NOTES

Transfer of Rhizobium japonicum Buchanan 1980 to Bradyrhizobium gen. nov., a Genus of Slow-Growing, Root Nodule Bacteria from Leguminous Plants

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Recent data indicate that the slow-growing, non-acid-producing root nodule bacteria of leguminous plants should be separated from the fast-growing, acid-producing strains and placed in a new genus. The separation is warranted by numerical taxonomy, deoxyribonucleic acid base ratio determinations, nucleic acid hybridization, ribosomal ribonucleic acid cistron similarities, serology, composition of extracellular gum, carbohydrate utilization and metabolism, bacteriophage and antibiotic susceptibilities, protein composition, and types of intracellular inclusion bodies in the bacteroid forms. The name proposed for the new genus is Bradyrhizobium. The type species of the genus is B. japonicum (Buchanan 1980) comb. nov. (basonym: Rhizobium japonicum Buchanan 1980), the type strain of which is ATCC 10324.

In the 8th edition of Bergey's Manual of Determinative Bacteriology, Jordan and Allen (14) subdivided the genus Rhizobium Frank 1889 into two groups on the basis of flagellar arrangement, growth rate in yeast extract-mannitol-mineral salts medium, guanosine plus cytosine content of the deoxyribonucleic acid (DNA), and the genera of host plants nodulated. This subdivision was based on 't Mannetje's (30) numerical taxonomy data and the need for additional investigations of the relationship between the slow- and fast-growing groups. The need to separate these two groups within the existing genus Rhizobium stems from recent findings involving numerical taxonomy (10, 20, 30), DNA base ratios (3, 34), nucleic acid hybridization (7, 12), ribosomal ribonucleic acid cistron similarities (4), serology (9, 13, 32, 33), composition of extracellular gum (5, 6, 15, 16), carbohydrate utilization (28) and metabolism (18), bacteriophage (21) and antibiotic susceptibilities (29), protein composition based on polyacrylamide gel electrophoresis (25), and types of intracellular inclusion bodies in the bacteroids within the root nodules (2).

The proposal to establish a new genus for the slow-growing, non-acid-producing rhizobia was initially approved by those members present at a meeting of the International Subcommittee on Agrobacterium and Rhizobium held at the Australian National University on 3 December 1980 during the IV International Symposium on Nitrogen Fixation. This proposal was subsequently approved by all the remaining members of the Subcommittee, as was the suggestion that the name of the new genus should incorporate the name 'rhizobium' and should be prefixed by an adjective signifying sluggish or slow. Both proposals received the unanimous support of the 23 specialists who attended an open meeting of the Subcommittee held on 6 December 1980 at the Australian National University.

A suitable name for the new genus is Bradyrhizobium (Gr. adj. bradus slow; M. L. neut. n. Rhizobium a bacterial generic name; M. L. neut. n. Bradyrhizobium the slow [growing] rhizobium). This name has the advantage of emphasizing the considerable agronomic importance of the root nodule bacteria and pays tribute to the long-standing and extensive literature on these microorganisms. It was such considerations which led the Judicial Commission of the International Committee on the Nomenclature of Bacteria (opinion 34, 1970, 11) to indicate the generic name of the nodule bacteria as Rhizobium Frank 1889 nom. gen. cons. rather than as Phytomyxa Schroeter 1886, which holds priority. The utilization of 'rhizobium' in the name of the proposed new genus also acknowledges the hypothesis of Norris (22, 23), which is not universally accepted, that the slow-growing, non-acid-producing strains of the root-nodule...
bacteria from tropical leguminous plants represent a survival of the ancestral type which ultimately gave rise to the fast-growing, acid-producing strains associated with the temperate-zone leguminous plants.

In 1964, Graham (10), after a taxonomic study of 83 strains of nodule bacteria, recommended that the slow-growing strains be grouped in the genus *Phytophthora*. This name, however, was proposed by Schroeter (26) to represent the root nodule bacteria in general. It has no priority as a generic name for the slow growers and it is illogical, considering that the name was originally proposed under the assumption that these bacteria were related to the slime molds.

The genus *Bradyrhizobium* represents an exceedingly heterogeneous group of nodule bacteria within which the taxonomic relationships are not well understood. It is proposed that, until such time as further species or biovars, or both, are established within the genus, there be only one designated species, *Bradyrhizobium japonicum* (Buchanan 1980) comb. nov. (synonym: *Rhizobium japonicum* Buchanan 1980 [1]). It is suggested that, for the present, the members of the genus *Bradyrhizobium* other than *B. japonicum* be referred to as *Bradyrhizobium* sp. with the name of the appropriate host plant in parentheses immediately following, for example, *Bradyrhizobium* sp. (Vigna) or *Bradyrhizobium* sp. (Lupinus). The species formerly designated *R. lupini* (14) is not being specified in the genus *Bradyrhizobium* since its only major distinguishing characteristic was a high degree of nodulation affinity for *Ornithopus* and *Lupinus* sp.

The taxonomic position of the nodule bacteria from *Lotononis* is uncertain. These organisms are similar to other strains of *Bradyrhizobium* in that they are monothrichous, grow slowly, and produce an alkaline reaction in mineral salts-mannitol medium after 28 days at 27°C. Do not produce 3-ketolactose, and exhibit negative hypertrophy-initiating ability (14).

Stimulate nodule production on roots of tropical and some temperate-zone leguminous plants (*Leguminosae*) such as those of the genera *Glycine*, *Vigna*, and *Macroptilium*. In addition to certain fast-growing strains, to be placed in the genus *Rhizobium*, cause root nodule production on *Lotus* (*L. uliginosus* and *L. pedunculatus*), *Lupinus*, *Ornithopus*, *Cicer*, *Sesbania*, *Leucaena*, *Mimosa*, *Lablab*, and *Acacia*. A highly specific nodulation of the nonleguminous plant *Parasponia* (*Trema*) by a strain of *Bradyrhizobium* sp. also occurs (31). Fix atmospheric nitrogen (dinitrogen) when in the symbiotic state within root nodules, although ineffective nodules (unable to fix nitrogen) are occasionally formed. Bacteria present in nodules as slightly swollen rods (containing polyphosphate inclusions) with rare branching or as coccus forms in *Arachis* nodules.

The guanine-plus-cytosine content of the DNA is 62 to 66 mol% (thermal denaturation). The type species is *B. japonicum* (Buchanan 1980) comb. nov. *Bradyrhizobium japonicum* (Buchanan 1980) comb. nov. The cells are gram-negative, nonsporeforming, short rods 0.5 to 0.9 μm by 1.2 to 3.0 μm. Motile by one polar or subpolar flagellum.

The colonies are circular, opaque, rarely translucent, white, and convex, and tend to be granular in texture. Growth on carbohydrate media usually accompanied by extracellular slime.

Most strains grow on a mineral salts medium containing yeast extract and glucose, galactose, gluconate, glycerol, fructose, arabinose, or mannitol. Maltose is utilized by about 10% of the strains, but lactose, rhamnose, raffinose, trehalose, sucrose, dulcitol, and dextrin are rarely utilized. Organic acids such as fumarate, malate, succinate, citrate, and pyruvate are utilized provided the basal medium has sufficient Ca²⁺ and Mg²⁺ to overcome the inhibitory chelating effects of these acids. Cellulose and starch are not utilized.

Some strains can utilize ammonium salts or nitrate as a sole source of nitrogen. Certain amino acids (glutamate, histidine, aspartate, and proline) serve as sole nitrogen sources, but in this respect they are inferior to vitamin-free casein hydrolysate. Casein and agar are not hydrolyzed. Peptone is poorly utilized.

Usually acid tolerant, most strains growing at pH 4.5. Over 30% of the strains will grow at pH 4.0 and a few as low as pH 3.5. Fails to grow above pH 9.0. An alkaline reaction is produced
in limus milk, without the production of a clear, upper "serum zone."

Fails to grow in media containing 2% NaCl and does not produce H₂S. Penicillinase production is common.

NADP⁺-linked 6-phosphogluconate dehydrogenase (EC 1.1.1.43), the key enzyme in the pentose phosphate pathway, is absent or virtually so (18). Glucose is metabolized largely by the Entner-Doudoroff route.

Usually has no requirement for extracellular vitamins, with the rare exception of biotin. Acidic heteropolysaccharides of the extracellular slime are heterogeneous in both structure and composition (5, 6) and contain d-galacturonic acid and, frequently, methylated sugars; neutral glycans of the slime also are heterogeneous.

Nitrogenase activity by free-living cells occurs in certain strains but only in media containing selected carbon sources and under reduced oxygen tension (17, 19, 24). Although typically chemoorganotrophic, some strains possess an active uptake hydrogenase which enables them to grow chemolithotrophically in an atmosphere of hydrogen, carbon dioxide, and low levels of oxygen (11).

Normally causes formation of effective root nodules on species of Glycine and on Macroptilium atropurpureum.

The guanine-plus-cytosine content of the DNA is 61 to 65 mol% (thermal denaturation).

Type strain: ATCC 10324 (27). In addition to the characteristics representative of all strains of the species, this strain grows on a mineral salts medium containing yeast extract and gluconate, glycerol, arabinose, or mannitol. It fails to utilize effectively glucose, galactose, fructose, maltose, lactose, rhamnose, raffinose, trehalose, sucrose, dulcitol, and dextrin. Ammonium salts and certain amino acids are utilized as sole sources of nitrogen. Growth occurs over the pH range 4.0 to 9.0. There is no demonstrated requirement for extracellular biotin. Nitrogenase activity can be demonstrated in free-living cells, and growth occurs chemolithotrophically in an atmosphere of hydrogen, carbon dioxide, and low levels of oxygen.

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**ADDENDUM IN PROOF**

Fast-growing root nodule bacteria, physiologically distinct from members of the genus *Bradyrhizobium*, recently have been obtained from soybean root nodules collected in China. Recent studies (H. H. Keyser, B. B. Boholoo, and T. S. Hu, unpublished data) indicate that these bacteria form effective root nodules on wild soybeans (*Glycine soja*) and on *Glycine max* cv. Peking, a black-seeded, genetically unimproved line of soybeans from China. However, ineffective nodules are produced on all commercial soybean cultivars thus far examined. The taxonomic status of these strains is currently unknown.

**REPRINT REQUESTS**

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**LITERATURE CITED**