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A revision of *Rhizobium* Frank 1889, with an emended description of the genus, and the inclusion of all species of *Agrobacterium* Conn 1942 and *Allorhizobium undicola* de Lajudie *et al.* 1998 as new combinations: *Rhizobium radiobacter*, *R. rhizogenes*, *R. rubi*, *R. undicola* and *R. vitis*

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***Rhizobium*, *Agrobacterium* and *Allorhizobium* are genera within the bacterial family *Rhizobiaceae*, together with *Sinorhizobium*. The species of *Agrobacterium*, *Agrobacterium tumefaciens* (syn. *Agrobacterium radiobacter*), *Agrobacterium rhizogenes*, *Agrobacterium rubi* and *Agrobacterium vitis*, together with *Allorhizobium undicola*, form a monophyletic group with all *Rhizobium* species, based on comparative 16S rDNA analyses. *Agrobacterium* is an artificial genus comprising plant-pathogenic species. The monophyletic nature of *Agrobacterium*, *Allorhizobium* and *Rhizobium* and their common phenotypic generic circumscription support their amalgamation into a single genus, *Rhizobium*. *Agrobacterium tumefaciens* was conserved as the type species of *Agrobacterium*, but the epithet *radiobacter* would take precedence as *Rhizobium radiobacter* in the revised genus. The proposed new combinations are *Rhizobium radiobacter*, *Rhizobium rhizogenes*, *Rhizobium rubi*, *Rhizobium undicola* and *Rhizobium vitis*.**

Keywords: *Rhizobiaceae*, phenetic, phylogenetic, polyphasic, taxonomy

INTRODUCTION

Nitrogen-fixing bacteria that form symbiotic associations with members of the Leguminosae, and related pathogenic bacteria, have been ascribed to the genera *Agrobacterium* Conn 1942, *Allorhizobium* de Lajudie *et al.* 1998b, *Azorhizobium* Dreyfus *et al.* 1988, *Bradyrhizobium* Jordan 1982, *Mesorhizobium* Jarvis *et al.* 1997, *Phyllobacterium* Knösel 1984, *Rhizobium* Frank 1889 and *Sinorhizobium* Chen *et al.* 1988. For some time, it has been clear that the nomenclature of *Rhizobium* and related genera does not accurately describe their natural classification and is in need of revision (Kersters & De Ley, 1984; Willems & Collins, 1993; Sawada *et al.*, 1993b; de Lajudie *et al.*, 1998b). In this paper, the relevant literature is reviewed and a nomenclature is proposed that aims to reflect, as closely as possible, the natural polyphasic and phenetic relationships of these taxa.

Abbreviations: ITS, internal transcribed spacer; LBP, local bootstrap probability; ME, minimum-evolution; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.

The original species nomenclature

The nomenclature of *Rhizobium* species was originally shaped by the belief that a natural classification could be based on the specificity of symbiotic plant range of bacterial strains and species. The recognition that nodulation and specificity were characters of strains carrying particular Sym plasmids, reviewed by Martínez-Romero & Palacios (1990), and therefore were taxonomically unreliable, has led to the aban-

donment of this concept. In *Rhizobium* species, non-symbiotic strains have been reported for different species (Jarvis *et al*., 1989; Laguerre *et al*., 1993; Segovia *et al*., 1991; Soberón-Chávez & Nájera, 1989), indicating that symbiotic plasmids can be lost in nature and are not essential for bacterial survival. Furthermore, functional plasmids can be transferred between members of *Rhizobium* and *Agrobacterium* species (Abe *et al*., 1998; Hooykaas *et al*., 1977; Martínez *et al*., 1987). Overviews of the relationships of bacterial nitrogen-fixing genera are given in Young (1994), Young & Haukka (1996) and Martínez-Romero & Caballero-Mellado (1996).

When they first proposed the names *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*, Smith & Townsend (1907) and Riker *et al*. (1930) followed the custom of giving names that reported a distinctive character of the species, in this case their pathogenic symptoms. *Agrobacterium tumefaciens* (Smith and Townsend 1907) Conn 1942 (type species) was the name given to strains of *Agrobacterium* capable of inducing tumorigenic reactions in a wide range of host plant species, although some of these tumorigenic strains isolated from *Vitis* spp. appeared to be specific to grape. *Agrobacterium rhizogenes* (Riker *et al*. 1930) Conn 1942 comprised strains capable of inducing a hairy-root (rhizogenic) reaction in host plants. *Agrobacterium rubi* (Hildebrand 1940) Starr and Weiss 1943 referred to strains capable of inducing tumorigenic reactions in the canes of *Rubus* spp. and appeared to have a relatively limited host range. Recently, the strains specific to *Vitis* spp., referred to above, were named *Agrobacterium vitis* Ophel and Kerr 1990. *Agrobacterium radiobacter* (Beijerinck and van Delden 1902) Conn 1942 comprised non-pathogenic *Agrobacterium* strains. Although Holmes & Roberts (1981) offered an alternative classification, the application of the names *Agrobacterium rhizogenes* and *Agrobacterium tumefaciens* on the basis of distinct pathogenic characters was supported by Kersters & De Ley (1984) and has since been used by many workers (examples are Jarvis *et al*., 1986; Sawada *et al*., 1992, 1993b; Weibgen *et al*., 1993; Bouzar *et al*., 1993).

Present classification of the family *Rhizobiaceae*

The rhizobia. A number of revisions and additions to the taxonomy of rhizobia (family *Rhizobiaceae*) have been made in the past 20 years. The *Approved Lists of Names of Bacteria* (Skerman *et al*., 1980) recorded in the genus *Rhizobium* all then-known bacteria capable of nodulation and nitrogen fixation in symbiotic relationships with plants in the family Leguminosae. Symbiotic species were *Rhizobium leguminosarum* (type species), *Rhizobium japonicum*, *Rhizobium lupini*, *Rhizobium meliloti*, *Rhizobium phaseoli* and *Rhizobium trifolii*. The *Agrobacterium* species *Agrobacterium tumefaciens*, *Agrobacterium rhizogenes* and *Agrobacterium rubi* and species in *Phyllobacterium* Knösel 1984 (*Phyllobacterium myrsinacearum* and *Phyllobacterium rubiacearum*) causing hypertrophies in plants as pathogenic (or oncogenic; meaning 'causing tumours') strains were also included in the family *Rhizobiaceae*. Since then, revisions have been made at both the generic and specific levels. Based on a summary of clustering analyses of phenotypic characters, DNA–DNA reassociation data and other data, Jordan (1982) revised the symbiotic nitrogen-fixing bacteria into two genera, in which fast-growing, acid-producing strains were retained in *Rhizobium* and slow-growing, alkali-producing strains were allocated to *Bradyrhizobium* as *Bradyrhizobium japonicum* Jordan 1982. Three species in *Rhizobium*, *R. leguminosarum* (amalgamating the former species of *R. leguminosarum*, *R. phaseoli* and *R. trifolii*), *R. meliloti* and *Rhizobium loti* were listed in the first edition of *Bergey's Manual of Systematic Bacteriology* (Jordan, 1984). Since then, the genus *Bradyrhizobium* has been shown to be on a phylogenetic branch distant from the *Rhizobium* species. Two species, *Bradyrhizobium elkanii* Kuykendall *et al*. 1993 and *Bradyrhizobium liaoningense* Xu *et al*. 1995, and other related slow-growing, symbiotic nitrogen-fixing strains have also been reported within this genus. The genus *Azorhizobium*, with a single species, *Azorhizobium caulinodans*, was proposed for stem-nodulating strains from *Sesbania rostrata* (Dreyfus *et al*., 1988). This taxon is also distantly related to other taxa with hypertrophying capabilities.

Other significant revisions have been made to taxa closely related to, and including, *Rhizobium* species. Chen *et al*. (1988) proposed a separate genus, *Sinorhizobium*, to include *Rhizobium fredii* Scholla and Elkan 1984 and the new species *Sinorhizobium xinjiangense* Chen *et al*. 1988. Subsequently, *R. meliloti* was transferred to the genus (de Lajudie *et al*. 1994) and *Sinorhizobium sahelense* de Lajudie *et al*. 1994, *Sinorhizobium terangae* de Lajudie *et al*. 1994, *Sinorhizobium medicae* Rome *et al*. 1996 and *Sinorhizobium arboris* and *Sinorhizobium kostiense* (Nick *et al*., 1999) have been proposed as new species. *R. loti* and some other recently described *Rhizobium* species are distinguished from *Rhizobium* and *Sinorhizobium* by comparative 16S rDNA sequence data, in having a slower growth rate (indicating underlying metabolic differences) and by a distinct fatty acid profile (Jarvis *et al*., 1996). For the species, Jarvis *et al*. (1997) proposed a new genus, *Mesorhizobium*, to include *Mesorhizobium loti* (type species), *Mesorhizobium ciceri* Nour *et al*. 1994, *Mesorhizobium huakuii* Chen *et al*. 1991, *Mesorhizobium mediterraneum* Nour *et al*. 1995 and *Mesorhizobium tianshanense* Chen *et al*. 1995. *Mesorhizobium amorphae* Wang *et al*. 1999 and *Mesorhizobium plurifarium* de Lajudie *et al*. 1998a are new species in the genus. Recently, a genus containing a single species, *Allorhizobium undicola* de Lajudie *et al*. 1998b, has been proposed for a population of nodulating, nitrogen-fixing strains that are more closely related to pathogenic *Agrobacterium* species than other nodulating *Rhizobium* species by comparative

sequence analysis of 16S rDNA. As well as these new genera, eight new species have been proposed within the genus *Rhizobium*: *Rhizobium etli* Segovia *et al.* 1993, *Rhizobium galegae* Lindström 1989, *Rhizobium gallicum* Amarger *et al.* 1997, *Rhizobium giardinii* Amarger *et al.* 1997, *Rhizobium hainanense* Chen *et al.* 1997, *Rhizobium huautlense* Wang *et al.* 1998, *R. mongolense* van Berkum *et al.* 1998 and *Rhizobium tropici* Martínez-Romero *et al.* 1991.

The agrobacteria. The natural species of the genus *Agrobacterium* have been investigated using numerical analysis of phenotypic characteristics (White, 1972; Kersters *et al.*, 1973; Holmes & Roberts, 1981), biochemical and physiological tests (Keane *et al.*, 1970; Kersters *et al.*, 1973; Kerr & Panagopoulos, 1977; Süle, 1978; Holmes & Roberts, 1981), fatty acid methyl ester profiles (Jarvis *et al.*, 1996; Sawada *et al.*, 1992), DNA–DNA reassociation (De Ley, 1972, 1974), measurements of the thermal stability of DNA–DNA hybrids (De Ley *et al.*, 1973) and comparison of electrophoregrams of soluble proteins (Kersters & De Ley, 1975). The results obtained by all methods indicated three genetically and phenotypically distinct groups or clusters, not including *Agrobacterium rubi*. These groups corresponded to biovars (or biotypes) 1, 2 and 3 of Keane *et al.* (1970), subsequently recognized as species (Holmes & Roberts, 1981; Bradbury, 1986; Sawada *et al.*, 1993b). [The term 'biovar' is usually applied to populations distinguished on the basis of their biochemical or physiological properties, distinguished as infrasubspecies (Lapage *et al.*, 1992). In *Agrobacterium*, it is generally agreed that biovar populations (*sensu* Keane *et al.*, 1970) have the status of species. In *Rhizobium*, the term biovar is generally applied to populations within species carrying particular Sym plasmids.] These three species, recognized on the basis of their overall phenotypic and genomic relatedness, are named according to the type strains allocated to each species population. (i) *Agrobacterium tumefaciens* corresponds to biotype 1 of Keane *et al.* (1970), group I of White (1972), cluster 1 of Kersters *et al.* (1973) and biovar 1 of Kersters & De Ley (1984), Willems & Collins (1993) and Sawada *et al.* (1993a). It includes the type strain of *Agrobacterium tumefaciens*, circumscribed according to its tumorigenic pathogenic characters, as well as the type strain of *Agrobacterium radiobacter*, a non-pathogenic strain. *Agrobacterium tumefaciens* is the conserved type species of *Agrobacterium* and therefore takes precedence over the earlier named *Agrobacterium radiobacter* (Judicial Commission, 1970). (ii) *Agrobacterium rhizogenes* corresponds to biotype 2 of Keane *et al.* (1970), biovar 2 of Kersters & De Ley (1984), Sawada *et al.* (1993a) and Willems & Collins (1993), group III of White (1972) and cluster 2 of Kersters *et al.* (1973). (iii) *Agrobacterium vitis* Ophel and Kerr 1990 was proposed as the name for the third biovar (Keane *et al.*, 1970; Kersters & De Ley, 1984, biotype 3; Kerr & Panagopoulos, 1977; Süle, 1978; Panagopoulos *et al.*, 1978). *Agrobacterium vitis* is found mainly on grapes but strains of this species have also been isolated from *Actinidia* spp. (Sawada & Ieki, 1992b). *Agrobacterium rubi* is a fourth species characterized in genotypic and phenotypic terms, usually isolated from *Rubus* spp., although strains have been isolated from other hosts (Bradbury, 1986) and it is capable of infecting a range of plant hosts (Sawada *et al.*, 1992).

Both the natural species *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*, named on the basis of the application of types, are represented by strains that may be either tumorigenic, rhizogenic or non-pathogenic, according to their plasmid complement. Such characters do not offer stable systematic classification at the species level. Though of practical use and widely supported, the special purpose nomenclature based on pathogenicity is inconsistent with the natural classification of *Agrobacterium* species as now understood.

Agrobacterium is a genus containing plant-pathogenic species closely related to *Rhizobium*. The amalgamation of these two genera has often been suggested (Graham, 1964; Heberlein *et al.*, 1967; De Ley, 1968; White, 1972; Graham, 1976; Kerr, 1992; Sawada *et al.*, 1993b). In spite of the artificial character of *Agrobacterium*, the convenience of the conventional nomenclature (described below) and the difficulty in applying species names to the plant pathogens without ambiguity seem to have been the reasons for the failure to make formal revisions.

Allorhizobium. de Lajudie *et al.* (1998b) proposed the name *Allorhizobium* (meaning *other Rhizobium*) for a monospecific genus, comprising the nitrogen-fixing species *Allorhizobium undicola*, established on the basis of comparative analysis of 16S rDNA sequence data that showed the organism as an outlying branch of the *Agrobacterium–Rhizobium* cluster. Its nearest neighbour is *Agrobacterium vitis*. Considering the description of *Allorhizobium undicola*, it is well supported as a species distinct from *Agrobacterium vitis* (and from all other members of *Agrobacterium–Rhizobium*) by DNA–DNA reassociation data, SDS-PAGE of proteins, PCR-RFLP of the internal transcribed spacer (ITS) region between the 16S and 23S rDNA and nutritional data. However, inspection of SDS-PAGE, ITS and nutritional data give no support for a closer relationship between *Agrobacterium vitis* and *Allorhizobium undicola* than to other species in *Agrobacterium*, *Rhizobium*, *Sinorhizobium* or *Mesorhizobium*. The generic relationships proposed between these species are therefore based entirely on the comparative 16S rDNA sequence data; on a perceived low percentage similarity value (95·5%) and the lack of bootstrap support for the branch. The reasons that de Lajudie *et al.* (1998b) gave for proposing the new genus were the unsettled state of *Agrobacterium* nomenclature and the evidence of heterogeneity in *Rhizobium*, which implied the need for the creation of a new genus separate from *Agrobacterium* and *Rhizobium*. However, *Allorhizobium undicola* shares a common generic circumscription with *Agrobacterium*

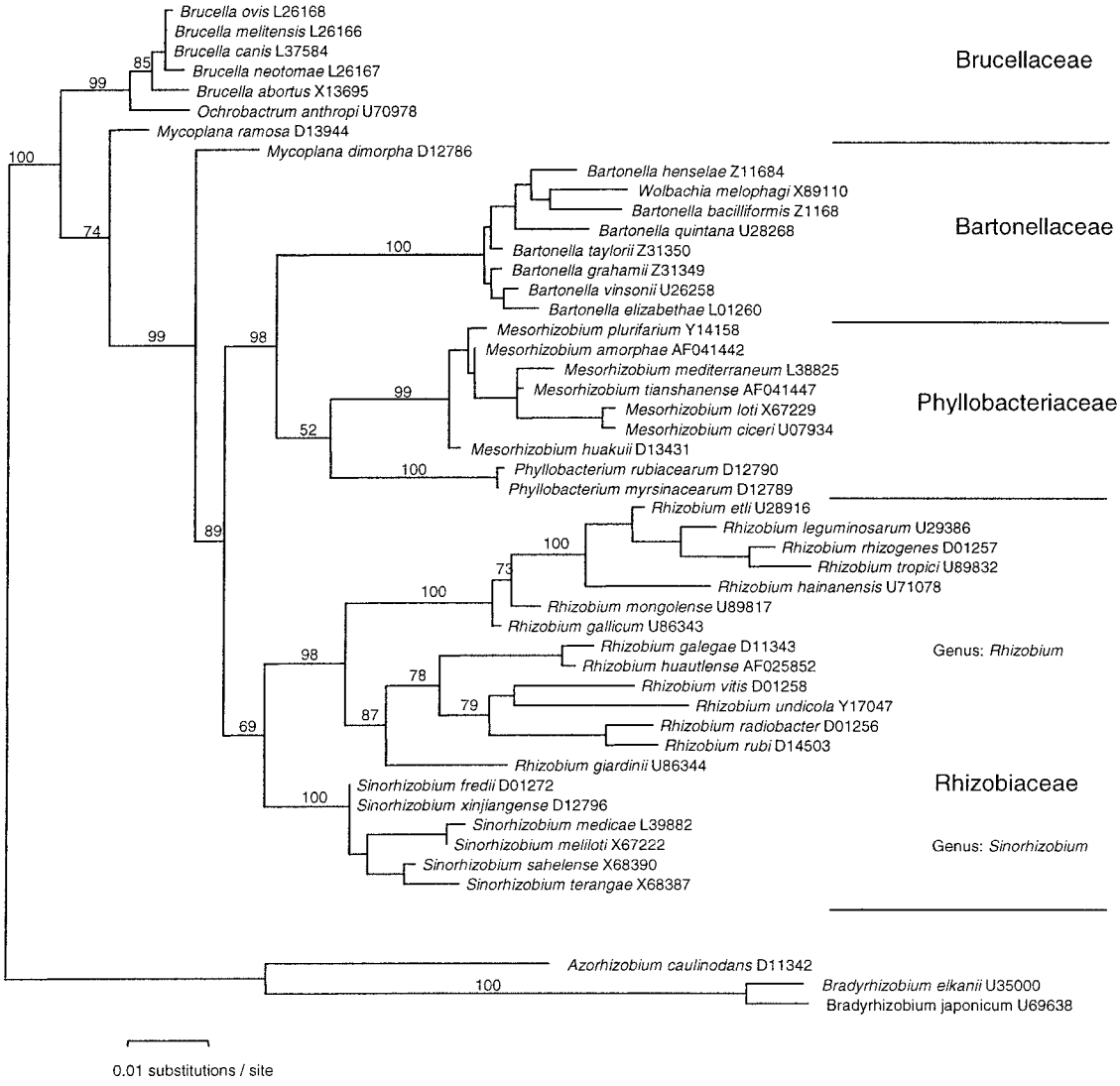

Fig. 1. Maximum-likelihood tree expressing the relationships among the *Rhizobiaceae* and their relatives, based on 16S rDNA sequences. Horizontal branch lengths are proportional to the estimated number of nucleotide substitutions and local bootstrap probabilities (as percentages) were determined for 1000 resamplings. Bacterial family names are those recorded on the website of the Bergey's Manual Trust (http://www.cme.msu.edu/Bergeys).

vitis and with all other members of *Agrobacterium* and *Rhizobium* (de Lajudie *et al.*, 1998b). Acceptance of sequence data alone based on the neighbour-joining analysis presented by de Lajudie *et al.* (1998b) as justification for the proposal of *Allorhizobium* invites reclassification of the outlying group of *Rhizobium* species and of *Agrobacterium vitis*, according to one of several possibilities: (i) proposal of the genus *Allorhizobium* to include *Allorhizobium undicola*, *Agrobacterium vitis*, *R. galegae* and *R. huautlense*; (ii) amalgamation of *Allorhizobium undicola* and *Agrobacterium vitis* in *Allorhizobium* and the creation of a new genus to recognize *R. galegae* and *R. huautlense*; (iii) proposal of a new genus for *Agrobacterium vitis* in a new monospecific sister genus with *Allorhizobium* (and the creation of a new genus to recognize *R. galegae* and *R. huautlense*); or (iv) proposal of monospecific genera for *Agrobacterium vitis*, *R. galegae* and *R. huautlense*. A similar iteration is possible following the ML and NJ analyses reported below. In these, *Allorhizobium undicola* shares a common branch with *Agrobacterium vitis*, which could be recognized as a species in the genus *Allorhizobium* or as a new sister genus. The conservation of *Agrobacterium tumefaciens* (syn. *radiobacter*) in the neighbouring branch forbids reclassification of this species, but *Agrobacterium rubi* could be renamed as a new genus. However, unless reference is made to the generic phenotype (that is to say, in the absence of distinct generic circumscriptions for the proposed genera), there is no rational way to choose between these alternatives. Alternatively, a revision accepting the inferred phylogeny of the taxon but which takes account of the supporting phenotypic data (which shows *Allorhizobium undicola* to be indistinguishable from *Rhizobium*) would see this species included in the genus *Rhizobium*.

METHODS

Analysis of 16S rDNA sequence data. Analyses were of 1444 base 16S rDNA sequences of *Rhizobium* species and species in the related families *Phyllobacteriaceae*, *Bartonellaceae* and *Brucellaceae*, with *Bradyrhizobium* species and *Azorhizobium caulinodans* as outlying taxa. Trees were constructed by using four tree-building methods, maximum-likelihood (ML) (Felsenstein, 1981), neighbour-joining (NJ) (Saitou & Nei, 1987), minimum-evolution (ME) (Rzhetsky & Nei, 1992) and maximum-parsimony (MP) (Swofford, 1993), as previously described in detail (Sawada *et al*., 1999).

To determine the ML tree topology, the local rearrangement searches of NucML (contained in the program package MOLPHY version 2.3) (Adachi & Hasegawa, 1996) were carried out, starting from the NJ tree topology and/or the topology obtained by the quick add OTUs search option as the initial trees. The HKY85 model (Hasegawa *et al*., 1985) (alpha/beta = 2·2; ln $L = -6993{\cdot}11$) was used for the base substitution process. The resulting tree is shown in Fig. 1.

Evolutionary distances (number of base substitutions) were estimated using the two-parameter method (Kimura, 1980) and the TN93 method (Tamura & Nei, 1993). Distances were then used to construct the NJ and ME trees using CLUSTAL W version 1.8 (Thompson *et al*., 1994) and MEGA2 (K. Tamura, personal communication), respectively. The tree resulting from the NJ method is shown in Fig. 2. The tree resulting from the ME method is identical in all essentials to the ML tree and is not shown.

PAUP version 3.1.1 (Swofford, 1993) and MEGA2 (K. Tamura, personal communication) were used for MP analysis and heuristic and branch-and-bound searches were used to ensure finding the most parsimonious trees. When two or more parsimonious trees were obtained, a strict consensus tree and a 50% majority-rule consensus tree were constructed.

As shown in Fig. 1 (ML tree) and Fig. 2 (NJ tree), the rate of nucleotide substitution differed among lineages. Because estimates based on maximum-parsimony are regarded as misleading when the rate of nucleotide substitution is not constant (Felsenstein, 1978; Hasegawa *et al*., 1991), the analysis is not shown here, although the topology was almost identical to that of ML tree.

To evaluate the reliability of the inferred ML tree topology, the local bootstrap probability (LBP) was calculated using the RELL method (Adachi & Hasegawa, 1996; Hasegawa & Kishino, 1994) with 1000 replications. For the NJ topology, the bootstrap probability (Felsenstein, 1985) was calculated by repeating the bootstrap resampling procedure 1000 times.

RESULTS AND DISCUSSION

Inferred phylogenetic relationships of the *Rhizobiaceae*

In terms of its phylogenetic relationships inferred from comparative sequence analysis of 16S rDNA, the family *Rhizobiaceae* is considered to be relatively closely related to the families *Bartonellaceae*, *Brucellaceae* and *Phyllobacteriaceae* in the α-*Proteobacteria*. This family terminology is that proposed in the forthcoming second edition of *Bergey's Manual of Systematic Bacteriology* and is presented on the website of the Bergey's Manual Trust (http://www.cme.msu.edu/Bergeys). Comparative analysis of 16S rDNA sequence data indicates that the genera *Bradyrhizobium*, in the *Bradyrhizobiaceae*, and *Azorhizobium* are distantly related to the other symbiotic nitrogen-fixing genera. Most or perhaps all species in the *Rhizobiaceae* and a number in the *Phyllobacteriaceae* (Wang *et al*., 1999; Xu & Murooka, 1995; Zou *et al*., 1997) appear to be distinct in their capacity to incorporate large functional plasmids or megaplasmids, which enable them to cause hypertrophies in plants as pathogenic tumours or symbiotic nitrogen-fixing nodules. Strains representing the *Bartonellaceae*, *Brucellaceae* and *Phyllobacteriaceae* (*Phyllobacterium* and *Mesorhizobium*) are sometimes represented as interspersed between the members of the *Rhizobiaceae* (de Lajudie *et al*., 1998a, b; Young & Haukka, 1996). In the analysis made here (Figs 1 and 2), the inclusion of sequences comprehensively representing the species of *Bartonella* (family *Bartonellaceae*) and of *Brucella* (family *Brucellaceae*), as well as sequences of other related genera, has resulted in a cladogram with family branches, including the family *Phyllobacteriaceae*. However, reliance cannot generally be placed on any particular sequence comparison, because analyses give differing results depending on the chosen algorithm and, most particularly, on the selection of included sequences, as shown by comparison of inferred phylogenies in recent reports (Amarger *et al*., 1997; Chen *et al*., 1997; de Lajudie *et al*., 1994, 1998a, b; Jarvis *et al*., 1997; Nour *et al*., 1995; Rome *et al*., 1996; Sawada *et al*., 1993b; Tan *et al*., 1997; van Berkum *et al*., 1998; Wang *et al*., 1998; Willems & Collins, 1993). Thus, Willems & Collins (1993) presented two unrooted phylogenetic trees in which the closer relationship of *Sinorhizobium* to *Rhizobium* or to *Mesorhizobium* depended upon whether an algorithmic or a parsimonious analysis was conducted. A similar result was reported by Laguerre *et al*. (1997) using mapped restriction site polymorphisms of 16S rDNA. It is noted here that, while high bootstrap values give confidence for some branches, such values do not indicate whether taxa are closely or distantly related. Also, low bootstrap values mean only that data are inadequate or not reliable for the purposes of inference. They do not mean, as is sometimes implied, that taxa under consideration are necessarily not closely related. In this study, the bootstrap values for the node linking the two *Rhizobium*/*Agrobacterium* clades using the ML, NJ, MP and ME methods were 98, 68, 49 and 74%, respectively.

The data reported here are consistent with earlier reports that species allocated to *Rhizobium*, *Allorhizobium*, *Agrobacterium* and *Sinorhizobium* are found in two or three clades. One clade corresponds to *Sinorhizobium*: *S. fredii* (the types species), *S. medicae*, *S. meliloti*, *S. sahelense*, *S. terangae* and *S. xinjiangense*. The second cluster is more heterogeneous and may be considered to be represented by two subclades: 2a, including *R. leguminosarum* (the type

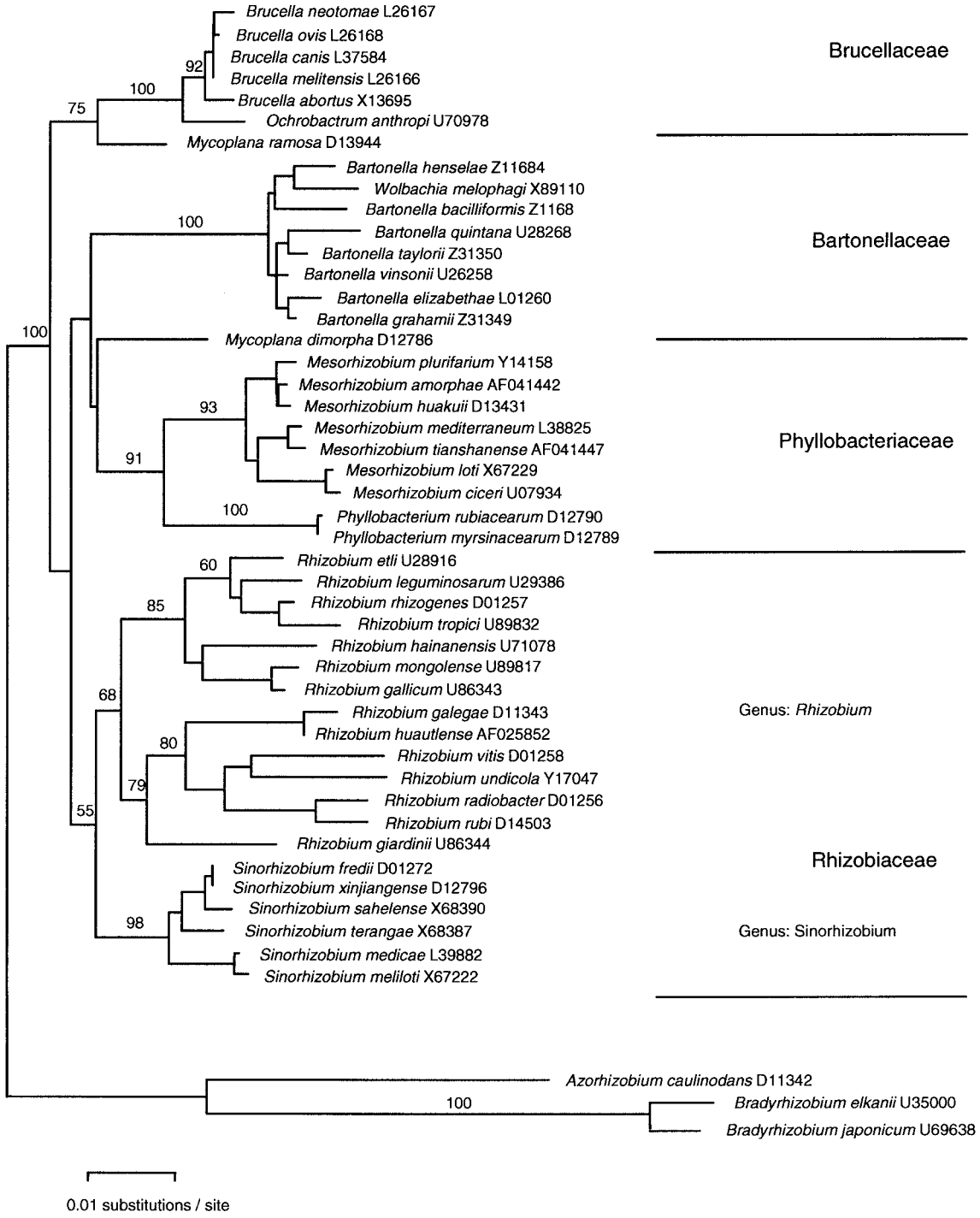

Fig. 2. Neighbour-joining tree expressing the relationships among the *Rhizobiaceae* and their relatives, based on 16S rDNA sequences. Sites that include gaps in more than one sequence were excluded. Horizontal branch lengths are proportional to the estimated number of nucleotide substitutions and bootstrap probabilities (as percentages) are determined from 1000 resamplings.

species), *R. etli*, *R. gallicum*, *R. giardinii*, *R. hainanense*, *R. mongolense*, *R. tropici* and *Agrobacterium rhizogenes*; and 2b, including *R. galegae*, *R. huautlense*, *Agrobacterium tumefaciens* (the type species), *Agrobacterium rubi*, *Agrobacterium vitis* and *Allorhizobium undicola*. They have differences amounting to less than 7% of the total sequence. The analyses reported here give no support for *Allorhizobium* as an outlying taxon/sequence. The extent of statistical support for individual branches and their relative positions depend on the form of phylogenetic analysis and the selection of sequences considered (Amarger *et al.*, 1997; Chen *et*

al., 1997; de Lajudie *et al*., 1994, 1998a, b; Jarvis *et al*., 1997; Nour *et al*., 1995; Rome *et al*., 1996; Sawada *et al*., 1993b; Tan *et al*., 1997; van Berkum *et al*., 1998; Wang *et al*., 1998; Willems & Collins, 1993). Young & Haukka (1996) and Eardly *et al*. (1996) note anomalies in sequence analyses attributable to recombination events between species, a conclusion supported but qualified by Wernegreen & Riley (1999). As yet, the significance and implications of recombination on the inference of phylogenetic relationships are unclear (Martínez-Romero & Caballero-Mellado, 1996).

Percentage differences based on pair-wise sequence comparisons were investigated (data not shown). Of rhizobial and agrobacterial sequences, 93% of pair-wise comparisons gave values greater than 94%. Between clusters, similarities ranged between 96·7% (*huautlense*/*gallicum*, *huautlense*/*mongolense* and *gallicum*/*galegae*) and 93·1% (*leguminosarum*/*undicola*). These data show a proportion of sequence pairs between clusters with percentage similarities as high as or higher than the percentage similarities of sequence pairs within clusters. This indicates relationships as close for species represented by sequence comparisons between clusters as those within clusters. Within cluster 1, percentage similarities ranged between 99·4 (*fredii*/*xinjiangense* and *gallicum*/*mongolense*), 97·5 (*terangae*/*medicae* and *terangae*/*meliloti*) and 96·2% (*hainanense*/*tropici*). Within cluster 2, percentage similarities ranged between 99·4 (*galegae*/*huautlense*) and 95·0% (*galegae*/*undicola*). In the absence of supporting phenotypic data or DNA reassociation data, it is possible that some of these species are synonyms. Stackebrandt & Goebel (1994) have noted the need to prove by other means the authenticity of species that share sequence similarities greater than 97%, in order to guard against synonymy.

High pair-wise percentage similarities between sequences representing members of the *Bartonellaceae* and the *Brucellaceae* and the *Rhizobiaceae* represent an anomaly for any coherent classification. *Brucella* and *Bartonella* species are semi-fastidious mammalian pathogens, growth of which is favoured by serum-based media. *Bartonella* species are polymorphic rickettsia-like organisms. In phenotypic terms, they share few obvious similarities with the rhizobia. Sequences representing these genera have relatively high pair-wise sequence similarity values with the rhizobia, usually exceeding 93%. Notably, several *Brucella* and *Sinorhizobium* species have similarity values of 96%. *Mycoplana* sequences have similarity values with *Sinorhizobium* of 96–97%. Rather than accepting such data uncritically as indicating close phylogenetic relationships, it is suggested that the limit of sensitivity for accurate phylogenetic inferences for these taxa using 16S rDNA sequence alone may have been reached. The justifications for classification for these genera need alternative supporting sequences and more refined methods of analysis than are routinely available at present. Sneath (1989) noted the problem of establishing phylogenetic relationships of closely related taxa at the family level and below using 16S rDNA sequence data. Although algorithmic methods (Swofford *et al*., 1996) provide support for branches of some closely related taxa, as yet there is no method universally applicable to all taxa.

Comparisons based on restriction analyses of 23S rDNA indicated that strains from *Rhizobium*, *Agrobacterium* and *Sinorhizobium* have similar fragmentation patterns (Selenska-Pobell & Evguenieva-Hackenberg, 1995). Terefework *et al*. (1998) and de Lajudie *et al*. (1998b) provide data showing that pathogenic and nodulating bacteria belonging to the genera *Agrobacterium*, *Rhizobium* and *Sinorhizobium* are interspersed on sub-branches of 16S or 23S rDNA phylogenetic trees.

Anomalous sequences

The family *Rhizobiaceae* also contains, as outliers to the rhizobial species, strains named *Blastobacter* spp. (Amarger *et al*., 1997; de Lajudie *et al*., 1998b; Young & Haukka, 1996) that do not have symbiotic or pathogenic characteristics. Other strains of *Blastobacter* spp. are to be found in the families *Methylobacteriaceae* (four strains), *Bradyrhizobiaceae* and *Sphingomonadaceae* (one strain each). The strains of *Blastobacter aggregatus* ATCC 43293 and *Blastobacter capsulatus* ATCC 43294 in the *Rhizobiaceae* are therefore perhaps incorrectly named or sequenced. Sequences such as *Mycoplana dimorpha* D12786 also need re-examination.

Generic definitions

There are no formal criteria for the definition of bacterial genera, but the suggested minimal standards for the description of new genera and species of root- and stem-nodulating bacteria (Graham *et al*., 1991) can be considered as a reference and the definition of species proposed by Wayne *et al*. (1987) for producing phylogenetic classification is also illustrative. Wayne *et al*. (1987) proposed a formal species definition based on genomic data, but emphasized the need for phenotypic support. Since then, the significance of this emphasis has become clear (Goodfellow & O'Donnell, 1993; Goodfellow *et al*., 1997). Genomic species, demonstrated by DNA–DNA reassociation data, are now accepted if circumscribed by phenotypic descriptions (using a selection of methods such as SDS-PAGE of proteins, fatty acid profiles, multilocus enzyme electrophoresis, nutritional, biochemical and morphological characters etc.) that distinguish them from other species. They are not recognized on the basis of genomic data alone (Ursing *et al*., 1995). In a similar way, genera may be indicated according to a phylogenetic principle using sequence data, but such genera, as taxa, also need to be differentiated in unique phenotypic terms if they are to satisfy any systematics

function other than understanding the historical relationships of the populations (Mayr, 1998). An important contribution to the discussion of the characterization of genera was provided by Murray *et al.* (1990), who noted the need for clarity of circumscription based on phenotypic properties at the level of genera, the impracticability of defining genera solely on the basis of phylogenetic data and the priority of phenotypic characterization over phylogenetic inference. As noted for *Allorhizobium*, there is no rational basis for making generic determinations unless there is phenotypic support for these taxa. Furthermore, proposed minimal standards for *Agrobacterium* and *Rhizobium* require that generic as well as species names should be based on both phenotypic and phylogenetic data (Graham *et al.*, 1991).

Multiple character analysis towards a phenetic (Goodfellow & O'Donnell, 1993) and polyphasic classification (*sensu* Vandamme *et al.*, 1996) aims to produce coherent taxa with relevant circumscriptions (Young *et al.*, 1992). A consideration of electrophoretic protein patterns and numerical analysis of nutritional and biochemical tests (de Lajudie *et al.*, 1994) give a discrimination of species that is inconsistent with their segregation into *Agrobacterium*, *Rhizobium* and *Sinorhizobium*. Recently, de Lajudie *et al.* (1998b) reported comparative studies of *Agrobacterium*, *Allorhizobium*, *Mesorhizobium*, *Rhizobium* and *Sinorhizobium* using protein patterns, ITS regions of 16S–23S rDNA, 16S rDNA and carbon-source utilization. None of these methods supported the discrimination of the species into existing generic groupings. Furthermore, morphological and biochemical characters of species give no support for discrimination of these genera (Table 1). There are no phenotypic single or multiple characteristics reported in the generic circumscriptions of *Agrobacterium*, *Allorhizobium*, *Rhizobium* and *Sinorhizobium* by which these taxa, as genera, can be differentiated. Analysis of fatty acid profiles showed that *Agrobacterium*, *Rhizobium* and *Sinorhizobium* were closely related (Jarvis *et al.*, 1996). 16S rDNA sequence data clearly show that *Agrobacterium* species (Willems & Collins, 1993) are closely allied with *Rhizobium* species such as *R. galegae* (Lindström, 1989) and the recently described *R. huautlense* (Wang *et. al.*, 1998) and *Allorhizobium undicola* (de Lajudie *et al.*, 1998b). *Agrobacterium rhizogenes* is in a clade with the majority of *Rhizobium* species. The notable unifying character of members of the genera *Agrobacterium* and *Rhizobium* is their capacity to exchange and operate the large Sym and Ti plasmids (Abe *et al.*, 1998; Hooykaas *et al.*, 1977; Martínez *et al.*, 1987). *Agrobacterium* can therefore be considered to be a polyphyletic genus that is an artificial amalgamation of plant-pathogenic species, similar in status to '*Phytomonas*'.

Proposed classification of *Rhizobium*

The three named genera *Agrobacterium*, *Allorhizobium* and *Rhizobium* do not represent distinct phenotypic entities; they do not have unique phenotypic generic circumscriptions. Furthermore, evidence of their phylogenetic differentiation is not compelling and depends on the choice of algorithm and sequences included in the analysis. We therefore propose their amalgamation in the single genus named *Rhizobium* as the senior subjective synonym (Rule 38, *International Code of Nomenclature of Bacteria*; Lapage *et al.*, 1992). The clades indicated, based only on branches from 16S rDNA comparative analyses, suggest an evolutionary divergence that could be interpreted as the incipient formation of new subgenera. *R. giardinii* is an outlying species that is not stably associated with any cluster. Acceptance of the genus *Allorhizobium* based on the criteria on which it was proposed would provide a justification for large numbers of alternative classifications for which there are no distinguishing criteria. *Allorhizobium* is considered to be an artificial genus only justified at present on the basis of the anomalous state of *Agrobacterium* nomenclature. *Allorhizobium undicola* should therefore be renamed *Rhizobium undicola*.

The phenotypic descriptions of species previously named in *Agrobacterium* are reported by Holmes & Roberts (1981), Kersters & De Ley (1984), Ophel & Kerr (1990), Sawada & Ieki (1992a), de Lajudie *et al.* (1994) and Amarger *et al.* (1997). The phenotypic description of *R. undicola* is given by de Lajudie *et al.* (1998b).

Comparative analyses of 16S rDNA sequence data indicate that *Mesorhizobium* may be more closely related to *Phyllobacterium* than to *Rhizobium* and *Sinorhizobium*, and their differing phenotypic generic characters (a slower growth rate, suggesting underlying metabolic differences, and distinct fatty acid profile; Jarvis *et al.*, 1996) are considered to justify their separation from other members of *Rhizobium*.

Sinorhizobium species could be considered as a subgeneric clade within the revised genus *Rhizobium* as proposed here. The absence of phenotypic support for separation of this genus from *Rhizobium* is justification for a proposal of synonymy. However, personal communications from colleagues suggest that phenotypic support for the genus may be pending and consideration needs to be given to possible publications before further revisions are made. In the meantime, this genus is retained as a separate taxon pending future consideration of its status.

Note on the nomenclature of *Agrobacterium* and pathogenic species

Bradbury (1986), Holmes & Roberts (1981), Holmes (1988) and Young *et al.* (1992) have supported the use of the names *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*, in accord with natural classification, recognizing pathogenicity of strains according

Table 1. Characteristics of *Rhizobium* (including *Agrobacterium* and *Allorhizobium*) and *Sinorhizobium* species

Taxa are indicated as: 1, *Rhizobium leguminosarum*; 2, *R. etli*; 3, *R. galegae*; 4, *R. gallicum*; 5, *R. giardinii*; 6, *R. hainanense*; 7, *R. huautlense*; 8, *R. mongolense*; 9, *R. radiobacter*; 10, *R. rhizogenes*; 11, *R. rubi*; 12, *R. tropici*; 13, *R. undicola*; 14, *R. vitis*; 15, *Sinorhizobium fredii*; 16, *S. medicae*; 17, *S. meliloti*; 18, *S. sahelense*; 19, *S. terangae*; and 20, *S. xinjiangense*. Data were taken from Graham & Parker (1964), Jordan (1984), Kersters & De Ley (1984), Kerr (1992) and the original species descriptions. +, More than 90% of strains expected to give a positive reaction; d, 10–90% of strains expected to give a positive reaction; −, fewer than 10% of strains expected to give a positive reaction; (+) weak growth in 2% NaCl.

Character	*Rhizobium*														*Sinorhizobium*					
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Polar flagella			1–2			1												⩾1	⩾1	
Peritrichous (or one subpolar) flagella	2–6		1–2					1	1–4	1–4	1–4	Yes	Yes	1–4	1–3		2–6	⩾1	⩾1	1–3
3-Ketolactose produced	−	−[a]*	−	−	−	−			+	−	−	−	−	−	−		−	−	−	
Growth factors required:	+	−	+						−	+	+	−[a]	−	+			+			
Biotin	d	−							−	+	+	−[a]	−	+			d			
Pantothenate	+	−	+				−		−		+	−[a]	−			−	−			
Thiamin	d	−	−				+		−			−[a]	−			−	−			
pH range for growth	4–9		5–9·5[b]	>4–8	<4–8·5	5–10	5–9	4–10		5–9		4–10			5–10·5	5–10	4·5–9·5			5–10·5
Growth in/at:																				
28 °C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
35 °C		+	+			+	+		+	−	−			−	+	+	+	+	+	+
40 °C	−	−	−	−	−	+	+			−	−	+		−	−	d	d	+	+	
1% NaCl	−		d[b]	−	d	+	+	−	+			−		+	+	+	+			+
2% NaCl	−		−	−	−	+	−	−	+	−	−	−		+	d	+	d			(+)
Luria–Bertani medium	−	−	−	−	−	+	−		+			+			−	+	+	−	−	−
Oncogenicity to few or many plant genera	−	−	−	−	−	−	−		Many	Many	Few	−	−	Few	−	−	−	−	−	−
Symbiotic nodulating/ nitrogen-fixing capacity	+	+	+	+	+	+	+	+	−	−	−	+	+	−	+	+	+	+	+	+

* Unpublished results (E. Martínez-Romero[a] or E.-T. Wang[b]) obtained using the methods of Wang *et al.* (1998).

to their 'tumorigenic', 'rhizogenic' and 'non-pathogenic' states. This use appears to pose problems of comprehension: the epithet *tumefaciens* is too closely associated with tumorigenicity to stand independently as a species name. Attempts to resolve the difficulty by recognizing *Agrobacterium radiobacter* in place of *Agrobacterium tumefaciens* (Kerr *et al*., 1978; Kersters & De Ley, 1984; Sawada *et al*., 1993b) cannot be adopted because *Agrobacterium tumefaciens* was conserved as the type species (Judicial Commission, 1970). Bouzar (1994) sought clarification of the proposal of Sawada *et al*. (1993b) which, with the response of H. Oyaizu and H. Sawada (Bouzar, 1994), the Judicial Commission of the ICSB deemed to have resolved the matter (L. G. Wayne, personal communication). The practical and usual solution to this nomenclatural confusion has been to use an artificial classification and irregular nomenclature, in which the species names *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* are applied to the pathogenic plasmid-borne states and the terms 'biotype' or 'biovar' are applied to the natural species groups. It is likely that this problem will persist for *Agrobacterium* nomenclature in the foreseeable future. Coincidentally, this problem is dissolved by the amalgamation of *Agrobacterium* and *Rhizobium* because the epithet *radiobacter* has chronological priority over *tumefaciens* and can be applied in the latter genus.

If the genus *Agrobacterium* is differentiated from *Rhizobium* in the future, the use of a natural classification that recognizes *Agrobacterium* species will probably require a radical change of nomenclature by application of the Code (Lapage *et al*., 1992). At least, the name *Agrobacterium tumefaciens* should be rejected (Rule 23a) as a *nomen ambiguum*; a name that has been used with different meanings and has thus become a source of error (Rule 56a). This would also involve the designation of a new type species. The obvious candidate is *Agrobacterium radiobacter*. For both these proposals, it would be necessary to make a Request for an Opinion to the Judicial Commission of the ICSB. A more radical option could involve the application of new names (and a new type species), extending the proposal of Kersters & De Ley (1984).

Elsewhere, the application of pathovars in terms of the Standards for Naming Pathovars (Dye *et al*., 1980) has been proposed (Kerr *et al*., 1978; Kersters & De Ley, 1984). However, the fact that most pathogenicity genes are carried on plasmids means that the pathogenic character of any strain is unstable. This lack of stability would make uncertain the application of pathovar names to particular strains, most notably to pathotype strains. For pathogenic strains in *Rhizobium*, therefore, this formal special purpose nomenclature (Dye *et al*., 1980) seems inappropriate. Species comprising pathogenic or non-pathogenic strains can be reported as tumorigenic (as a 'Ti strain' or 'Ti'), as a rhizogenic 'Ri strain' or 'Ri', or as non-pathogenic strains of the species, where relevant.

Emended description of the genus *Rhizobium* Frank 1889, 338[AL] (*Agrobacterium* Conn 1942, 359[AL]; *Allorhizobium* de Lajudie, Laurent-Fulele, Willems, Torck, Coopman, Collins, Kersters, Dreyfus and Gillis 1998b, 1288[VP])

Rhizobium (Rhi.zo′bi.um. Gr. n. *rhiza* a root; Gr. n. *bios* life; M. L. neut. n. *Rhizobium* that which lives in a root).

Cells are rods, 0·5–1·0 × 1·2–3·0 μm. Non-spore-forming. Gram-negative. Motile by one to six flagella. Insertion usually peritrichous or peritrichous/subpolar. Fimbriae have been described on some strains. Aerobic, possessing a respiratory type of metabolism with oxygen as the terminal electron acceptor. Optimum temperature for growth is 25–30 °C; some species can grow at temperatures above 40 °C. Optimum pH, 6–7; range, pH 4–10. Generation times of *Rhizobium* strains are 1·5–3·0 h. Colonies are usually white or beige, circular, convex, semi-translucent or opaque, raised and mucilaginous, usually 2–4 mm in diameter within 3–5 d on yeast/mannitol/mineral salts agar (YMA). Growth on carbohydrate media is usually accompanied by copious amounts of extracellular polysaccharide slime. Pronounced turbidity develops after 2 or 3 d in aerated or agitated broth. Chemoorganotrophic, utilizing a wide range of carbohydrates and salts of organic acids as sole carbon sources, without gas formation. Cellulose and starch are not utilized. Produce an acidic reaction in mineral salts medium containing mannitol or other carbohydrates. Ammonium salts, nitrate, nitrite and most amino acids can serve as nitrogen sources. Strains of some species will grow in a simple mineral salts medium with vitamin-free casein hydrolysate as the sole source of both carbon and nitrogen, but strains of many species require one or more growth factors such as biotin, pantothenate or nicotinic acid. Peptone is poorly utilized. Casein, starch, chitin and agar are not hydrolysed. Members of *Rhizobium* are distinguished from those in the related genera, *Mesorhizobium* and *Phyllobacterium*, by differences in growth rate, fatty acid profiles and 16S rDNA sequence. Closely related in terms of 16S rDNA sequence similarity, all known *Rhizobium* species include strains that induce hypertrophisms in plants. Hypertrophisms in most species are root nodules either with or without symbiotic nitrogen fixation, while in other species they occur as unregulated oncogenic (tumorigenic or rhizogenic) growths. Some cells of symbiotic bacterial species enter root hair cells of leguminous plants (family Leguminosae) via invagination or by wounds ('crack entry') and elicit the production of root nodules, wherein the bacteria engage as intracellular symbionts, usually fixing nitrogen. Many well-defined nodulation (*nod*) and nitrogen fixation (*nif*) genes are clustered on large or megaplasmids (pSyms). Plant-host specificity is usually for a few legume genera but may, in some strains, extend to many legume genera and is largely determined by the chemical structure of lipochito-

oligosaccharide Nod factors produced (Dénarié *et al*., 1992, 1996). These highly specific 'cell-wall-like' molecules induce nodule organogenesis in the absence of bacteria. Strains of plant-pathogenic *Rhizobium* (previously *Agrobacterium*) species invade the crown, roots and stems of many dicotyledonous and some gymnospermous plants via wounds. Self-proliferating tumours are induced by the genetic transfer of a small DNA region carried on large tumour-inducing Ti or hairy-root-inducing Ri plasmids into the host plant genome. Plasmid transfer between species results in the expression and stable inheritance of the particular plant-interactive properties of the plasmid-donor species.

The G+C content of the DNA is 57–66 mol% (T_m). The type species is *Rhizobium leguminosarum* (Frank 1879) Frank 1889, 338^{AL}.

Emended description of *Rhizobium radiobacter* (Beijerinck and van Delden 1902) comb. nov. (*Agrobacterium radiobacter* (Beijerinck and van Delden 1902) Conn 1942, 359^{AL}; *Agrobacterium tumefaciens* (Smith and Townsend 1907, 672) Conn 1942, 359^{AL})

The phenotypic description of *Rhizobium radiobacter* is reported by Holmes & Roberts (1981), Kersters & De Ley (1984), Sawada & Ieki (1992a), de Lajudie *et al*. (1994) and Amarger *et al*. (1997).

Ti or Ri plasmids determine the pathogenic status of strains. The species comprises pathogenic or non-pathogenic strains, both of which can be reported as tumorigenic; as a 'Ti strain' or 'Ti', as a rhizogenic 'Ri strain' or 'Ri' or as non-pathogenic strains of the species where relevant. Pathogenic strains have a wide and perhaps complex host range.

The epithet *tumefaciens* was conserved as the type species for *Agrobacterium*. The epithet *radiobacter* takes precedence over *tumefaciens* as the senior subjective synonym in *Rhizobium*.

The type strain is ATCC 19358^T (= DSM 30147^T; = IFO 13532^T; = ICMP 5785^T; = NCIB 9042^T; = NCPPB 3001^T).

Emended description of *Rhizobium rhizogenes* (Riker, Banfield, Wright, Keitt and Sagen 1930) comb. nov. (*Agrobacterium rhizogenes* (Riker, Banfield, Wright, Keitt and Sagen 1930) Conn 1942, 359^{AL})

The phenotypic description of *Rhizobium rhizogenes* is reported by Holmes & Roberts (1981), Kersters & De Ley (1984), Sawada & Ieki (1992a), de Lajudie *et al*. (1994) and Amarger *et al*. (1997).

Ti or Ri plasmids determine the pathogenic status of strains. The species comprises pathogenic or non-pathogenic strains that can be reported as tumorigenic, as a 'Ti strain' or 'Ti', as a rhizogenic 'Ri strain' or 'Ri' or as non-pathogenic strains of the species where relevant. Pathogenic strains have a wide and perhaps complex host range.

The type strain is ATCC 11325^T (= DSM 30148^T; = ICMP 5794^T; = IFO 13257^T).

Emended description of *Rhizobium rubi* (Hildebrand 1940) comb. nov. (*Agrobacterium rubi* (Hildebrand 1940) Starr and Weiss 1943, 316^{AL})

The phenotypic description of *Rhizobium rubi* is reported by Holmes & Roberts (1981), Kersters & De Ley (1984), Sawada & Ieki (1992a), de Lajudie *et al*. (1994) and Amarger *et al*. (1997).

The species comprises pathogenic and non-pathogenic strains that can be reported as tumorigenic, as a Ti strain or Ti, or as non-pathogenic strains of the species where relevant. Isolated from above-ground cane galls on *Rubus* spp. (black raspberry, boysenberry). The host range is not limited to *Rubus* spp. (Anderson & Moore, 1979).

The type strain is ATCC 13335^T (= CFBP 1317^T; = ICMP 6428^T; = IFO 13261^T; = LMG 156^T; = NCPPB 1854^T).

Emended description of *Rhizobium undicola* (de Lajudie, Laurent-Fulele, Willems, Torck, Coopman, Collins, Kersters, Dreyfus and Gillis 1998) comb. nov. (*Allorhizobium undicola* de Lajudie *et al.* 1998, 1288^{VP})

The phenotypic description of *Rhizobium undicola* is reported by de Lajudie *et al*. (1998b).

Members of this species fix nitrogen when in a natural symbiotic association with *Neptunia natans*. They also form symbiotic associations with *Acacia* spp., *Faidherbia* spp. and *Lotus arabicus*, but not with *Medicago* spp. or *Sesbania* spp. in artificial inoculation.

The type strain is ORS992^T (= LMG 11875^T).

Emended description of *Rhizobium vitis* (Ophel and Kerr 1990) comb. nov. (*Agrobacterium vitis* Ophel and Kerr 1990, 240^{VP})

The phenotypic description of *Rhizobium vitis* is reported by Ophel & Kerr (1990).

Ti plasmids determine the pathogenic status of strains. The species comprises pathogenic or non-pathogenic strains that can be reported as tumorigenic (Ti strain) or non-pathogenic strains of the species where relevant. Strains are generally isolated from *Vitis* spp. (grape), but they have occasionally been isolated from other dicotyledonous plant species.

The type strain is ATCC 49767^T (= ICMP 10752^T; = NCPPB 3554^T).

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