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Rhizobium galegae, a New Species of Legume Root Nodule Bacteria

KRISTINA LINDSTRÖM

Department of Microbiology, University of Helsinki, SF-00710 Helsinki, Finland

Studies of root nodule bacteria isolated from *Galega orientalis* and *Galega officinalis* are reviewed, and as a result a new species, *Rhizobium galegae*, is proposed. The type strain of the species is *R. galegae* HAMBI 540 (= ATCC 43677), which forms nitrogen-fixing root nodules on *G. orientalis*. Deoxyribonucleic acid homology distinguishes *R. galegae* from other rhizobia, whereas nodulation of *Galega* sp. and sensitivity to phage gal 1/R phenotypically separate the strains currently included in the species from other members of the genus *Rhizobium*. Other criteria supporting the formation of a new species include ribosomal ribonucleic acid-deoxyribonucleic acid homology data, protein and lipopolysaccharide patterns, serological properties, and numerical taxonomy data.

Root nodule bacteria are able to penetrate the roots of primarily leguminous plants. As a result of the infection, a specific structure, the root nodule, is formed. Once established within a nodule, the bacteria reduce atmospheric nitrogen to ammonia, which is transported to the plant. The present classification of root nodule bacteria, as described in *Bergey's Manual of Systematic Bacteriology*, separates the fast- and slow-growing organisms into two genera, *Rhizobium* and *Bradyrhizobium* (4). The fast-growing rhizobia are divided into three species, *Rhizobium leguminosarum*, *Rhizobium meliloti*, and *Rhizobium loti*, with *R. leguminosarum* comprising three biovars (*R. leguminosarum* biovar *trifolii*, *R. leguminosarum* biovar *phaseoli*, and *R. leguminosarum* biovar *viciae*). In addition, fast-growing rhizobia that nodulate soybeans have recently been assigned to a new species, *Rhizobium fredii* (12). The taxonomic criteria and methods used currently in the classification of the family *Rhizobiaceae* include numerical taxonomy, guanine-plus-cytosine content, deoxyribonucleic acid (DNA) homology, protein patterns, serology, phage typing, composition of extracellular polysaccharides, plasmid transfer, and plant infection. The cross-inoculation test, which was used previously as a major taxonomic criterion, is now used only to complement other methods (4).

Fast-growing root nodule bacteria which nodulate the plant species *Galega orientalis* and *Galega officinalis* (goat's rue) were first studied by Hauke-Pacewiczowa (2) and Proctor (10); these authors claimed that the *Galega* rhizobia belong to *R. leguminosarum* and "the fast-growing lupine-cowpea complex" (now recognized as *R. loti*), respectively. However, results from more recent and extensive studies, conducted because of the potential agricultural properties of *G. orientalis* (5, 8, 13), disagree with those of Hauke-Pacewiczowa (2) and Proctor (10). DNA homology, ribosomal ribonucleic acid-DNA hybridization, lipopolysaccharide and protein pattern, serology, phage typing, and cross-nodulation experiments all have shown that the *Galega* rhizobia form a homologous group of fast-growing rhizobia which is not closely related to the currently recognized *Rhizobium* species (3, 6, 7, 9, 9a, 9b, 15).

***Rhizobium galegae* sp. nov.** *Rhizobium galegae* (ga.le'gae. M.L. fem. gen. n. *galegae*, of *Galega*). Short, gram-negative, nonsporeforming rods which are motile by means of one or two polar or subpolar flagella (Fig. 1). In nitrogen-fixing nodules the cells are elongated bacteroids, and some are branched (6, 9a). Colonies on Vincent yeast-mannitol agar (14) are more than 1.0 mm in diameter after 7 days at 28°C. The maximum growth temperature is 33 to 37°C. The cells grow in the presence of 0.5% NaCl but not in the presence of 2.0% NaCl. A majority of the strains studied form a serum zone and give an alkaline reaction in litmus milk. They hydrolyze urea but do not precipitate calcium glycerophosphate, nor do they reduce nitrate. They require calcium panthotenate but not thiamine for growth. The following carbon sources are readily utilized in defined medium (def 9 medium [7]), with the formation of acidic end products: D-galactose, lactose, L-arabinose, dulcitol, raffinose, D-xylose, trehalose, L-rhamnose, sorbitol, maltose, D-glucose, D-fructose, and mannitol. The response to sucrose and citrate is weaker. The intrinsic antibiotic resistance patterns of *R. galegae* strains show fairly high resistance to trimethoprim and bacitracin and low resistance to neomycin and chloramphenicol (7). Numerical taxonomy separates the *R. galegae* strains from the previously described rhizobial species (7).

The guanine-plus-cytosine content of the DNA of *R. galegae* is 63 mol% (7). Data from DNA hybridizations in which DNAs from strains HAMBI 540^T (T = type strain), HAMBI 1141, and galNW3 are used show that the *R. galegae* strains form a uniform DNA homology group, which

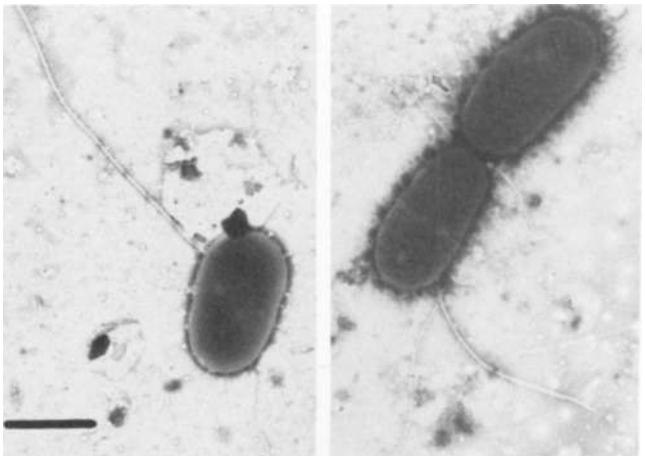

FIG. 1. Electron micrographs of *R. galegae* HAMBI 540^T. Negative staining of 16-h-old cells from a nonagitated broth culture. Bar = 1 μm.

TABLE 1. Mean relative levels of DNA homology between *R. galegae* reference strains HAMBI 540^T, HAMBI 1141, and galNW3 and other rhizobia and bradyrhizobia

Taxon	% Homology with reference strain:		
	HAMBI 540$^{T a}$	HAMBI 1141^b	galNW3^b
R. galegae	77 ± 9^c	79 ± 14	85 ± 9
R. meliloti		8 ± 6	22 ± 1
R. leguminosarum	22 ± 4	22 ± 7	44 ± 1
R. loti	16 ± 4	10	16 ± 2
Rhizobium sp. (*Coronilla*)		14	25
Rhizobium sp. (*Leucaena*)		10 ± 2	24
Bradyrhizobium sp.	20 ± 9	2	12 ± 5

[a] Data from reference 6.
[b] Data calculated from reference 15.
[c] Mean ± standard deviation.

is not closely related to other *Rhizobium* or *Bradyrhizobium* species (Table 1) (6, 15). Thus, DNA homology can be used to distinguish *R. galegae* strains from other rhizobial species. Ribosomal ribonucleic acid-DNA hybridization data place *R. galegae* in a separate group (group III) within the genus *Rhizobium* (3); *R. meliloti*, *R. fredii*, and *R. leguminosarum* form group I, and *R. loti* forms group II. All of the *R. galegae* strains tested contain at least one very large plasmid, and some strains also contain additional smaller plasmids (Fig. 2).

All strains produce extracellular polysaccharide (unpublished data). The soluble protein and lipopolysaccharide

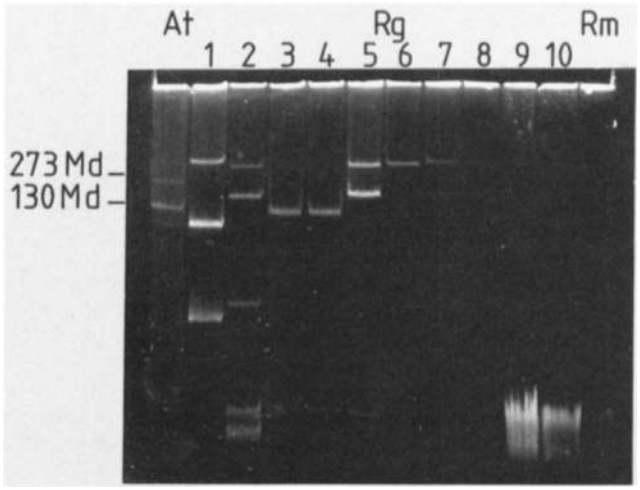

FIG. 2. Plasmid profiles of *R. galegae* strains. Lane 1, *R. galegae* HAMBI 1146; lane 2, *R. galegae* HAMBI 1145; lane 3, *R. galegae* HAMBI 1143; lane 4, *R. galegae* HAMBI 1141; lane 5, *R. galegae* HAMBI 1183; lane 6, *R. galegae* HAMBI 1185; lane 7, *R. galegae* HAMBI 503; lane 8, *R. galegae* HAMBI 490; lane 9, *R. galegae* HAMBI 1147; lane 10, *R. galegae* HAMBI 540^T. The controls used were *Agrobacterium tumefaciens* C58 (lane At) and *R. meliloti* Rm 1021 (lane Rm). Md, Megadalton. The method used was the Eckhardt method (1) as modified by Rosenberg et al. (11).

TABLE 2. *Galega* rhizobia included in the species *R. galegae*

Strain[a]	Geographic origin or source[b]	Reference(s)
HAMBI 540^T (= gal1261^T = ATCC 43677^T)[c]	Finland	3, 5–7, 9b
HAMBI 1174 (= gal1261R)	Sm^r and Spc^r derivative of HAMBI 540^T	7–9a
HAMBI 1155 (= galE)	USSR	6, 7
HAMBI 1147 (= gal129)	Finland	3, 6, 7, 15
HAMBI 1460 (= gor3)	Finland	
HAMBI 1462 (= gal302)	USSR	9b
HAMBI 1428 (= K-092)	USSR	9b
HAMBI 1141 (= gal1)	New Zealand	6, 7, 9b, 15
HAMBI 1207 (= gal1s)	Sm^r derivative of HAMBI 1141	
HAMBI 1143 (= gal3)	New Zealand	6, 7, 15
HAMBI 1208 (= gal3s)	Sm^r derivative of HAMBI 1143	
HAMBI 1144 (= gal7)	New Zealand	6, 7, 15
HAMBI 1145 (= gal12)	New Zealand	6, 7, 9b, 15
HAMBI 1146 (= gal14)	New Zealand	6, 7, 15
HAMBI 1122 (= NZP 5067)	New Zealand	6
HAMBI 1151 (= NZP 5068)	New Zealand	6, 7, 9b
HAMBI 490 (= galB7i)	Finland	6, 7, 9–9b, 15
HAMBI 1209 (= galB7is)	Sm^r derivative of HAMBI 490	
HAMBI 503 (= 59A2)	United States	6, 7, 15
HAMBI 1183 (= G6)	England	7, 9b
HAMBI 1184 (= G8)	England	7
HAMBI 1185 (= G9)	England	7
HAMBI 1186 (= G10)	England	
HAMBI 1187 (= G11)	England	
HAMBI 1189 (= G12)	England	
HAMBI 1190 (= G15)	England	
HAMBI 1191 (= G16)	England	
galNW1	New Zealand	15
galNW2	New Zealand	15
galNW3	New Zealand	15

[a] HAMBI, Culture Collection of the Department of Microbiology, University of Helsinki, Helsinki, Finland; NZP, Division of Scientific and Industrial Research, Palmerston North, New Zealand.
[b] Sm^r, Streptomycin resistant; Spc^r, spectinomycin resistant.
[c] Reference strain.

patterns of *R. galegae* strains differ from those of other rhizobia. Antiserum raised against strain HAMBI 540^T (isolated from *G. orientalis*) reacts with lipopolysaccharides from all of the *R. galegae* strains tested. Immunoblotting of whole-cell soluble proteins reveals two protein bands which are unique to *R. galegae* strains (9b).

Cells can be infected by bacteriophages which are host range specific. Susceptibility to phage gal 1/R can be used to distinguish *R. galegae* strains from other fast- and slow-growing root nodule bacteria (6, 7).

Nitrogen-fixing (effective) nodules are formed by strains isolated from *G. orientalis* on that plant and by strains isolated from *G. officinalis* on their original host. Strains isolated from *G. orientalis* form ineffective nodules on *G. officinalis* and vice versa. *R. galegae* strains do not infect the other leguminous plants tested. Plants of *Galega* spp. are only occasionally infected by other rhizobia, but when infection does take place, no nitrogen fixation occurs (6, 9, 9a). Thus, in addition to typing with phage gal 1/R, effective nodulation of *G. orientalis* or *G. officinalis* phenotypically distinguishes *R. galegae* from other *Rhizobium* species.

The strains which presently can be included in the species *R. galegae* are listed in Table 2.

The type strain of *R. galegae* is strain HAMBI 540 (isolated from *G. orientalis*), a culture of which has been deposited in the American Type Culture Collection as strain ATCC 43677. This strain conforms to the description given above for the species with respect to morphology, physiology, guanine-plus-cytosine content, DNA and ribosomal ribonucleic acid homology, serology, and cross-nodulation. It is a Finnish field isolate derived from strain HAMBI 1155 (= galE), which was originally isolated by Debora Gurfel in Estonia. It is used as an inoculant in field experiments with goat's rue (5). It is lysed by phages gal 1/R, gal 1/OW, gal 3/R, and gal 3/OW (6) and is the isolation host of phages gal 1261/M and gal 1261/V (7; J. J. Patel and K. Lindström, Abstr. Global Impacts Appl. Microbiol. VII, abstr. no. B16, p. 86, 1985). It is not lysed by bacteriophages which lyse *R. leguminosarum*, *R. meliloti*, and *R. loti* (6, 7).

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