repens* (CC275e), *Phaseolus vulgaris* (NZP5459), *Lotus corniculatus* (CC811), *Lotus maroccanus* (CC809a), *Coronilla varia* (CC401), and *Medicago sativa* (SU47) were used to provide labeled reference DNAs. These reference DNAs were reassociated with unlabeled DNA from up to 122 strains of rhizobia and with DNA from *Escherichia coli* K-12. The relative homology values were used to organize the strains into groups showing similar genetic homologies, and these are listed in Table 2.

Genetic homology in group 1. The common features of the 64 strains in group 1 were the high mean homology values of 51, 65, and 54% with reference DNAs from NU125, CC275e, and NZP5459, respectively, and the low mean homology values of 7, 9, 23, and 14% with reference strains CC811, CC809a, CC401, and SU47, respectively. The geographic and plant origins of these strains were diverse. Thirty-one *Rhizobium trifolii* strains originated from 16 clover (*Trifolium*) species, 16 *Rhizobium leguminosarum* strains originated from various *Pisum*, *Vicia*, and *Lathyrus* species, 13 *Rhizobium phaseoli*

TABLE 1. *Origin and plant specificities of strains used*^a

Group and strain no.	Geographic origin	Plant origin	Forms effective nodules ^b	Forms ineffective nodules ^b	Does not nodulate
Group 1					
NU125	Uruguay ^c	<i>T. polymorphum</i>		<i>T. repens</i> , <i>T. subterraneum</i>	<i>T. repens</i> , <i>T. subterraneum</i>
NU126 ^d	Uruguay ^c	<i>T. polymorphum</i>			
NU132	Uruguay ^c	<i>T. polymorphum</i>		<i>T. repens</i> , <i>T. subterraneum</i>	
NU134	Uruguay ^c	<i>T. polymorphum</i>	<i>T. repens</i> , <i>T. subterraneum</i>		
674A	South Africa ^c	<i>T. africanum</i>		<i>T. repens</i> , <i>T. subterraneum</i> , <i>T. africanum</i> , <i>T. semipilesum</i> , <i>T. lugardii</i>	<i>T. repens</i>
SA3	South Africa ^c	<i>T. africanum</i>	<i>T. africanum</i> , <i>T. lugardii</i>	<i>T. subterraneum</i>	
CB727	South Africa ^c	<i>T. burchellianum</i>	<i>T. lugardii</i>	<i>T. repens</i> , <i>T. subterraneum</i>	
CB774	Tanzania ^c	<i>T. rueppellianum</i>	<i>T. lugardii</i>		
408A	East Africa ^c	<i>T. rueppellianum</i>	<i>T. lugardii</i>	<i>T. subterraneum</i> , <i>T. africanum</i> , <i>T. repens</i>	
CB778	Kenya ^c	<i>T. semipilosum</i>	<i>T. semipilosum</i>		
CB758	Tanzania ^c	<i>T. tembense</i>	<i>T. lugardii</i>		
CB775	Tanzania ^c	<i>T. usambarensis</i>	<i>T. lugardii</i>		
39	United States	<i>T. arvense</i>	<i>T. repens</i> , <i>T. subterraneum</i>		
3D1j12	United States ^c	<i>T. beckwithii</i>	<i>T. repens</i> , <i>T. subterraneum</i>		
41	United States ^c	<i>T. carolinianum</i>		<i>T. repens</i> , <i>T. subterraneum</i>	
3D1j14	United States ^c	<i>T. rydbergii</i>		<i>T. repens</i> , <i>T. subterraneum</i>	
NZP550/2	United States	<i>T. vesiculosum</i>	<i>T. repens</i> , <i>T. subterraneum</i>		
CC227	Turkey ^c	<i>T. ambiguum</i>	<i>T. ambiguum</i>	<i>T. repens</i> , <i>T. subterraneum</i>	
CC229 ^c	Turkey ^c	<i>T. ambiguum</i>	<i>T. ambiguum</i>		
CC231a	Turkey ^c	<i>T. ambiguum</i>	<i>T. repens</i> , <i>T. subterraneum</i>		
SU202	Wales ^c	<i>T. pratense</i>		<i>T. repens</i> , <i>T. subterraneum</i>	<i>L. leucocephala</i> , <i>V. hirsuta</i> , <i>C. varia</i> , <i>O. vicifolia</i> , <i>L. corniculatus</i> , <i>M. sativa</i>
CC275e	Tasmania	<i>T. repens</i>	<i>T. repens</i> , <i>T. subterraneum</i>	<i>C. puniceus</i> , <i>L. nissolia</i> , <i>T. lupinaster</i>	
NSP562a	New Zealand	<i>T. repens</i>	<i>T. repens</i> , <i>T. subterraneum</i>		
NZP564	New Zealand	<i>T. repens</i>		<i>T. repens</i> , <i>T. subterraneum</i>	
NZP565	New Zealand	<i>T. repens</i>	<i>T. repens</i> , <i>T. subterraneum</i>		
NZP566	New Zealand	<i>T. repens</i>	<i>T. repens</i>	<i>T. subterraneum</i>	
NZP567	New Zealand	<i>T. repens</i>	<i>T. repens</i>	<i>T. subterraneum</i>	
K8	Netherlands ^c	<i>T. repens</i>	<i>T. repens</i>	<i>T. subterraneum</i>	

TABLE 1—Continued

Group and strain no.	Geographic origin	Plant origin	Forms effective nodules ^b	Forms ineffective nodules ^b	Does not nodulate
CC511 ^c	Australia	<i>P. vulgaris</i>	<i>P. vulgaris</i>	<i>C. angustata</i> , <i>M. atropurpureum</i>	<i>L. leucocephala</i> , <i>S. microphylla</i> , <i>C. puniceus</i> , <i>V. hirsuta</i> , <i>P. communis</i> , <i>C. varia</i>
3644	England	<i>P. vulgaris</i>	<i>P. vulgaris</i>	<i>M. atropurpureum</i> , <i>O. vicifolia</i>	<i>L. leucocephala</i> , <i>S. microphylla</i> , <i>R. pseudoacacia</i> , <i>C. angustata</i> , <i>V. hirsuta</i> , <i>C. varia</i> , <i>L. corniculatus</i> , <i>T. repens</i> , <i>C. puniceus</i> , <i>M. sativa</i>
4001	Brazil	<i>P. vulgaris</i>	<i>P. vulgaris</i>	<i>P. vulgaris</i>	
F300	Brazil	<i>P. vulgaris</i>		<i>T. repens</i> , <i>C. angustata</i> , <i>C. puniceus</i> , <i>P. communis</i> , <i>O. vicifolia</i>	<i>P. vulgaris</i>
F310 ^d	Brazil	<i>P. vulgaris</i>		<i>T. subterraneum</i>	<i>V. hirsuta</i>
NZP5065	New Zealand ^c	<i>Carmichaelia</i> sp. ^f		<i>A. sophorae</i> , <i>R. pseudoacacia</i> , <i>L. nissolia</i> , <i>C. angustata</i> , <i>C. puniceus</i> , <i>O. vicifolia</i> , <i>M. atropurpureum</i>	<i>L. corniculatus</i> , <i>M. sativa</i>
NZP5279	New Zealand ^c	<i>Carmichaelia</i> sp. ^f	<i>T. repens</i>	<i>A. sophorae</i> , <i>R. pseudoacacia</i> , <i>C. angustata</i> , <i>C. puniceus</i> , <i>O. vicifolia</i> , <i>L. nissolia</i> , <i>M. atropurpureum</i>	<i>M. sativa</i>
CB2001	Australia ^c	<i>N. gracilis</i>	<i>L. leucocephala</i> , <i>S. microphylla</i> , <i>P. communis</i> , <i>P. vulgaris</i>		
CB2002	Australia ^c	<i>N. gracilis</i>	<i>L. leucocephala</i> , <i>S. microphylla</i> , <i>P. communis</i> , <i>P. vulgaris</i>		
Group 2					
CC401	United States	<i>C. varia</i>	<i>L. leucocephala</i> , <i>A. sophorae</i> , (<i>S. microphylla</i>), <i>P. vulgaris</i> , <i>C. varia</i> , <i>O. vicifolia</i> , (<i>R. pseudoacacia</i>)	<i>C. angustata</i> , <i>C. puniceus</i> , <i>P. communis</i> , <i>M. atropurpureum</i>	<i>L. nissolia</i> , <i>M. sativa</i> , <i>L. corniculatus</i>
3145	United States	<i>C. varia</i>	<i>L. leucocephala</i> , <i>A. sophorae</i> , (<i>S. microphylla</i>), <i>P. vulgaris</i> , <i>C. varia</i> , <i>O. vicifolia</i> , <i>M. atropurpureum</i> , (<i>R. pseudoacacia</i>)	<i>C. angustata</i> , <i>C. puniceus</i> , <i>P. communis</i> , <i>M. atropurpureum</i>	<i>L. nissolia</i> , <i>T. repens</i> , <i>M. sativa</i> , <i>L. corniculatus</i>

116A5	United States	<i>O. viciifolia</i>	<i>L. leucocephala</i> , <i>S. microphylla</i> , <i>P. vulgaris</i> , <i>C. varia</i> , <i>O. viciifolia</i> , <i>C. angustata</i> , (<i>L. corniculatus</i>)	<i>A. sophorae</i> , <i>C. puniceus</i> , <i>P. communis</i> , <i>M. atropurpureum</i> , <i>R. pseudoacacia</i> , <i>L. nissolia</i>	<i>T. repens</i> , <i>M. sativa</i>
116A14	United States	<i>O. viciifolia</i>	<i>L. leucocephala</i> , (<i>S. microphylla</i>), (<i>P. vulgaris</i>), (<i>C. varia</i>), <i>O. viciifolia</i>	<i>A. sophorae</i> , <i>C. angustata</i> , <i>C. puniceus</i> , <i>P. communis</i> , <i>M. atropurpureum</i> , <i>R. pseudoacacia</i>	<i>L. nissolia</i> , <i>T. repens</i>
114B2 ^c	United States ^c	<i>S. formosa</i>	<i>L. leucocephala</i> , <i>A. sophorae</i> , <i>S. microphylla</i> , <i>C. varia</i> , <i>O. viciifolia</i> , <i>P. vulgaris</i>	<i>C. angustata</i> , <i>C. puniceus</i> , <i>P. communis</i> , <i>M. atropurpureum</i> , <i>R. pseudoacacia</i>	<i>L. corniculatus</i> , <i>M. sativa</i>
114A1	United States	<i>S. secundiflora</i>	<i>L. leucocephala</i> , <i>A. sophorae</i> , <i>S. microphylla</i> , <i>P. vulgaris</i> , <i>C. varia</i> , <i>O. viciifolia</i>	<i>C. angustata</i> , <i>C. puniceus</i> , <i>P. communis</i> , <i>M. atropurpureum</i> , <i>R. pseudoacacia</i>	<i>L. nissolia</i> , <i>T. repens</i> , <i>M. sativa</i>
Group 3 CC2093 SU47 CB1170	Australia Australia United States	<i>M. lacinata</i> <i>M. sativa</i> <i>M. sativa</i>	<i>M. sativa</i> <i>M. sativa</i>	<i>M. sativa</i>	<i>T. repens</i> , <i>L. leucocephala</i> , <i>L. corniculatus</i> , <i>O. viciifolia</i> , <i>M. atropurpureum</i> <i>A. sophorae</i> , <i>L. leucocephala</i> , <i>R. pseudoacacia</i> , <i>O. viciifolia</i> , <i>M. atropurpureum</i> <i>R. pseudoacacia</i> , <i>L. nissolia</i> , <i>T. repens</i> , <i>L. corniculatus</i> , <i>C. varia</i> , <i>P. communis</i>
PDDCC1322	New Zealand	<i>M. sativa</i>	<i>M. sativa</i>	<i>C. angustata</i> , <i>C. puniceus</i>	
U45	Uruguay	<i>M. sativa</i>	<i>M. sativa</i>	<i>C. angustata</i> , <i>P. vulgaris</i>	<i>S. microphylla</i> , <i>P. communis</i>
3DOa13 Balsac CC2017	United States Canada Australia ^c	<i>M. sativa</i> <i>M. sativa</i> <i>T. suavisima</i>	<i>M. sativa</i> <i>M. sativa</i> <i>T. suavisima</i>	<i>C. angustata</i> , <i>C. puniceus</i>	
Group 4 Ca.Wisc. ^c	United States	<i>C. arborescens</i>	<i>C. arborescens</i> , <i>S. microphylla</i> , (<i>R. pseudoacacia</i>), <i>C. angustata</i> , <i>C. puniceus</i> , <i>L. corniculatus</i> , <i>S. flavesens</i>	<i>L. leucocephala</i> , <i>P. vulgaris</i> , <i>O. viciifolia</i> , <i>M. atropurpureum</i>	<i>C. varia</i> , <i>P. communis</i> , <i>M. sativa</i>

TABLE 1—Continued

Group and strain no.	Geographic origin	Plant origin	Forms effective nodules ^b	Forms ineffective nodules ^b	Does not nodulate
27A5	United States	<i>C. arietinum</i>	<i>C. arietinum</i>	<i>C. angustata</i> , <i>C. puniceus</i> , <i>M. sativa</i>	<i>O. viciifolia</i> , <i>L. leucocephala</i> , <i>L. nissolia</i> , <i>S. microphylla</i> , <i>M. atropurpureum</i> , <i>P. communis</i> , <i>R. pseudoacacia</i>
3HOa1	United States	<i>C. arietinum</i>	<i>C. arietinum</i>	<i>C. angustata</i> , <i>C. puniceus</i> , <i>M. sativa</i>	<i>L. leucocephala</i> , <i>L. nissolia</i> , <i>T. repens</i> , <i>P. communis</i> , <i>O. viciifolia</i> , <i>C. varia</i>
Revadim	Israel ^c	<i>C. arietinum</i>	<i>C. arietinum</i>		<i>L. leucocephala</i> , <i>L. nissolia</i> , <i>P. communis</i>
CC1005	Chile ^c	<i>Hosackia</i> sp.	<i>L. corniculatus</i>	<i>L. pedunculatus</i> , <i>M. atropurpureum</i>	<i>L. leucocephala</i> , <i>C. varia</i> , <i>O. viciifolia</i> , <i>S. microphylla</i>
CB1274	Sarawak	<i>L. leucocephala</i>	<i>L. leucocephala</i> , (<i>R. microphylla</i>), (<i>R. pseudoacacia</i>), <i>C. angustata</i> , <i>C. puniceus</i>	<i>A. sophorae</i> , <i>P. vulgaris</i> , <i>L. nissolia</i> , <i>P. communis</i> , <i>C. varia</i> , <i>O. viciifolia</i> , <i>M. atropurpureum</i>	<i>T. repens</i> , <i>M. sativa</i> , <i>L. corniculatus</i>
461	Canada	<i>Lotus</i> sp.	<i>L. corniculatus</i>	<i>L. pedunculatus</i>	
SU343	United States	<i>L. corniculatus</i>	<i>L. corniculatus</i> , <i>L. leucocephala</i> , <i>L. pedunculatus</i>	<i>L. angustifolius</i> , <i>C. puniceus</i>	<i>M. atropurpureum</i>
CC811	United States	<i>L. corniculatus</i>	<i>L. corniculatus</i>	<i>L. pedunculatus</i>	<i>M. atropurpureum</i>
Lc265Da	Ireland ^c	<i>L. corniculatus</i>	<i>L. corniculatus</i> , (<i>R. pseudoacacia</i>)	<i>L. leucocephala</i> , <i>L. pedunculatus</i> , <i>A. sophorae</i> , <i>C. angustata</i> , <i>C. puniceus</i> , <i>P. vulgaris</i>	<i>P. communis</i> , <i>O. viciifolia</i> , <i>C. varia</i> , <i>S. microphylla</i> , <i>L. nissolia</i> , <i>M. atropurpureum</i>
NZP2037	New Zealand	<i>L. divaricatus</i>	<i>L. corniculatus</i> , <i>L. pedunculatus</i> , <i>L. leucocephala</i>	<i>A. sophorae</i> , <i>C. angustata</i> , <i>C. puniceus</i> , <i>P. vulgaris</i>	<i>S. microphylla</i> , <i>R. pseudoacacia</i> , <i>P. communis</i> , <i>O. viciifolia</i> , <i>T. repens</i>
CC809a	Morocco ^c	<i>L. maroccanus</i>	<i>L. corniculatus</i> , <i>L. maroccanus</i>	<i>L. pedunculatus</i>	<i>M. atropurpureum</i>
NZP2213	New Zealand	<i>L. tenuis</i>	<i>L. corniculatus</i>	<i>L. pedunculatus</i>	<i>M. atropurpureum</i>
NZP2260	New Zealand	<i>L. tenuis</i>	<i>L. corniculatus</i>	<i>L. pedunculatus</i> , <i>M. atropurpureum</i>	<i>S. microphylla</i> , <i>T. repens</i>
L. densiflorus	United States ^c	<i>L. densiflorus</i>	<i>L. tenuis</i>	<i>L. corniculatus</i> , <i>M. atropurpureum</i>	<i>L. pedunculatus</i> , <i>S. microphylla</i> , <i>R. pseudoacacia</i> , <i>C. puniceus</i> , <i>P. communis</i> , <i>T. repens</i>
3G2c2a	United States	<i>O. viciifolia</i>	(<i>O. viciifolia</i>), <i>S. microphylla</i> , <i>C. angustata</i> , <i>C. puniceus</i> , (<i>P. communis</i>), <i>S. flavesces</i>	<i>A. sophorae</i> , <i>R. pseudoacacia</i> , <i>L. leucocephala</i> , <i>C. varia</i> , <i>M. atropurpureum</i> , <i>P. vulgaris</i>	<i>L. corniculatus</i> , <i>T. repens</i> , <i>L. nissolia</i>

NZP5361	New Zealand	<i>P. communis</i>	<i>P. communis</i> , <i>S. microphylla</i> , <i>S. flavescens</i>	<i>A. sophorae</i> , <i>R. pseudoacacia</i> , <i>C. angustata</i> , <i>C. puniceus</i> , <i>O. viciifolia</i>	<i>C. varia</i> , <i>L. leucocephala</i> , <i>L. nissolia</i> , <i>L. corniculatus</i> , <i>M. sativa</i>
CB712 ^c	Australia ^c	<i>P. eriantha</i>	<i>P. pinnata</i>		
NZP5302	United States ^c	<i>R. pseudoacacia</i>	<i>R. pseudoacacia</i>		
J2061	Japan ^c	<i>S. angustifolia</i>	<i>S. microphylla</i> , <i>A. sophorae</i> , <i>C. angustata</i> , <i>C. puniceus</i> , <i>S. flavescens</i>	<i>P. communis</i> , <i>P. vulgaris</i>	All other hosts tested <i>L. leucocephala</i> , <i>R. pseudoacacia</i> , <i>L. nissolia</i> , <i>T. lupinaster</i> , <i>L. corniculatus</i> , <i>M. atropurpureum</i>
NZP5275	New Zealand ^c	<i>S. microphylla</i>	<i>S. microphylla</i> , <i>S. flavescens</i>	<i>C. angustata</i> , <i>C. puniceus</i> , <i>P. communis</i> , <i>O. viciifolia</i> , <i>P. vulgaris</i>	<i>L. leucocephala</i> , <i>C. varia</i> , <i>L. nissolia</i> , <i>L. corniculatus</i> , <i>T. repens</i>
NZP5057	New Zealand ^c	<i>S. tetraptera</i>	<i>S. microphylla</i>	<i>C. angustata</i> , <i>C. puniceus</i> , <i>P. communis</i> , <i>O. viciifolia</i> , <i>A. sophorae</i>	<i>L. leucocephala</i> , <i>C. varia</i> , <i>T. repens</i>
TLN3(NZP504)	Japan ^c	<i>T. lupinaster</i>	<i>T. lupinaster</i> , <i>S. flavescens</i>	(<i>T. repens</i>), <i>T. subterraneum</i> , <i>C. angustata</i> , <i>P. vulgaris</i> , <i>R. pseudoacacia</i> , <i>O. viciifolia</i> , <i>C. puniceus</i>	<i>L. nissolia</i> , <i>L. leucocephala</i> , <i>C. varia</i> , <i>M. sativa</i>
TLN3(NZP505)	Japan ^c	<i>T. lupinaster</i>	<i>T. lupinaster</i> , <i>S. flavescens</i>	<i>T. repens</i> , <i>T. subterraneum</i> , <i>C. angusta</i> , <i>C. puniceus</i>	<i>S. microphylla</i> , <i>L. leucocephala</i> , <i>P. communis</i> , <i>M. sativa</i>
Group 5 3F6s1	United States	<i>A. onobrychis</i>	<i>A. sophorae</i> , <i>L. leucocephala</i> , <i>S. microphylla</i> , <i>C. puniceus</i> , <i>C. angustata</i> , <i>S. flavescens</i> , (<i>L. corniculatus</i>)	<i>R. pseudoacacia</i> , <i>P. communis</i> , <i>C. varia</i> , <i>P. vulgaris</i> , <i>M. atropurpureum</i>	
J2040	Japan	<i>C. chamlagu</i>	<i>C. puniceus</i> , (<i>L. corniculatus</i>), (<i>T. lupinaster</i>), <i>S. flavescens</i> , <i>C. arborescens</i> , <i>S. microphylla</i> , <i>C. angustata</i>	<i>P. vulgaris</i> , <i>P. communis</i>	<i>C. varia</i> , <i>M. sativa</i>
CB81	Australia	<i>L. leucocephala</i>	<i>L. leucocephala</i> , <i>A. sophorae</i> , <i>S. microphylla</i> , <i>R. pseudoacacia</i> , <i>C. puniceus</i>	<i>C. angustata</i> , <i>P. communis</i> , <i>O. viciifolia</i> , <i>P. vulgaris</i>	<i>M. atropurpureum</i> , <i>M. sativa</i> , <i>L. nissolia</i> , <i>L. corniculatus</i> , <i>T. repens</i>
CB2919	Australia	<i>L. leucocephala</i>	<i>L. leucocephala</i> , (<i>O. viciifolia</i>)	<i>P. vulgaris</i> , <i>M. atropurpureum</i>	<i>M. sativa</i> , <i>L. nissolia</i> , <i>P. communis</i>

TABLE 1—Continued

Group and strain no.	Geographic origin	Plant origin	Forms effective nodules ^b	Forms ineffective nodules ^b	Does not nodulate
NGR8	New Guinea	<i>L. leucocephala</i>	<i>L. leucocephala</i> , <i>S. microphylla</i>	<i>A. sophorae</i> , <i>R. pseudoacacia</i> , <i>C. angustata</i> , <i>C. puniceus</i> , <i>M. atropurpureum</i> , <i>P. communis</i>	<i>O. viciifolia</i> , <i>M. sativa</i> , <i>L. nissolia</i> , <i>L. corniculatus</i>
X18b	Brazil ^c	<i>M. caesalpiniaefolia</i>		<i>L. leucocephala</i> , <i>R. pseudoacacia</i> , <i>C. angustata</i> , <i>P. vulgaris</i> , <i>C. puniceus</i>	<i>M. sativa</i>
J1010	Indonesia ^c	<i>M. invisa</i>	<i>L. leucocephala</i> , <i>S. microphylla</i> , <i>C. varia</i>	<i>L. nissolia</i> , <i>C. angustata</i> , <i>C. puniceus</i> , <i>P. communis</i> , <i>R. pseudoacacia</i> , <i>P. vulgaris</i> , <i>M. atropurpureum</i>	<i>M. sativa</i> , <i>L. corniculatus</i>
NZP5299	New Zealand	<i>P. communis</i>	<i>P. communis</i> , <i>S. microphylla</i> , (<i>R. pseudoacacia</i>)	<i>C. angustata</i> , <i>C. puniceus</i> , <i>O. viciifolia</i>	<i>L. leucocephala</i> , <i>T. repens</i> , <i>M. sativa</i>
CB362	Australia ^c	<i>P. eriantha</i>	<i>P. pinnata</i>	<i>C. angustata</i> , <i>P. vulgaris</i>	<i>L. leucocephala</i> , <i>S. microphylla</i> , <i>R. pseudoacacia</i> , <i>C. puniceus</i> , <i>P. communis</i> , <i>C. varia</i> , <i>O. viciifolia</i>
NZP5355 Vr5 ^d Group 6 ES1	New Zealand Brazil ^c Brazil ^c	<i>R. pseudoacacia</i> <i>V. racemosa</i> <i>E. speciosa</i>	<i>R. pseudoacacia</i> <i>M. atropurpureum</i>		All other hosts tested All hosts tested
NZP2192	New Zealand	<i>L. corniculatus</i>	<i>L. pedunculatus</i> , <i>M. atropurpureum</i>		
NZP2257	New Zealand	<i>L. corniculatus</i>	<i>L. pedunculatus</i> , <i>L. corniculatus</i> , <i>M. atropurpureum</i> , <i>A. sophorae</i>	<i>P. vulgaris</i>	<i>T. repens</i> , <i>P. communis</i> , <i>M. sativa</i> , <i>C. puniceus</i>
NZP2076	New Zealand	<i>L. suaveolens</i>	<i>L. pedunculatus</i> , <i>M. atropurpureum</i>		
NZP5087 WU425 NZP5113 94	New Zealand Australia New Zealand United States ^c	<i>L. angustifolius</i> <i>O. compressus</i> <i>O. perpusillus</i> <i>P. acutifolius</i>	<i>L. pedunculatus</i> <i>L. pedunculatus</i> <i>L. pedunculatus</i> <i>M. atropurpureum</i>	<i>M. atropurpureum</i> (Siratro) <i>M. atropurpureum</i> (Siratro) <i>M. atropurpureum</i> (Siratro) <i>P. vulgaris</i>	

100	United States	<i>P. aureus</i>	<i>M. atropurpureum</i>	<i>P. vulgaris</i>
^a	Abbreviations: <i>A. sophorae</i> , <i>Acacia sophorae</i> ; <i>A. onobrychis</i> , <i>Astragalus onobrychis</i> ; <i>C. arborescens</i> , <i>Caragana arborescens</i> ; <i>C. chamlagu</i> , <i>Caragana chamlagu</i> ; <i>C. angustata</i> , <i>Carmichaelia angustata</i> ; <i>C. arietinum</i> , <i>Cicer arietinum</i> ; <i>C. puniceus</i> , <i>Cilanthus puniceus</i> ; <i>C. varia</i> , <i>Coronilla varia</i> ; <i>E. speciosa</i> , <i>Erythrina speciosa</i> ; <i>L. japonicus</i> , <i>Lathyrus japonicus</i> ; <i>L. latifolius</i> , <i>Lathyrus latifolius</i> ; <i>L. nissolia</i> , <i>Lathyrus nissolia</i> ; <i>L. sativus</i> , <i>Lathyrus sativus</i> ; <i>L. leucocephala</i> , <i>Leucaena leucocephala</i> ; <i>L. corniculatus</i> , <i>Lotus corniculatus</i> ; <i>L. divaricatus</i> , <i>Lotus divaricatus</i> ; <i>L. maroccanus</i> , <i>Lotus maroccanus</i> ; <i>L. pedunculatus</i> , <i>Lotus pedunculatus</i> ; <i>L. suaveolens</i> , <i>Lotus suaveolens</i> ; <i>L. tenuis</i> , <i>Lotus tenuis</i> ; <i>L. angustifolius</i> , <i>Lupinus angustifolius</i> ; <i>L. densiflorus</i> , <i>Lupinus densiflorus</i> ; <i>M. atropurpureum</i> , <i>Macroptilium atropurpureum</i> (Stratrol); <i>M. lacinata</i> , <i>Medicago lacinata</i> ; <i>M. sativa</i> , <i>Medicago sativa</i> ; <i>M. caesalpiniaefolia</i> , <i>Mimosa caesalpiniaefolia</i> ; <i>M. invisa</i> , <i>Mimosa invisa</i> ; <i>N. gracilis</i> , <i>Neptunia gracilis</i> ; <i>O. viciifolia</i> , <i>Onobrychis viciifolia</i> ; <i>O. compressus</i> , <i>Ornithopus compressus</i> ; <i>O. perspusillus</i> , <i>Ornithopus perspusillus</i> ; <i>P. communis</i> , <i>Parochetus communis</i> ; <i>P. acutifolius</i> , <i>Phaseolus acutifolius</i> ; <i>P. aureus</i> , <i>Phaseolus aureus</i> ; <i>P. vulgaris</i> , <i>Phaseolus vulgaris</i> ; <i>P. arvense</i> , <i>Pisum arvense</i> ; <i>P. hortense</i> , <i>Pisum hortense</i> ; <i>P. sativum</i> , <i>Pisum sativum</i> ; <i>P. eriantha</i> , <i>Psoralea eriantha</i> ; <i>P. pinnata</i> , <i>Psoralea pinnata</i> ; <i>R. pseudoacacia</i> , <i>Robinia pseudoacacia</i> ; <i>S. angustifolia</i> , <i>Sophora angustifolia</i> ; <i>S. flavescens</i> , <i>Sophora flavescens</i> ; <i>S. formosa</i> , <i>Sophora formosa</i> ; <i>S. microphylla</i> , <i>Sophora microphylla</i> ; <i>S. secundiflora</i> , <i>Sophora secundiflora</i> ; <i>S. tetraptera</i> , <i>Sophora tetraptera</i> ; <i>T. africanum</i> , <i>Trifolium africanum</i> ; <i>T. ambiguum</i> , <i>Trifolium ambiguum</i> ; <i>T. arvense</i> , <i>Trifolium arvense</i> ; <i>T. beckwithii</i> , <i>Trifolium beckwithii</i> ; <i>T. burchellianum</i> , <i>Trifolium burchellianum</i> ; <i>T. carolinianum</i> , <i>Trifolium carolinianum</i> ; <i>T. lugardii</i> , <i>Trifolium lugardii</i> ; <i>T. lupinaster</i> , <i>Trifolium lupinaster</i> ; <i>T. polymorphum</i> , <i>Trifolium polymorphum</i> ; <i>T. pratense</i> , <i>Trifolium pratense</i> ; <i>T. repens</i> , <i>Trifolium repens</i> ; <i>T. rueppellianum</i> , <i>Trifolium rueppellianum</i> ; <i>T. rydbergii</i> , <i>Trifolium rydbergii</i> ; <i>T. semipilosum</i> , <i>Trifolium semipilosum</i> ; <i>T. subterraneum</i> , <i>Trifolium subterraneum</i> ; <i>T. tembense</i> , <i>Trifolium tembense</i> ; <i>T. vesiculosum</i> , <i>Trifolium vesiculosum</i> ; <i>T. suavisima</i> , <i>Trigonella suavisima</i> ; <i>V. angustifolia</i> , <i>Vicia angustifolia</i> ; <i>V. faba</i> , <i>Vicia faba</i> ; <i>V. hirsuta</i> , <i>Vicia hirsuta</i> ; <i>V. racemosa</i> , <i>Vicia racemosa</i> .			

^c Host plant indigenous to country of origin.

^d The culture, as received from overseas, did not nodulate the test hosts.

^e The organism used in the investigation and grown from a single colony was found to be a nonnodulating variant of the original strain; nodulation data for the original strain are given.

^f Isolated from an ineffective nodule on this plant host.

TABLE 2. Relative hybridization of DNA from 130 strains of *Rhizobium* with DNA from reference strains of fast-growing rhizobia after incubation at 65°C and Cat 250°

Source of unlabeled DNA (strain no., plant host)	Relative hybridization (%) of the following reference strains with their plant origins:						
	NU125, <i>Trifolium</i> <i>polymorphum</i>	CC275e, <i>Trifolium</i> <i>repens</i>	NZP5459, <i>Phaseolus</i> <i>vulgaris</i>	CC811, <i>Lotus</i> <i>corniculatus</i>	CC808a, <i>Lotus</i> <i>maroccanus</i>	CC401, <i>Coronilla</i> <i>varia</i>	SU47, <i>Medicago</i> <i>sativa</i>
Group 1							
NU125, <i>Trifolium polymorphum</i>	100	52	71	12	5	26	15
NU126, <i>T. polymorphum</i>	76	50	74	9	5	26	10
NU132, <i>T. polymorphum</i>	79	49	68	6	3	16	11
NU134, <i>T. polymorphum</i>	76	47	66	6	5	20	10
674A, <i>T. africanum</i>	53	64	58	11	9	28	16
SA3, <i>T. africanum</i>	59	60	61	5	7	26	4
CB727, <i>T. burchellianum</i>	40	33	38	5	6	19	5
CB774, <i>T. rueppellianum</i>	57	ND	63	ND	ND	ND	ND
408A, <i>T. rueppellianum</i>	57	53	66	11	8	24	17
CB778, <i>T. semipilosum</i>	49	ND	49	ND	ND	ND	ND
CB758, <i>T. tembense</i>	60	ND	58	ND	ND	ND	ND
CB775, <i>T. usambarense</i>	63	ND	62	ND	ND	ND	ND
39, <i>T. arvense</i>	53	74	51	3	10	26	16
3Dj12, <i>T. bechuithii</i>	47	75	59	6	12	24	13
41, <i>T. carolinianum</i>	50	50	44	13	13	26	16
3Dj14, <i>T. rydbergii</i>	55	86	51	8	10	22	20
NZP550/2, <i>T. vesiculosum</i>	42	62	41	3	5	15	0
CC227, <i>T. ambiguum</i>	30	74	34	3	6	11	7
CC229, <i>T. ambiguum</i>	50	73	53	4	2	20	11
CC231a, <i>T. ambiguum</i>	40	66	46	5	3	9	7
SU202, <i>T. pratense</i>	53	93	58	9	6	29	18
CC275e, <i>T. repens</i>	57	100	55	8	7	27	17
NZP562a, <i>T. repens</i>	47	89	45	6	6	23	13
NZP564, <i>T. repens</i>	55	84	51	9	6	32	11
NZP565, <i>T. repens</i>	52	76	52	9	5	25	15
NZP566, <i>T. repens</i>	52	67	50	6	12	25	15
NZP567, <i>T. repens</i>	55	70	54	3	11	26	19
K8, <i>T. repens</i>	39	68	44	4	2	17	7
CC2480a, <i>T. subterraneum</i>	54	72	52	13	17	29	16
WU290, <i>T. subterraneum</i>	53	80	51	10	14	26	16
WU351, <i>T. subterraneum</i>	52	83	49	11	13	27	16
NZP5470, <i>Lathyrus latifolius</i>	54	82	57	9	5	30	18
92AA1, <i>L. japonicus</i>	54	76	51	12	16	25	16
92AA3, <i>L. japonicus</i>	50	82	53	12	11	26	17
NZP5469, <i>L. sativus</i>	52	61	52	6	12	28	17

CB596, <i>Pisum</i> sp.	47	84	55	8	12	30	16
SU391, <i>Pisum</i> sp.	55	71	55	6	9	31	15
TA101, <i>P. arvense</i>	38	67	37	13	8	25	13
63, <i>P. arvense</i>	54	66	52	15	16	27	17
52, <i>P. hortense</i>	67	57	76	8	5	25	13
A1, <i>P. sativum</i>	46	69	57	5	8	20	11
F3, <i>P. sativum</i>	47	49	41	10	10	23	13
NZP525, <i>Vicia angustifolia</i>	48	86	52	8	28	23	15
3HOg2, <i>V. faba</i>	52	67	51	3	8	28	16
V73m5, <i>V. faba</i>	40	71	52	5	4	19	10
3HOg1, <i>V. faba</i>	47	72	51	6	9	25	13
3HOc3, <i>V. villosa</i>	52	72	53	5	13	32	18
CIAT75, <i>Phaseolus vulgaris</i>	44	ND	51	9	12	20	ND
CIAT137, <i>P. vulgaris</i>	63	ND	70	6	12	22	ND
CIAT161, <i>P. vulgaris</i>	50	ND	49	12	14	23	ND
CIAT225, <i>P. vulgaris</i>	45	ND	52	2	5	21	ND
CIAT632, <i>P. vulgaris</i>	60	ND	63	3	13	20	ND
NZP5459, <i>P. vulgaris</i>	68	58	100	8	13	24	16
CB971, <i>P. vulgaris</i>	50	47	66	6	5	15	10
NZP5097, <i>P. vulgaris</i>	57	48	63	3	3	2	14
CC511, <i>P. vulgaris</i>	36	46	54	7	3	23	16
3644, <i>P. vulgaris</i>	39	60	50	7	4	20	14
4001, <i>P. vulgaris</i>	51	45	58	7	8	24	14
F300, <i>P. vulgaris</i>	24	30	33	12	7	17	12
F310, <i>P. vulgaris</i>	45	32	46	7	5	26	12
NZP5065, <i>Carmichaelia</i> sp. (ineffective nodule)	47	68	52	5	4	23	15
NZP5279, <i>Carmichaelis</i> sp. (ineffective nodule)	49	100	63	1	12	23	15
CB2001, <i>Neptunia gracilis</i>	42	47	54	15	13	30	17
CB2002, <i>N. gracilis</i>	47	52	52	5	11	28	16
Mean relative homology	51	65	54	7	9	23	14
Group 2							
CC401, <i>Coronilla varia</i>	22	26	25	2	7	100	16
31A5, <i>C. varia</i>	19	29	23	9	9	106	12
116A5, <i>Onobrychis viciifolia</i>	21	29	21	8	8	78	18
116A14, <i>O. viciifolia</i>	18	23	20	4	4	93	12
146B2, <i>Sophora formosa</i>	23	30	24	0	9	100	17
146A1, <i>Sophora secundiflora</i>	21	30	24	5	7	103	18
Mean relative homology	21	28	23	5	7	96	16
Group 3							
CC2093, <i>Medicago lacinata</i>	12	14	14	4	8	12	68
SU47, <i>Medicago sativa</i>	15	19	11	9	8	8	100

TABLE 2—Continued

Source of unlabeled DNA (strain no., plant host)	Relative hybridization (%) of the following reference strains with their plant origins:						
	NU125, <i>Trifolium</i> <i>polymorphum</i>	CC275e, <i>Trifolium</i> <i>repens</i>	NZP5459, <i>Phaseolus</i> <i>vulgaris</i>	CC811, <i>Lotus</i> <i>corniculatus</i>	CC809a, <i>Lotus</i> <i>maroccanus</i>	CC401, <i>Coronilla</i> <i>varia</i>	SU47, <i>Medicago</i> <i>sativa</i>
CB1170, <i>M. sativa</i>	13	14	13	12	14	12	95
PDDCC1322, <i>M. sativa</i>	12	ND	10	3	11	17	101
U45, <i>M. sativa</i>	14	ND	10	3	13	16	96
3DOa13, <i>M. sativa</i>	8	ND	15	9	8	17	103
Balsac, <i>M. sativa</i>	15	ND	13	11	21	14	103
CC2017, <i>Trigonella suavissima</i>	11	15	14	8	6	14	78
Mean relative homology	13	16	13	7	11	14	92
Group 4							
Ca.Wisc., <i>Caragana arborescens</i>	9	13	12	52	62	6	10
27A5, <i>Cicer arietinum</i>	3	3	8	41	51	6	8
3HOa1, <i>C. arietinum</i>	4	5	7	41	85	3	5
Revadim, <i>C. arietinum</i>	11	8	9	51	88	8	9
CC1005, <i>Hosackia</i> sp.	9	12	9	35	43	8	11
CB1274, <i>Leucaena leucocephala</i>	14	14	14	45	39	8	12
461, <i>Lotus</i> sp.	9	15	9	62	51	10	15
SU343, <i>Lotus corniculatus</i>	8	11	11	74	40	7	10
CC811, <i>L. corniculatus</i>	13	11	9	100	51	6	13
Le265Da, <i>L. corniculatus</i>	11	7	12	68	32	8	12
NZP2037, <i>Lotus divaricatus</i>	9	4	11	60	46	8	10
CC809a, <i>L. maroccanus</i>	5	9	9	46	100	5	9
NZP2213, <i>L. tenuis</i>	6	10	8	60	47	5	5
NZP2260, <i>L. tenuis</i>	5	7	9	51	59	9	8
L.densif85, <i>Lupinus densiflorus</i>	10	2	13	44	49	7	10
3G2c2a, <i>Onobrychis viciifolia</i>	8	6	8	51	71	6	8
NZP5361, <i>Parochetus communis</i>	9	7	10	58	41	2	7
CB712, <i>Psoralea eriantha</i>	8	14	13	39	38	10	14
NZP5302, <i>Robinia pseudoacacia</i>	7	6	15	47	51	7	7
J2061, <i>Sophora angustifolia</i>	12	11	9	45	39	9	13
NZP5275, <i>Sophora microphylla</i>	8	8	11	59	52	7	9
NZP5057, <i>Sophora tetralpura</i>	8	9	8	37	36	7	8
TL3(NZP504), <i>Trifolium lupinaster</i>	9	12	16	64	47	9	11
TLN(NZP505), <i>T. lupinaster</i>	4	6	8	46	37	6	6
Mean relative homology	8	9	10	51	50	7	10

Group 5									
3F6s1, <i>Astragalus onobrychis</i>	4	7	6	33	27	4	5		
J2040, <i>Caragana chamlagu</i>	8	9	8	32	18	7	8		
CB81, <i>Leucaena leucocephala</i>	9	11	6	22	27	3	12		
CB2919, <i>L. leucocephala</i>	10	6	7	6	4	8	16		
NGR8, <i>L. leucocephala</i>	14	15	16	8	5	17	33		
X18b, <i>Mimosa caesalpiniaeifolia</i>	3	0	2	5	10	2	2		
J1010, <i>Mimos invisa</i>	17	19	18	7	7	18	17		
NZP5299, <i>Parochetus communis</i>	1	2	5	29	14	3	1		
CB362, <i>Psoralea eriantha</i>	8	12	8	30	30	8	14		
NZP5355, <i>Robinia pseudoacacia</i>	3	4	39	39	12	6	3		
Vr5, <i>Vigna racemosa</i>	20	30	24	6	4	23	13		
Mean relative homology	9	10	13	20	14	9	11		
Group 6									
ES1, <i>Erythrina speciosa</i>	4	3	3	4	5	3	4		
NZP2192, <i>Lotus corniculatus</i>	9	ND	9	6	5	6	ND		
NZP2257, <i>L. corniculatus</i>	3	ND	3	4	4	3	ND		
NZP2076, <i>Lotus suaveolens</i>	4	ND	0	2	4	3	ND		
NZP5087, <i>Lupinus angustifolius</i>	2	ND	0	2	3	5	ND		
WU425, <i>Ornithippus compressus</i>	4	ND	5	8	5	6	ND		
NZP5113, <i>Ornithippus perpusillus</i>	6	ND	5	6	5	5	ND		
94, <i>Phaseolus acutifolius</i>	6	9	5	5	5	3	5		
100, <i>Phaseolus aureus</i>	5	7	6	5	4	4	6		
Mean relative homology	5	6	4	5	4	4	5		
Control									
<i>Escherichia coli</i> K-12	0	0	0	0	1.0	0	0		

^a ND, Not done.

coli strains originated from bean (*Phaseolus vulgaris*) species, and 2 *Rhizobium* strains each originated from *Carmichaelia* sp. and 2 from *Neptunia gracilis*.

Genetic diversity and plant specificity in group 1. As seen in Table 2, there was considerable genetic diversity within the group. The calculation of mean homology values for strains from the same plant host or geographic location indicated that some subgroups existed. For example, the mean homology values of clover rhizobia with respect to the reference DNA from NU125, CC275e, and NZP5459 were 83, 50, 70%, respectively, for the 4 *Trifolium polymorphum* strains, 55, 53, and 57%, respectively, for the 3 African *Trifolium* strains (674A through CB775), and 49, 78, and 50%, respectively, for the remaining 19 strains from European, Caucasian, and North American *Trifolium* species.

The mean homologies for rhizobia from European, Caucasian, and North American clovers were very similar to those for pea rhizobia (50, 71, 53%, respectively) and indicated that plant specificity among clover and pea rhizobia was not reflected in base-sequence homology.

Strains designated *Rhizobium phaseoli* showed some base sequence divergence from *Rhizobium trifolii* and *Rhizobium leguminosarum* and a lower average homology with reference DNA from *Rhizobium phaseoli* NZP5459 than did *Rhizobium trifolii* or *Rhizobium leguminosarum* strains with reference DNA from *Rhizobium trifolii* strain CC275e. Four strains of *Rhizobium* from *Trifolium polymorphum* showed a higher mean homology (70%) with reference DNA from *Rhizobium phaseoli* NZP5459 than did other bean rhizobia (58%). However, when a strain of *Rhizobium* from *Trifolium polymorphum* (NU125) was used to provide reference DNA, the average homology with *Rhizobium phaseoli* strains was only 49%, and the range was 24 to 68%. These results indicate that although some strains of *Rhizobium* from clover and beans have considerable base-sequence homology, strains designated *Rhizobium phaseoli* are a genetically diverse group which contains members which are not closely related to *Rhizobium trifolii* and *Rhizobium leguminosarum*.

The two *Rhizobium* strains from *Carmichaelia* were obtained from ineffective nodules on *Carmichaelia flagelliformis* seedlings used as trap hosts for isolating rhizobia from soil samples. Both strains behaved as clover rhizobia, nodulating clover species freely, and strain NZP5279 fixed nitrogen on white clover. In contrast, rhizobia effective on *Carmichaelia* spp. did not nodulate clovers (11) and showed low DNA homology with *Rhizobium trifolii* (16).

The DNA homology of the two strains (Table 2) confirms that the strains are members of *Rhizobium trifolii*.

The two strains of rhizobia from *Neptunia gracilis* had homology values within the range for the clover, pea, and bean rhizobia. The inclusion of these two strains in group 1 was not expected on the basis of plant specificity. Although they both fixed some nitrogen on *Phaseolus vulgaris* and nodulated *Lathyrus nissolia*, in most respects they were quite distinct in host range from other strains in group 1. Thus, none of the *Rhizobium leguminosarum*, *Rhizobium phaseoli*, or *Rhizobium trifolii* strains that were tested nodulated *Leucaena leucocephala* or fixed nitrogen with *Sophora microphylla* or *Parochetus communis*.

Two strains included in group 1 showed comparatively low homology with reference strains NU125, CC275e, and NZP5459. Strain CB727, from *Trifolium burchellianum*, showed host specificity typical of strains obtained from African clovers. It could fix some nitrogen on *Trifolium lugardii*, and it also nodulated white and subterranean clovers. Strain F300, on the other hand, although received as a strain of *Rhizobium phaseoli*, was found to nodulate *Phaseolus vulgaris* but not to fix nitrogen on this host. There is, therefore some doubt whether F300 should be regarded as a typical *Rhizobium phaseoli* strain.

The DNA homology values for F300 support this as the values are 33% or less with the three group 1 reference strains (NU125, CC275e, and NZP5459). However, this strain showed less homology with the other four reference strains and, therefore, is placed in the group 1 homology grouping because, traditionally, this strain would be called *Rhizobium phaseoli*. Further study, however, may result in this strain being grouped with some of the strains from group 5.

A wide range of *Rhizobium* strains is able to nodulate *Phaseolus vulgaris* (14), including, as determined in the investigation reported here, strains from several different homology groups given in Tables 1 and 2.

Genetic homology in group 2. The six strains in group 2 (Table 2) formed a distinct genetic cluster which had a mean homology of 96% (range 78 to 106) with reference DNA from strain CC401 isolated from *Coronilla varia*. They showed low DNA homology (18 to 30%) with reference DNAs from NU125, CC175e, and NZP5459, and very little homology (0 to 9%) with reference DNAs from CC811 and CC809a. The six strains were isolated in the United States from four hosts, two of them indigenous to North America and two naturalized in the United States but originally from Europe. Nevertheless,

all of the six *Rhizobium* strains were closely similar in plant specificity. They all fixed nitrogen with *Leucaena leucocephala*, *Sophora microphylla*, *Phaseolus vulgaris*, *Coronilla varia*, and *Onobrychis vicifolia*; no other strains used in this investigation formed effective nodules on all five species. They did, however, differ among themselves in nodulation and nitrogen fixation with certain other hosts (e.g. *Acacia sophorae*, *Macroptilium atropurpureum* (Siratro), and *Lathyrus nissolia*), as indicated in Table 1.

Genetic homology and plant specificity in group 3. Only strains belonging to the alfalfa cross-inoculation group (*Rhizobium meliloti*) were found in group 3. These strains showed a range of homology values of from 68 to 103% with DNA from the alfalfa reference strain SU47, and little homology (3 to 17%) with other reference DNAs. Six of the strains showed a homology of 95% or greater with SU47 reference DNA. The remaining two strains, which showed 68% and 78% homology, were isolated from *Medicago laciniata* and *Trigonella sauvissima*, species with very specific rhizobial requirements (1). Rhizobia not belonging to the alfalfa cross-inoculation group showed low homology with respect to the SU47 reference DNA (0 to 19%) except for NGR8 (group 5), a strain from *Leucaena leucocephala*, which showed 33% homology. Although in this investigation no symbiotic relationship was found between *Medicago sativa* and *Leucaena leucocephala* rhizobia, Trinick (25) reported that some strains from *Leucaena leucocephala*, but not NGR8, were able to form ineffective nodules on *Medicago sativa*.

Genetic homology in group 4. The strains placed in group 4 showed relatively high mean DNA homologies (51 and 50%) with the two reference strains CC811 and CC809a from *Lotus* spp. and low homologies (7 to 10%) with DNAs from the other reference strains. Group 4 is genetically diverse, since homology values with reference DNAs from *Lotus* rhizobia ranged from 36 to 88%. Two *Rhizobium* strains, TL3 and TLN3 from *Trifolium lupinaster*, would be classified as *Rhizobium trifolii* on the basis of their plant origins and cross-inoculation groups; the remainder have not been previously classified.

The six strains associated with *Lotus corniculatus* and *Lotus tenuis* had a mean homology of 63% relative to strain CC811 from *Lotus corniculatus* a value appreciably higher than the mean of 48% for the remaining strains of group 4. The mean homology of the same six strains relative to strain CC809a from a different *Lotus* species was 47% compared with 51% for the remaining group 4 strains. These results suggest that a distinguishable subgroup may be associ-

ated with *Lotus corniculatus* and *Lotus tenuis*. These are closely related plant species.

Plant specificity in group 4. Group 4 strains were isolated from many different hosts, with species from 12 genera, and they were also of diverse geographic origin. They differed greatly in host specificity, including the range of hosts that they could nodulate. Thus, strain NZP5302 (obtained from an American peat culture for *Robinia pseudoacacia*) was not found to nodulate any legumes other than *Robinia pseudoacacia*, but strains CaWisc, CB1274, and 3G2c2a could fix nitrogen with five or more unrelated legumes, and nodulate many others. On the basis of host specificity, there was no reason to place these strains in one group or to separate them from the strains placed in group 5, which includes several isolates from the same or related hosts.

Group 5. Group 5 does not necessarily represent a genetic group, but it contains strains of fast-growing rhizobia that did not fall into any of the above-mentioned groups. All of these strains showed significantly less than 40% homology with any of the seven reference DNAs. One strain (Vr5) showed homologies with group 1 reference DNAs from NU125, CC275e, and NZP5459, which indicated some genetic relationship with this group. This strain was not placed in group 1 because group 2 strains which showed the same or higher homology values with group 1 reference DNAs were identified as a distinct genetic group with reference DNA from strain CC401. Similarly, strains J2040, 3F6S1, and CB81 were placed in group 5 even though they showed greater similarity to the two *Lotus* reference strains than to the other five reference strains. From these examples it is seen that some strains with intermediate homology values cannot be clearly placed in any particular group. Such strains might be classified more accurately if homology values were obtained with additional reference DNAs.

The *Rhizobium* strain NZP5355 obtained from *Robinia pseudoacacia* and placed in group 5 differed from all of the other strains studied as it showed moderate (39%) DNA homology with NZP5459 and CC811 reference strains and low values (<11%) with the remaining reference strains. The other *Rhizobium* strain, NZP5302, taken from *Robinia pseudoacacia* was a typical group 4 strain since it showed 47 and 51% homologies with the two *Lotus* sp. reference DNAs and a low value (15%) with DNA and NZP5459. Both strains were found to nodulate *Robinia pseudoacacia* only.

Group 6. All of the nine slow-growing strains listed in Table 2 showed low DNA homology (<10%) with all seven fast-growing reference

strains. This result supports the generally recognized distinction between fast- and slow-growing rhizobia, but gives no indication of the genetic relationships among slow-growing rhizobia.

Reciprocal matches and genome sizes. The values for the reciprocal matches which can be extracted from Table 2 give a mean error of $\pm 1.4\%$ with the greatest error being $\pm 4\%$ for strains CC401 and SU47. Genome sizes were measured for reference strains CC275e, CC811, CC809a, and SU47. The respective values were 3.14×10^9 , 3.54×10^9 , 3.14×10^9 and 3.31×10^9 daltons with a mean of 3.28×10^9 daltons. The narrow range of genome sizes and the small error between reciprocal matches suggest that the homology values can be taken as a direct indication of shared similar cistrons.

Relationships between groups 1 through 4 and 6 (Table 2) are summarized in Fig. 1 by using the mean homologies between groups to construct a dendrogram.

Group 6 contains slow-growing rhizobia whose mean homology with all reference DNAs was 4%. Consequently it is joined to the fast-growing rhizobia at that level.

The mean homologies between groups 4 and 1, 4 and 3, and 1 and 3 were 8, 10, and 14%, respectively. The mean homologies between the first two groups were not significantly different and were lower than that between groups 1 and 3. Accordingly, the dendrogram shows group 4 branching from the remaining strains at 9% and group 3 branching from the remainder at 14%.

The mean homologies between groups 4 and 1, 3 and 2, and 1 and 2 were 5, 15, 24%, respectively. Consequently, group 2 branches from group 1 at 24%.

Within group 1, *Rhizobium leguminosarum* strains were assigned to the same cluster as temperate zone clovers because of their similar homology patterns. *Trifolium polymorphum* rhizobia and *Rhizobium phaseoli* had mean homologies with reference strains CC275e, NU125, and NZP5459, from within their cluster, of 79, 83, and 58%, respectively, whereas the mean intercluster homology was 50%. DNA from some bean rhizobia has considerable homology with DNA from *Trifolium polymorphum* rhizobia, and therefore these groups were placed in the same cluster; however, it must be recognized that rhizobia which nodulate beans are a genetically diverse group. The mean homologies of African rhizobia with reference strains CC275e, NU125, and NZP5459 were 55, 52, and 57%, respectively. Consequently, they were included in group 1 but were not assigned to either cluster.

DISCUSSION

The strains whose properties are described in this paper were selected to confirm and extend results reported in a previous communication (15). That paper described relationships among strains of *Rhizobium trifolii* and between *Rhizobium trifolii* and several strains of *Rhizobium leguminosarum* and *Rhizobium phaseoli*. Considerable genetic homology was reported within *Rhizobium trifolii* and between *Rhizobium trifolii* and *Rhizobium leguminosarum*, but rhizobia from *Trifolium lupinaster*, *Trifolium semipilosum*, and *Phaseolus vulgaris* showed less homology with the remaining clover rhizobia.

Homology among strains of *Rhizobium trifolii*. In this study, 33 *Rhizobium* strains from

FIG. 1. Genetic relationship of *Rhizobium* strains described in this paper.

Trifolium sp., including 21 strains not used previously, were examined to see if DNA homology was correlated with plant or geographic origin. The *Rhizobium* strains from European and Caucasian clovers all had similar homologies with reference strains NU125, CC275e, and NZP5459, and this confirms the earlier observation (15) made with different *Rhizobium* strains from similar plant origins. Three *Rhizobium* strains from indigenous North American *Trifolium* species were tested, and these showed similar homologies to those of *Rhizobium* strains associated with European clovers, except for the comparatively low homology of the strain from *Trifolium carolinianum* with CC275e DNA. It would be necessary to test a number of additional *Rhizobium* strains isolated from indigenous North American clovers in undisturbed natural habitats to determine whether this last result indicated that there is a genetically distinct subgroup among rhizobia associated with certain of these clovers.

The mean DNA homologies of the eight *Rhizobium* strains from African *Trifolium* spp. with reference DNA from NU125, CC275e, and NZP5459, although somewhat depressed by the low values for CB727, do support the proposal that rhizobia from African clovers are genetically distinguishable from temperate-zone clover rhizobia (15). This proposition is in agreement with cross-inoculation information in which the distinction is more marked.

The mean DNA homologies for the four strains from the South American clover *Trifolium polymorphum* show that these are also genetically distinct. These strains had been obtained on three separate occasions from two different localities, all from unimproved pasture on virgin soil. One of the strains was found to fix some nitrogen on white and subterranean clovers (Table 1), but, nevertheless, DNA homology showed that it was related to the other strains from *Trifolium polymorphum* rather than to strains effective on white or subterranean clover. It seems possible that genes conferring the ability to fix nitrogen on European clovers may have been transferred naturally from introduced *Rhizobium trifolii* strains to this strain associated with *Trifolium polymorphum*.

Rhizobia from *Trifolium lupinaster* were reported to have a low homology with temperate-zone clover and with bean rhizobia (15). DNA reassociation studies with additional reference strains confirmed this observation (Table 2) and showed that these rhizobia had considerably greater homology with fast-growing *Lotus* rhizobia (Group 4) than with reference strains

taken from *Trifolium* sp. With the acquisition from Moscow of a few seeds of *Trifolium lupinaster*, it was possible to confirm that these strains could fix nitrogen on this clover. Thus, the strains undoubtedly belong to *Rhizobium trifolii*, as presently defined, although a recent observation that the strains could fix nitrogen on *Sophora flavescens* may indicate that their plant specificity differs markedly from that of most other *Rhizobium trifolii* strains. A strain from *Sophora angustifolia* (often classified as a subspecies or variety of *S. flavescens*) did not nodulate *Trifolium lupinaster*; however, strain J20400 from *Caragana chamlagu* was found to nodulate and fix nitrogen on one of three seedlings of *Trifolium lupinaster*, indicating that *Trifolium lupinaster* may be anomalous in its strain specificity. The natural range of *Trifolium lupinaster* extends westward from Japan across northern Asia into eastern Europe, and it would be valuable to determine whether isolates from *Trifolium lupinaster* in Poland or Czechoslovakia at the western end of its natural range are similar in DNA homology and plant specificity to the Japanese strains or show a closer relationship with strains associated with other European clovers.

DNA homology between *Rhizobium trifolii* and *Rhizobium leguminosarum*. The mean DNA homologies between rhizobia from several species of *Lathyrus*, *Vicia*, and *Pisum* and reference strains NU125, CC275e, and NZP5459 (Table 2) were 50, 71, and 53%, respectively. These values are very similar to those observed with European, Caucasian, and North American clovers. They confirm the earlier observation of relationship based on the homology of four strains of pea rhizobia with clover rhizobia (15) and extend it to rhizobia from a wider range of host plants in the *Rhizobium leguminosarum* cross-inoculation group. All strains were, however, from host species of European origin except *Lathyrus japonicus*, which, in its various forms, occurs also in European and Eastern Asia. It would be interesting to know whether rhizobia associated with species of *Vicia* and *Lathyrus* from South America and species of these genera confined to eastern Asia would show homologies similar to those of strains from *Trifolium polymorphum* and *Trifolium lupinaster*, respectively.

DNA homology between *Rhizobium trifolii* and *Rhizobium phaseoli*. *Rhizobium phaseoli* is here restricted to those strains isolated from *Phaseolus vulgaris*, and it does not include the other strains listed in Table 1 as able to nodulate *Phaseolus vulgaris*. DNA from *Rhizobium phaseoli* strains showed moderate to low

homology (mean 46%) with reference DNA from *Rhizobium trifolii* strain CC275e. This lack of close homology between *Rhizobium phaseoli* strains and rhizobia (*Rhizobium trifolii*) from temperate-zone clovers was first reported by Gibbins and Gregory (7) and subsequently by Jarvis et al. (15). The mean homology with DNA from reference strain NU125 was slightly higher (49%), and a few strains, particularly NZP5459, showed relatively high homology with this reference strain. The genetic diversity of the *Rhizobium phaseoli* strains is also evident from the comparison of genetic homologies between the *Rhizobium phaseoli* reference DNA (NZP5459), other strains of *Rhizobium phaseoli*, and strains of *Rhizobium trifolii* and *Rhizobium leguminosarum* in group 1. The mean homology of *Rhizobium phaseoli* strains (58%) was not significantly greater than that of *Rhizobium trifolii* strains (54%) or *Rhizobium leguminosarum* strains (53%). It is concluded that *Rhizobium phaseoli* strains do not represent a sufficiently distinct genetic population to be designated as a separate species. In the future, it may be possible to resolve them into distinct genetic groups. For the present we believe they should be combined with *Rhizobium trifolii* and *Rhizobium leguminosarum* to form one species of fast-growing rhizobia designated *Rhizobium leguminosarum* Frank. If this is accepted, the homology between rhizobia from temperate-zone pasture clovers, from African clovers, and from *Trifolium polymorphum* is such that they must also be included in this species. Consolidation of *Rhizobium trifolii*, *Rhizobium leguminosarum*, and *Rhizobium phaseoli* as one species was first recommended by Graham (9) after an examination of 100 strains of *Rhizobium* by numerical taxonomy.

DNA homology between consolidated *Rhizobium leguminosarum* and *Rhizobium meliloti*. The average DNA homologies of seven *Rhizobium* strains from *Medicago* sp. and *Trigonella suavissima* with the *Rhizobium leguminosarum* reference strains NU125, CC275e, and NZP5459 were 13, 16, and 12%, respectively. Conversely, the *Rhizobium meliloti* reference strain SU47 had an average homology with the consolidated *Rhizobium leguminosarum* strains of 14%. In addition, the homology of *Rhizobium meliloti* strains with respect to SU47 was much greater than that of *Rhizobium leguminosarum* with any of the reference strains we have used. It is concluded that *Rhizobium meliloti* is a separate, homogenous group. This view is supported by several independent studies (9, 16, 23, 24, 27, 28) and contrasts with a report (7) that two strains of *Rhizobium phaseoli* were more

closely related to *Rhizobium meliloti* than to *Rhizobium leguminosarum*. The genetic variability we have found among strains designated as *Rhizobium phaseoli* is a possible explanation for this observation, but our experience suggests that extensive homology between strains designated *Rhizobium phaseoli* and *Rhizobium meliloti* is uncommon.

DNA homology between a consolidated *Rhizobium leguminosarum*, *Rhizobium meliloti*, and *Lotus* sp. rhizobia. Little homology was shown between the DNA of fast-growing rhizobia from *Lotus* sp. (CC811 and CC809a) used as reference strains and DNA from strains of a consolidated *Rhizobium leguminosarum* (group 1) or *Rhizobium meliloti* (group 3). Similarly, there was little homology between DNA from strains in group 4 and reference DNAs from strains CC275e, NU125, NZP5459, and SU47. These results confirm and extend the earlier report (16) which indicated a lack of homology between rhizobia from *Lotus* sp., *Carmichaelia* sp., and two strains of clover rhizobia. Group 4 includes rhizobia from 12 genera of host plants to which can be added *Carmichaelia* sp. and *Clanthus puniceus* (16). The existence of a widely branched cross-inoculation group containing species from at least nine plant genera was reported by Jensen (17). Group 4 contains rhizobia from several of the plant hosts mentioned by Jensen and provides evidence for the existence of a corresponding bacterial homology group among the fast-growing rhizobia. Clearly, this genetic group cannot be characterized by its plant specificity. At present it can only be recognized by DNA reassociation.

In an earlier study (16) the mean homology of nine *Rhizobium* strains from *Carmichaelia* sp. and *Clanthus puniceus* with reference strains CC811 was reported as 62%. This was only slightly increased (to 65%) when one of the strains from *Carmichaelia* spp. (NZP5105) was used as the reference strain. Such a moderate level of homology among strains obtained in a country as geographically isolated as New Zealand with a limited leguminous flora (most species of which are confined to these islands [13]), suggest that some genetic diversity is inherent among group 4 strains. If this is the case, it is unlikely that group 4 contains subgroups with mean homologies as high as those found in the consolidated species *Rhizobium leguminosarum* (group 1).

DNA homology between named species and previously unclassified strains. Two strains of rhizobia from *Neptunia gracilis* showed homologies with reference DNAs from strains in group 1 (Table 2), which suggested

that they should be included in a consolidated *Rhizobium leguminosarum*. It should be noted that only two strains from *Neptunia gracilis* have been studied compared with the large number of strains derived from clovers, peas, and beans. Classifying these *Neptunia* strains with group 1 does not imply that all other strains from *Neptunia* sp. will be genetically homologous with group 1 rhizobia.

The six strains comprising group 2 are closely similar genetically and in plant specificity, although they were isolated from four different hosts. It remains to be seen whether the present clear demarcation of this group is maintained in the future when the DNA homologies and plant specificities of further strains isolated from the same and other hosts have been determined. Although all strains fixed nitrogen with both *Leucaena leucocephala* and *Phaseolus vulgaris*, they were very different from typical strains isolated from either of these hosts, both in DNA homology and in plant specificity. One further strain, taken from *Onobrychis viciifolia* (3G2c2a) and which differed in plant specificity from the group 2 strains, showed very low homology with the reference strain CC401; strains from other *Sophora* sp. were also very distinct. To confirm this, it would be necessary to test strains of European origin isolated from *Coronilla* and *Onobrychis* sp. Neither genus is indigenous to North America (13), but a related genus, *Hedysarum*, does have some North American species.

The 11 group 5 strains showed little DNA homology with any of the reference strains, although 6 of the 11 strains were from plant species which also gave strains included in group 4. Six of the group 5 strains, were isolated from tropical or subtropical localities, and this could indicate that further groups may exist among the little known fast-growing rhizobia from the tropics. Whether these tropical fast-growing strains showed any greater homology with slow-growing strains of group 6 than to the strains in groups 1 to 4 remains to be determined.

The distinction between fast and slow-growing rhizobia. The division of *Rhizobium* into two groups characterized as fast growers and slow growers is well established (2, 10, 18, 20, 22, 26, 27). It has been proposed that the difference between these groups is large enough to justify splitting the rhizobia into two genera, *Rhizobium* and *Phytomyxa* (9, 21, 26). Counter arguments, seeking to retain one genus for all legume root-nodule bacteria, center on the lack of information about slow-growers (2, 24) or on the guanine-plus-cytosine contents of the DNA and flagellation in strains which infect lupins

(*Rhizobium lupini*), soya beans (*Rhizobium japonicum*), or cowpeas (5). The slow-growing rhizobia described in this paper each had a mean homology of 10% or less with the seven fast-growing reference strains used. This low-level homology with fast-growing rhizobia strongly supports a division of root bacteria into two related genera. It also indicates a measure of relationship which is inconsistent with the proposition that fast-growing and slow-growing rhizobia should be placed in different taxonomic orders (26).

Our results suggest that the fast-growing rhizobia can be classified into four species corresponding with the four genetic groups we have identified: group 1 comprises *Rhizobium trifolii*, *Rhizobium leguminosarum*, and *Rhizobium phaseoli*, all consolidated under the name *Rhizobium leguminosarum*; group 2 comprises the *Rhizobium* strains from *Coronilla* sp. and some strains from *Sophora* and *Onobrychis* sp.; group 3 comprises *Rhizobium meliloti*; and group 4 comprises strains from a variety of hosts including *Lotus corniculatus*, *Lotus tenuis*, and *Lupinus densiflorus*. Some of the previously unclassified rhizobia (group 5) do not show close homology with any of the reference strains we have used, and it is probable that further genetic groups of fast-growing rhizobia remain to be identified.

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