Rhizobium loti, a New Species of Legume Root Nodule Bacteria

B. D. W. JARVIS,¹ C. E. PANKHURST,² AND J. J. PATEL²

Department of Microbiology and Genetics, Massey University,¹ and Applied Biochemistry Division,
Department of Scientific and Industrial Research,² Palmerston North, New Zealand

Comparative studies of fast-growing *Lotus* rhizobia were reviewed, and as a result a new species, *Rhizobium loti*, is proposed. The type strain of this species, which was isolated from a root nodule on *Lotus corniculatus* (bird's-foot trefoil), is NZP 2213 (= ATCC 33669). Plant specificity, phage relationships, the soluble-protein pattern, and deoxyribonucleic acid base sequence homology distinguished *R. loti* from currently recognized *Rhizobium* species.

Rhizobium species have been defined in terms of plant cross-inoculation groups (16). However, it is generally recognized that this approach is inadequate since cross-inoculation groups are not mutually exclusive (9, 11, 31) and plant specificity is probably a plasmid-borne character (4, 6, 21, 29). Furthermore, many of the strains studied have never been designated by a species name, including the *Lotus* rhizobia, which nodulate a variety of leguminous plants belonging to the genera *Lupinus*, *Ornithopus*, *Anthyllis*, *Caragana*, and *Astragalus* (13, 14).

A revised classification of the genus (D. C. Jordan, personal communication) has been proposed for the forthcoming edition of *Bergey's Manual of Determinative Bacteriology*, in which the fast-growing species *Rhizobium trifolii*, *Rhizobium phaseoli*, and *Rhizobium leguminosarum* are combined as one species, designated *R. leguminosarum* (Frank) Frank, comprising three biovars (*trifolii*, *phaseoli*, and *viceae*). *Rhizobium meliloti* Dangeard is retained as a separate species in the genus *Rhizobium*, and those rhizobia which grow slowly on yeast extract media are transferred to a separate genus, *Bradyrhizobium* (15). Only one species is recognized in this genus, *Bradyrhizobium japonicum* (Kirchner) Jordan 1982. As part of this revision it is proposed that the fast-growing *Lotus* rhizobia and related strains be recognized as a new species, *Rhizobium loti*.

Two groups of *Lotus* rhizobia have been recognized in comparative studies of bacteroid ultrastructure (7), internal antigens (23, 32), extracellular polysaccharide composition (2), enzymatic complement (17–19), gel electrophoresis of soluble proteins (27, 28), growth rate and acid production on yeast extract media (1, 5, 20), susceptibility to flavolans and isoflavonoids (24, 25), susceptibility to antibiotics (22), phage relationships (26), deoxyribonucleic acid (DNA) homology (8, 12), and plant cross-nodulation (1, 8,

10, 13, 14, 22). Several of these criteria indicate that the fast-growing, acid-producing *Lotus* rhizobia are also clearly distinguishable from *R. leguminosarum* and *R. meliloti* (8, 12, 18, 24, 25, 28, 29, 32).

Jensen and Hansen (13, 14) discovered an extensive cross-inoculation group involving the plant genera *Lupinus*, *Ornithopus*, *Lotus*, *Anthyllis*, *Caragana*, *Astragalus*, *Ononis*, *Genista*, and *Mimosa*, which were nodulated by both fast- and slow-growing rhizobia. Recent DNA-DNA hybridization studies have shown that fast-growing *Lotus* rhizobia are genetically related to rhizobia obtained from a variety of plant hosts, including *Lotus corniculatus*, *Lotus divaricatus*, *Lotus maroccanus*, *Lotus tenuis*, *Caragana arborescens*, *Cicer arietinum*, *Luecaena leucocephala*, *Lupinus densiflorus*, *Onobrychis viciifolia*, *Parochetus communis*, *Psoralea eriatha*, *Robinia pseudoacacia*, *Sophora angustifolia*, *Sophora microphylla*, *Sophora tetraptera*, and *Trifolium lupinaster* (8), as well as *Carmichaelia* species (12). Consequently, we expect that although the description of *R. loti* given below is based on characteristics of well-studied root nodule bacteria (Table 1) from *Lotus corniculatus* and *Lotus tenuis*, the description probably applies to rhizobia from the wider range of plant species indicated above.

Rhizobium loti sp. nov. (M.L. noun *Lotus* generic name; M.L. gen. noun *loti* of *Lotus*). Short, gram-negative, nonsporeforming rods which are motile predominantly by means of one polar or subpolar flagellum. In nodules the cells are bacteroids (i.e., club shaped and branched) and contain inclusion bodies composed of poly- β -hydroxybutyric acid. Other inclusions, such as polyphosphate bodies, glycogen granules, and lipid droplets, are not present.

Colonies on Vincent yeast mannitol agar (30) are more than 1.0 mm in diameter after 7 days at 28°C, but growth is inhibited at 42°C. Growth at

TABLE 1. Fast-growing *Lotus* rhizobia included as members of *R. loti* and references in which these strains are described

Strain ^a	Reference(s)	Other sources of information ^b
NZP 2014	2, 7, 23, 26	Patel; Pankhurst
NZP 2024	2, 7, 23	Pankhurst
NZP 2034	2, 8, 23	
NZP 2037	2, 7, 8, 22, 23, 26	Patel; Pankhurst
NZP 2042	2, 7, 23	Patel
NZP 2048	2, 7, 22, 23	Patel
NZP 2079	2, 23	Pankhurst
NZP 2985	2, 7, 23	
NZP 2150	2, 7, 23	Pankhurst
NZP 2171	2, 7, 23	
NZP 2196 (= SU343)	2, 7, 8, 23	Patel
NZP 2205	7, 23, 26	Patel
NZP 2213	7, 8, 22, 23, 26	Patel; Pankhurst
NZP 2227 (= strain 461, University of Guelph)	7, 8, 23, 26	Patel
NZP 2230 (= CC809a)	2, 8, 23	
NZP 2234 (= CC811)	2, 7, 23	
NZP 2235 (= CC812a)	23	Patel
NZP 2238 (= Lc265Da)	7, 8, 22, 23, 26	Patel
NZP 2260	8, 23, 26	Patel

^a NZP, New Zealand Department of Scientific and Industrial Research, Applied Biochemistry Division.

^b Patel, J. J. Patel (unpublished data from a survey of bacteriophage host specificity involving five phage races and 270 *Rhizobium* strains); Pankhurst, C. D. Pankhurst, unpublished data on the responses of 24 *Rhizobium* strains in 28 physiological tests.

28°C on yeast mannitol agar is inhibited by 3% NaCl and by pH values of less than 4.0 or more than 10.0. On Vincent medium without mannitol, all strains utilize one or more of the following carbohydrates and form acidic end products: glucose, galactose, fructose, arabinose, xylose, rhamnose, maltose, sucrose, lactose, trehalose, raffinose, mannitol, and dulcitol.

The electrophoretic patterns of the soluble proteins from strains of fast-growing *Lotus* rhizobia are similar to one another and are distinguishable from those of other species of fast-growing rhizobia.

All strains produce extracellular polysaccharides which contain uronic acid, galactose, and glucose. Somatic antigens are highly strain specific, but internal antigens show a reaction of identity between strains and can be used to distinguish *R. loti* from slow-growing *Lotus* rhizobia and from other *Rhizobium* nomenspecies.

Cells can be infected by bacteriophages belonging to Bradley morphological types B and C (3). These phages are host range specific, and susceptibility to ϕ 2037/1 may be used to distinguish *R. loti* from slow-growing *Lotus* rhizobia and from other *Rhizobium* nomenspecies.

The guanine-plus-cytosine content of the DNA of *R. loti* is 59 to 64 mol%. DNA-DNA hybridizations with reference DNAs from *R. trifolii* strain CC275e (5 to 13%; mean, 8%), *R. meliloti* strain SU47 (5 to 15%; mean, 10%),

Rhizobium sp. strain CC811 from *Lotus corniculatus* (46 to 74%; mean, 60%), and *Rhizobium* sp. strain CC809a from *Lotus maroccanus* (32 to 59%; mean, 47%) indicate that *R. loti* can be clearly distinguished from other fast-growing rhizobia. Strains of rhizobia from a variety of other plants have similar genetic relationships, indicating that *R. loti* forms part of a larger group of root nodule bacteria.

Nitrogen-fixing (effective) nodules are normally formed on the roots of *Lotus corniculatus* (bird's-foot trefoil), *Lotus tenuis* (slender bird's-foot trefoil), *Lupinus densiflorus* (lupine), and *Anthyllis vulneraria* (kidney vetch). A few strains nodulate effectively on *Lotus pedunculatus* (*Lotus uliginosus*; big trefoil) or *Leucaena leucocephala*. Ineffective nodules may be formed on *Lotus pedunculatus* (*Lotus uliginosus*), *Lotus angustissimus* (narrow-leaved *Lotus*), *Leucaena leucocephala*, *Clianthus puniceus* (red kowhai), *Acacia sophorea* (*Acacia longifolia*; sallow wattle), *Carmichaelia angustata* (large-flowered broom), *Phaseolus vulgaris* (kidney bean), *Macroptilium atropurpureum* (siratro), and *Ornithopus sativus* (serradella).

The type strain of *R. loti* is NZP 2213, a culture of which has been deposited in the American Type Culture Collection under the number ATCC 33669. This strain conforms to the description given above for the species with respect to morphology, physiology, antigenic

relationships, and plant specificity. It is lysed by bacteriophages 1 through 7 of the 2037 series and by ϕ 2205 but not by any bacteriophage from slow-growing *Lotus* rhizobia (26). The percent DNA-DNA homologies with *R. trifolii* CC275e, *R. meliloti* SU47, *R. loti* CC811, and *R. loti* CC809a are 10, 5, 60, and 47%, respectively. Nitrogen-fixing nodules are formed on the roots of the following plants: *Lotus corniculatus*, *Lotus tenuis*, *Lotus japonicus*, *Lotus krylovii*, *Lotus filicalius*, and *Lotus schoelleri*. Nodules which cannot fix nitrogen are formed on *Lotus pedunculatus*, *Lotus hispidus* (*Lotus suave-dens*), and *Lotus angustissimus*.

REPRINT REQUESTS

Address reprint requests to: Dr. B. D. W. Jarvis, Department of Microbiology and Genetics, Massey University, Palmerston North, New Zealand.

LITERATURE CITED

1. Abdel-Chaffar, A. S., and H. L. Jensen. 1966. The rhizobia of *Lupinus densiflorus* Benth., with some remarks on the classification of root nodule bacteria. *Arch. Microbiol.* 54:393-405.
2. Bailey, R. W., R. M. Greenwood, and A. Craig. 1971. Extracellular polysaccharides of *Rhizobium* strains associated with *Lotus* species. *J. Gen. Microbiol.* 65:315-324.
3. Bradley, D. E. 1967. Ultrastructure of bacteriophages and bacteriocins. *Bacteriol. Rev.* 31:230-314.
4. Brewin, N. J., J. E. Beringer, and A. W. B. Johnston. 1980. Plasmid-mediated transfer of host-range specificity between two strains of *Rhizobium leguminosarum*. *J. Gen. Microbiol.* 120:413-420.
5. Brockwell, J., S. K. Asuo, and G. A. Rea. 1966. Acid production by rhizobia from the genera *Trifolium* and *Lotus*. *J. Aust. Inst. Agric. Sci.* 32:295-297.
6. Buchanan-Wollaston, A. V., J. E. Beringer, N. J. Brewin, P. R. Hirsch, and A. W. B. Johnston. 1980. Isolation of symbiotically defective mutants in *Rhizobium leguminosarum* by insertion of the transposon Tn5 into a transmissible plasmid. *Mol. Gen. Genet.* 178:185-190.
7. Craig, A. S., R. M. Greenwood, and K. I. Williamson. 1973. Ultrastructural inclusions of rhizobial bacteroids of *Lotus* nodules and their taxonomic significance. *Arch. Microbiol.* 89:23-32.
8. Crow, V. L., B. D. W. Jarvis, and R. M. Greenwood. 1981. Deoxyribonucleic acid homologies among acid-producing strains of *Rhizobium*. *Int. J. Syst. Bacteriol.* 31:152-172.
9. Dixon, R. O. D. 1969. Rhizobia, with particular reference to relationships with host plants. *Annu. Rev. Microbiol.* 23:137-158.
10. Erdman, L. W., and U. M. Means. 1949. Strains of *Rhizobium* effective on the trefoils *Lotus corniculatus* and *Lotus uliginosus*. *Soil Sci. Soc. Am. Proc.* 14:170-175.
11. Graham, P. H. 1976. Identification and classification of root nodule bacteria, p. 99-112. *In* P. S. Nutman (ed.), *Symbiotic nitrogen fixation in plants*. Cambridge University Press, London.
12. Jarvis, B. D. W., T. S. MacLean, I. G. C. Robinson, and G. R. Fanning. 1977. Phenetic similarity and DNA base sequence homology of root nodule bacteria from New Zealand native legumes and *Rhizobium* strains from agricultural plants. *N.Z. J. Agric. Res.* 20:235-248.
13. Jensen, H. L. 1967. Mutual host plant relationships in two groups of legume root nodule bacteria (*Rhizobium* spp.). *Arch. Microbiol.* 59:174-179.
14. Jensen, H. L., and A. L. Hansen. 1968. Observations on host plant relations in root nodule bacteria of the *Lotus-Anthyllis* and *Lupinus-Ornithopus* groups. *Acta Agric. Scand.* 18:135-142.
15. Jordan, D. C. 1982. Transfer of *Rhizobium japonicum* Buchanan 1980 to *Bradyrhizobium* gen. nov., a genus of slow-growing, root nodule bacteria from leguminous plants. *Int. J. Syst. Bacteriol.* 32:136-139.
16. Jordan, D. C., and O. N. Allen. 1974. Family III. *Rhizobiaceae* Conn, 1938, p. 261-264. *In* R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
17. Martinez-de Drets, G., and A. Arias. 1972. Enzymatic basis for differentiation of *Rhizobium* into fast- and slow-growing groups. *J. Bacteriol.* 109:467-470.
18. Martinez-de Drets, G., A. Arias, and M. Rovira de Cutinella. 1974. Fast- and slow-growing rhizobia: differences in sucrose utilization and invertase activity. *Can. J. Microbiol.* 20:605-609.
19. Murphy, P. M., and C. L. Masterson. 1970. Determination of multiple forms of esterases in *Rhizobium* by paper electrophoresis. *J. Gen. Microbiol.* 61:121-129.
20. Norris, N. O. 1965. Acid production by *Rhizobium* a unifying concept. *Plant Soil* 22:143-166.
21. Nuti, M. P., A. A. Lepidi, R. K. Prakash, R. A. Schilperoot, and F. C. Cannon. 1979. Evidence for nitrogen fixation (*nif*) genes on indigenous *Rhizobium* plasmids. *Nature (London)* 282:533-535.
22. Pankhurst, C. E. 1977. Symbiotic effectiveness of antibiotic-resistant mutants of fast- and slow-growing strains of *Rhizobium* nodulating *Lotus* species. *Can. J. Microbiol.* 23:1026-1033.
23. Pankhurst, C. E. 1979. Some antigenic properties of cultured cell and bacteroid forms of fast- and slow-growing strains of *Lotus* rhizobia. *Microbios* 24:19-28.
24. Pankhurst, C. E., and D. R. Biggs. 1980. Sensitivity of *Rhizobium* to selected isoflavonoids. *Can. J. Microbiol.* 26:542-545.
25. Pankhurst, C. E., and W. T. Jones. 1979. Effectiveness of *Lotus* root nodules. II. Relationship between root nodule effectiveness and *in vitro* sensitivity of fast-growing *Lotus* rhizobia to flavolans. *J. Exp. Bot.* 30:1095-1107.
26. Patel, J. J. 1976. Morphology and host range of virulent phages of *Lotus* rhizobia. *Can. J. Microbiol.* 22:204-212.
27. Peterson, P. J., R. M. Greenwood, G. B. Belling, and N. O. Bathurst. 1971. The electrophoretic movement of soluble proteins and the production of unusual amino acids in *Rhizobium* isolates as taxonomic criteria. *Plant Soil*, p. 111-114.
28. Roberts, G. P., W. T. Leps, L. E. Silver, and W. J. Brill. 1980. Use of two-dimensional polyacrylamide gel electrophoresis to identify and classify *Rhizobium* strains. *Appl. Environ. Microbiol.* 39:414-422.
29. Spitzbarth, M., A. Puhler, and W. Heumann. 1979. Characterization of plasmids isolated from *Rhizobium meliloti*. *Arch. Microbiol.* 121:1-7.
30. Vincent, J. M. 1970. A manual for the practical study of root nodule bacteria. Blackwell Scientific Publications, Oxford.
31. Vincent, J. M. 1977. *Rhizobium*: general microbiology, p. 277-366. *In* R. W. F. Hardy and W. S. Silver (ed.), *A treatise on dinitrogen fixation*, Sect. 3. Biology. John Wiley & Sons, Inc., New York.
32. Vincent, J. M., and B. Humphrey. 1970. Taxonomically significant group antigens in *Rhizobium*. *J. Gen. Microbiol.* 63:379-382.