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The taxonomy of rhizobia: an overview

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Abstract

The taxonomy of rhizobia, bacteria capable of nodulating leguminous plants, has changed considerably over the last 20 years, with the original genus *Rhizobium*, a member of the alpha-Proteobacteria, now divided into several genera. The study of new geographically dispersed host plants, has been a source of many new species and is expected to yield many more. Here we provide an overview of the history of the rhizobia, but focus on the *Rhizobium–Allorhizobium–Agrobacterium* relationship. Finally, we review recent reports of nodulation and nitrogen fixation with legume hosts by bacteria that are outside the traditional rhizobial phylogenetic lineages. They include species of *Methylobacterium* and *Devosia* in the alpha-Proteobacteria and of *Burkholderia* and *Ralstonia* in the beta-Proteobacteria.

Introduction

The term "rhizobia", in the strictest sense, refers to members of the genus *Rhizobium*. Over the years, however, the term has come to be used for all the bacteria that are capable of nodulation and nitrogen fixation in association with legumes and that belong to a genus that was at one time part of the genus *Rhizobium* or closely related to it.

The family *Rhizobiaceae* in the 1984 edition of Bergey's Manual of Systematic Bacteriology, is composed of the rhizobia (at that time just including *Rhizobium* and *Bradyrhizobium*), *Agrobacterium* and *Phyllobacterium* (Jordan, 1984).

History

By the end of the 19th century, it was realized that atmospheric nitrogen was being assimilated through the root-nodules of legume plants. In 1888, Beijerinck reported isolation of the root-nodule bacteria and established that they were responsible for this process of nitrogen fixation. He named these bacteria *Bacillus radicicola* (Beijerinck, 1888). Later, Frank changed the name to *Rhizobium* with originally just one species, *R. leguminosarum* (Frank, 1889).

Extensive testing of nodulation of diverse legume hosts by different bacteria in the beginning of the 20th century, led to the establishment of cross-inoculation groups, with rhizobia from one plant in a cross-inoculation group supposed to nodulate all other plants in the group (Fred et al., 1932). This concept was also used in rhizobial taxonomy, but later it was abandoned as an unreliable taxonomic marker (Graham, 1964; Wilson, 1944), in part because of aberrant cross-infection among plant groups. Beginning in the early 1960s, bacteriologists started using a large diversity of morphological, nutritional and metabolic characters (Graham, 1964; Moffet and Colwell, 1968; 'tMannetje, 1967), as well as serology (Graham, 1963; Vincent and Humphrey, 1970) and simple DNA characteristics (De Ley and Rassel, 1965) in numerical taxonomy studies. This

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demonstrated the relatedness of *Rhizobium* and *Agrobacterium* and led to a clear distinction between the fast and slow growing rhizobia (Graham, 1964), with the latter group subsequently placed in a separate genus, *Bradyrhizobium* (Jordan, 1982).

From the 80s on, with the introduction of more genetic characteristics (DNA–DNA and DNA–rRNA hybridizations, rRNA catalogues, rDNA sequencing) more diversity was discovered among the rhizobia and their relationships with other groups of bacteria became apparent. This led to a gradual increase in the number of genera (Table 1). In parallel, there has also been a significant increase in the number of validly published species (Table 1), with 48 species of rhizobia now recognized.

Two main reasons for this increase in the number of genera and species are:

(1) Many different legume species have now been studied. This in contrast to original efforts, which emphasized those legumes that were important food and pasture species crops, mostly from the Western world. As an example, consider Table 2, where the *Mesorhizobium* species are listed together with the host plants from which they were reported. Even now, only about 20% of the total of about 18,000 species and 57% of about 650 genera of legume plants have been studied for nodulation (Sprent, 1995). This leaves a large number of legume species to be studied and potentially many more species and genera of rhizobia to be described.

Table 1. Rising number of species in the genera of the rhizobia

Genus	Original publication	Number of species					
		Before 1980	81–85	86–90	91–95	96–00	01–06
Agrobacterium	Cohn (1942)	4	4	5	5	5	5
Rhizobium	Frank (1889)	4	5	5	10	10	16
Bradyrhizobium	Jordan (1982)		1	1	3	3	7
Sinorhizobium	Chen et al. (1988)			2	5	8	11
Azorhizobium	Dreyfus et al., (1988)			1	1	1	2
Mesorhizobium	Jarvis et al. (1997)					7	11
Allorhizobium	de Lajudie et al. (1998a)					1	1
Total		8	9	13	23	34	53

Table 2. Overview of the species of *Mesorhizobium* (Jarvis et al., 1997) and the plants they were isolated from

Name	Year[a]	Host plants	Reference
M. loti	1982	*Lotus*, *Lupinus*, *Anthyllis*, *Leucaena*	Jarvis et al. (1982)
M. huakuii	1991	*Astragalus* (China)	Chen et al. (1991)
M. ciceri	1994	*Cicer arietinum* (Spain, USA, India, Russia, Turkey, Morocco, Syria)	Nour et al. (1994)
M. tianshanense	1995	*Glycyrrhiza*, *Sophora*, *Caragana*, *Halimodendron*, *Swainsonia*, *Glycine* (China)	Chen et al. (1995)
M. mediterraneum	1995	*Cicer arietinum* (Spain, Syria, India, Lebanon, Syria, Tunesia)	Nour et al. (1995)
M. plurifarium	1998	*Acacia*, *Prosopis*, *Chamaecrista*, *Leucaena* (Senegal, Sudan, Brazil)	de Lajudie et al. (1998b)
M. amorphae	1999	*Amorpha fruticosa* (China)	Wang et al. (1999)
M. chacoense	2001	*Prosopis* (Argentina)	Velázquez et al. (2001)
M. septentrionale	2004	*Astragalus adsurgens* (China)	Gao et al. (2004)
M. temperatum	2004	*Astragalus adsurgens* (China)	Gao et al. (2004)
M. thiogangeticum	2006	Rhizosphere of *Clitoria ternatea* (India)	Gosh and Roy (2006)

[a]Year of first description.

(2) The other reason for increasing numbers of rhizobial species is the ongoing evolution of taxonomic research. Improvements and new developments in the methods to study cell DNA and RNA have led to a more detailed characterization resulting in phylogenetic and polyphasic classifications. Currently an increasing number of total bacterial genomes are becoming available. This will undoubtedly have a further major impact on bacterial taxonomy. Most recent taxonomic studies have made use of a polyphasic approach (Graham et al., 1991;Vandamme et al., 1996), with genetic, phenotypic, chemotaxonomic, phylogenetic data combined to establish a comprehensive picture of the relationships of the bacteria, and to propose a suitable classification.

Sinorhizobium

Chen et al. (1988) proposed a separate genus for the fast-growing soybean rhizobia, renaming *R. fredii* as *Sinorhizobium fredii*, and proposing a second species, *S. xinjiangense* (the original spelling *S. xinjiangensis* was later corrected [Euzéby, 1998]). This new genus was controversial at first since genetic evidence to justify its creation and to separate it from *R. fredii* was not presented at the time (Jarvis et al., 1992). Later, phylogenetic data were presented to support a third genus of rhizobia, not restricted to the fast-growing soybean rhizobia (de Lajudie et al., 1994) and the genus definition was emended. *R. meliloti* was transferred to *Sinorhizobium* as *S. meliloti* and two additional species, *S. saheli* and *S. terangae* (the original spelling *S. teranga* was later corrected [Trüper and De'Clari, 1997]), were proposed for isolates from *Acacia* and *Sesbania* from Senegal. The genus *Sinorhizobium* is now widely accepted and currently has 11 valid species (Table 3). New genetic evidence in support of the separation of *S. xinjiangense* and *S. fredii* has been presented (Peng et al., 2002). However, this strongly relies on DNA–DNA hybridizations performed with DNA from the *S. fredii* type strain USDA 205^T the quality of which was not validated by homologous hybridization with *S. fredii* strains.

It has recently become evident from 16S rDNA comparisons that *Ensifer adhaerens* is also phylogenetically a member of the *Sinorhizobium* lineage (Balkwill, 2005). This organism is a soil bacterium that can adhere to and lyse other soil bacteria, and that was initially described mostly on the basis of phenotypic data (Casida Jr, 1982). Our own polyphasic studies have shown that a small group of four diverse rhizobial isolates and two soil isolates cannot be distinguished clearly from *Ensifer adhaerens* on the basis of DNA–DNA hybridizations and phenotypic features and we should therefore include these rhizobia in *Ensifer*. Phylogenetically, *Ensifer* and *Sinorhizobium*

Table 3. Species of *Sinorhizobium*

Name	Year[a]	Host plants	Reference
S. meliloti	1926	*Melilotus*, *Medicago*, *Trigonella*	Dangeard (1926)
S. fredii	1984	*Glycine*, *Vigna*, *Cajanus*	Scholla and Elkan (1984)
S. xinjiangense	1988	*Glycine*	Chen et al. (1988)
S. saheli	1994	*Sesbania*, *Acacia* (Senegal)	de Lajudie et al. (1994)
S. terangae	1994	*Sesbania* (Senegal)	de Lajudie et al. (1994)
S. medicae	1996	*Medicago* (Syria, France)	Rome et al. (1996)
S. arboris	1999	*Acacia*, *Prosopis* (Sudan, Kenya)	Nick et al. (1999
S. kostiense	1999	*Acacia*, *Prosopis* (Sudan)	Nick et al. (1999)
S. kummerowiae	2002	*Kummerowia stipulacea*	Wei et al. (2002
S. morelense	2002	*Leucaena leucocephala* (Mexico)	Wang et al. (2002)
"*S. adhaerens*"[b]	2003	*Medicago sativa* (Spain), *Leucaena leucocephala* (Brazil), *Pithecellobium dulce* (Brazil)	Willems et al. (2003)
S. americanum	2003	*Acacia* spp. (Mexico)	Toledo et al. (2003)

[a]Year of first description.
[b]*Ensifer adhaerens* was proposed to belong *Sinorhizobium*, however the name "*Sinorhizobium adhaerens*" remains not valid pending a judicial opinion (Willems et al., 2003).

form a single group in the 16S rDNA dendrogram of the alpha-Proteobacteria and may therefore be regarded as a single genus. This has important nomenclatural consequences because the older name *Ensifer* would have precedence. There are several reasons why a change from *Sinorhizobium* to *Ensifer* may not be the best solution, and allowing an exception to Rule 38 may be more appropriate. We have therefore proposed the creation of the species *Sinorhizobium adhaerens* comb. nov. and submitted a request for Opinion on the conservation of *Sinorhizobium adhaerens* over *Ensifer adhaerens* (Willems et al., 2003). This proposal was regarded unjustified by Young (2003) who proposed that all *Sinorhizobium* species should be transferred to *Ensifer* instead. While the request for opinion is pending the combination "*Sinorhizobium adhaerens*" is not valid and *Ensifer adhaerens* remains the correct name.

Mesorhizobium

The genus *Mesorhizobium* was proposed for five rhizobial species (*R. loti*, *R. huakuii*, *R. ciceri*, *R. mediterraneum* and *R. tianshanense*) that are phylogenetically related and distinct from the large phylogenetic grouping that includes *Rhizobium*, *Agrobacterium* and *Sinorhizobium* (Jarvis et al., 1997). They are characterized by a growth rate intermediate between the fast- and slow-growing rhizobia. On the basis of 16S rDNA sequence data, *Mesorhizobium* is phylogenetically separated from the fast-growing rhizobia by the genera *Bartonella*, *Defluvibacter*, *Aquamicrobium* *Phyllobacterium*, *Aminobacter* and *Pseudaminobacter* (Figure 1).

Bradyrhizobium

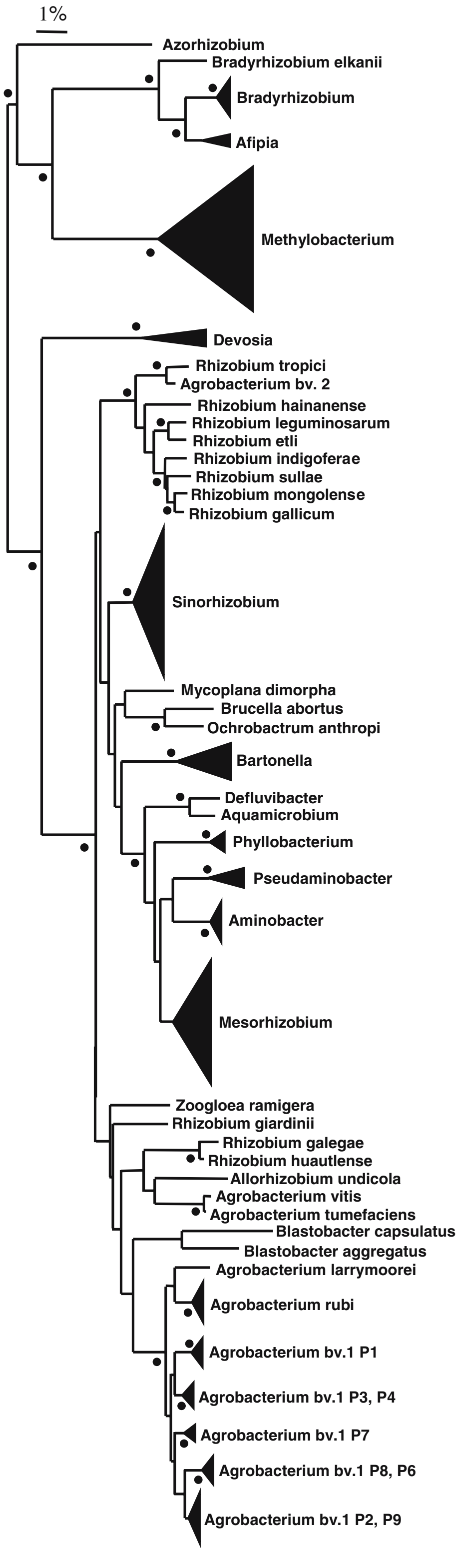
Bradyrhizobium was created for the slow-growing species *Rhizobium japonicum* (Jordan, 1982). Originally the soybean-nodulating *B. japonicum* was the only species described, although it was recognized that slow-growing strains occur on various legume genera (Elkan and Bunn, 1992). To date, five additional species have been validly named in this genus, two of them nodulating *Glycine* (*B. elkanii* [Kuykendall et al., 1992] and *B. liaoningense* [Xu et al., 1995]), *B. yuanmingense* nodulating *Lespedeza* (Yao et al., 2002),

Figure 1. 16S rDNA phylogeny of rhizobia and relatives in the alpha-Proteobacteria. The tree was calculated with the neighbor joining method, using Kimura-2 corrections. A bootstrap analysis was performed on 500 replicates and the groupings that were recovered in 95 or more percent of trees are marked in the dendrogram by a black dot at the branching point. Numbers P1–P9 refer to the *Agrobacterium* DNA groups of Popoff et al. (1984).

B. betae from the roots of *Beta vulgaris* afflicted with tumor-like deformations (Rivas et al., 2004), *B. canariense* from genistoid legumes from the Canary Islands (Vinuesa et al., 2005). In addition to the species subdivision, a number of serogroups have been described among slow-growing soybean symbionts (Date and Decker, 1965). Many other slow-growing rhizobia have been isolated from other legume hosts and are commonly referred to as *Bradyrhizobium* sp., followed by the name of the legume host. A special feature of the *Bradyrhizobium*–legume symbiosis is that some bradyrhizobia can form stem nodules on some plant species, produce bacteriochlorophyll and perform photosynthesis (Alazard, 1985; Evans et al., 1990; Molouba et al., 1999). Some photosynthetic bradyrhizobia have also been reported as endophytes of African wild rice (Chaintreuil et al., 2000).

A major factor complicating the evaluation of the taxonomic status and interrelationships of bradyrhizobia is the high similarity of 16S rDNA gene sequences. Many strains have 16S rDNA sequence divergences of 0.1–2.0%. Only sequences for *B. elkanii* and related strains differ by up to 4% from those of other bradyrhizobia (Willems et al., 2001a). A further complicating factor is the very slow growth of these organisms, often precluding the use of the standard phenotypic test procedures (e.g. Biolog, API systems). As a consequence many bradyrhizobia have been characterized more thoroughly by genotypic methods. Our own work using AFLP, DNA–DNA hybridizations and 16S–23S internal transcribed spacer (ITS) analyses has resulted in the delineation of at least 11 *Bradyrhizobium* genospecies, including the named species (Willems et al., 2001c).

From 16S rDNA phylogeny, the genera *Afipia*, *Rhodopseudomonas* and *Nitrobacter* also appear closely related to the bradyrhizobia, with *B. ekanii* occupying a more peripheral phylogenetic position (Willems et al., 2001a). This is in contrast to ITS



sequence data that show that *B. elkanii* is more closely related to the bradyrhizobia than are the three non-rhizobial genera. Based on ITS sequence data, the photosynthetic bradyrhizobia isolated from stem-nodules of *Aeschynomene*, form a distinct group closely related to *Blastobacter denitrificans* (Willems et al., 2001b; van Berkum and Eardly, 2002). As a result of a comprehensive study of both groups, van Berkum et al. (2006) recently proposed to transfer *Blastobacter denitrificans* to *Bradyrhizobium* and unite the species with the isolates from *Aeschynomene indica* as the species *Bradyrhizobium denitrificans*.

The Rhizobium–Allorhizobium–Agrobacterium issue

Figure 1 provides an overview of the phylogeny of the rhizobia and relatives in the alpha-Proteobacteria based on 16S rDNA sequence data. *Bradyrhizobium* and *Azorhizobium* are quite separate. *Sinorhizobium* and *Mesorhizobium* also form separate clusters, but it is clear that *Agrobacterium*, *Allorhizobium* and *Rhizobium* are rather more closely related. The recent proposal (Young et al., 2001) to abandon the genera *Agrobacterium* and *Allorhizobium* and incorporate them in *Rhizobium* has not met with universal approval (Farrand et al., 2003).

The genus *Allorhizobium* contains a single species, *Al. undicola*, for isolates from nodules of *Neptunia natans* from Senegal (de Lajudie et al., 1998a). Phylogenetically (Figure 1), it takes a separate position in the large *Agrobacterium–Rhizobium* 16S rDNA cluster, with *A. vitis* (96.3% 16S rDNA sequence similarity), *R. galegae* (95.1%) and *R. huautlense* (95.3%) as its nearest neighbors. In view of its remoteness from the *Rhizobium* type species, *R. leguminosarum*, and the confused taxonomic situation in *Agrobacterium* (see below) and because the *Neptunia* isolates can be distinguished phenotypically and genotypically from related taxa, it was considered most appropriate that they be placed in a separate genus (de Lajudie et al., 1998a). This genus may need emendation or revision in a future scheme to correct the classification and nomenclature of *Agrobacterium* and *Rhizobium* species, in particular *A. vitis*, *R. galegae* and *R. huautlense*.

The genus *Rhizobium* currently has 15 species, from various hosts (Table 4 – not including *Sinorhizobium* and *Agrobacterium*). *Agrobacterium*, a genus proposed in 1942 (Conn, 1942) that comprises bacteria responsible for various kinds of hypertrophies in plants, has six valid species. The oldest species were – for practical purposes – described mainly on the basis of phytopathological properties. For example, *A. tumefaciens* groups those strains that cause tumors on plants; *A. radiobacter* unites strains that are not pathogenic and *A. rhizogenes* comprises strains that cause hairy root growth (Conn, 1942). Of the other species, *A. rubi* is pathogenic on *Rubus* (Starr and Weiss, 1943), *A. vitis* on grapevine (Ophel and Kerr, 1990) and *A. larrymoorei* on *Ficus* (Bouzar and Jones, 2001).

Using a polyphasic approach, various authors have recognized three large groups or biovars among strains assigned to *A. tumefaciens*, *A. rhizogenes* and *A. radiobacter* in the official (phytopathology-based) classification (summarized in Kersters and De Ley, 1984). The taxonomic situation is complicated by the fact that these biovars do not correspond to the existing species, with biovar 1 containing strains of *A. tumefaciens*, *A. rhizogenes* and *A. radiobacter*. Among these strains are the type strains of *A. tumefaciens* and *A. radiobacter*. Biovar 2 also contains strains of all three species, including the type strain of *A. rhizogenes*, whereas biovar 3 contains *A. tumefaciens* and *A. vitis* strains. Furthermore, *Agrobacterium* biovar 1 has been shown to contain several genospecies by DNA–DNA hybridizations (De Ley, 1974; Popoff et al., 1984), some of which contain clinical isolates that do not have virulence genes but were originally named *Agrobacterium* because of biochemical features (Lautrop, 1967; Riley and Weaver, 1977). A further complicating factor is that the genus *Agrobacterium* was declared conserved by the Judicial Commission, with *A. tumefaciens* as the type species (Judicial Commission, 1970). However, as Young et al. (2006) recently pointed out, the species name *A. tumefaciens* is not a conserved name as some authors may previously have believed.

The phytopathology-based taxonomy and the polyphasic classification (Kersters and De Ley, 1984) of the genus *Agrobacterium* is shown in Table 5. No species names were proposed for the biovars described by Kersters and De Ley (1984) because the rules of the Bacteriological Code

Table 4. Species of *Rhizobium*

Name	Year[a]	Host plant(s)	Reference
R. leguminosarum	1879	*Pisum*, *Lathyrus*, *Vicia*, *Lens*, *Phaseolus*, *Trifolium*	Frank (1879)
R. lupinii[b]	1886	*Lupinus*, *Ornithopus*	Schroeter (1886)
R. galegae	1989	*Galega*	Lindström (1989)
R. tropici	1991	*Phaseolus vulgaris*, *Leucaena*	Martínez-Romero et al. (1991)
R. etli	1993	*Phaseolus vulgaris*	Segovia et al. (1993)
R. gallicum	1997	*Phaseolus vulgaris*	Amarger et al. (1997)
R. giardinii	1997	*Phaseolus vulgaris*	Amarger et al. (1997)
R. hainanense	1997	*Desmodium*, *Stylosanthes*, *Centrosema*, *Tephrosia*, *Acacia*, *Zornia*, *Macroptilium*	Chen et al. (1997)
R. mongolense	1998	*Medicago ruthenica*	van Berkum et al. (1998)
R. huautlense	1998	*Sesbania herbacea*	Wang et al., (1998)
R. yanglingense	2001	*Coronilla*, *Gueldenstaedtia*, *Amphicarpaea*	Tan et al. (2001)
R. sullae	2002	*Hedysarum coronarium*	Squartini et al. (2002)
R. indigoferae	2002	*Indigofera*	Wei et al. (2002)
R. loessense	2003	*Astragalus*	Wei et al. (2003)
R. daejeonense	2005	*Medicago*	Quan et al. (2005)

[a]Year of first description.
[b]The relationships of *R. lupinii* (Kuykendall et al., 2005) are unclear because of doubts on the purity of the type strain, but the species was included on the approved lists.

Table 5. Official and polyphasic classification of *Agrobacterium*

Official classification[a] (phytopathology)	Polyphasic classification[b] (Kersters and De Ley, 1984)
A. tumefaciens (tumors)	Biovar 1: tumorigenic (*A. tumefaciens*), rhizogenic (*A. rhizogenes*) and avirulent (*A. radiobacter*) strains, includes type strains of *A. tumefaciens* and *A. radiobacter*
A. radiobacter (no symptoms)	Biovar 2: tumorigenic (*A. tumefaciens*), rhizogenic (*A. rhizogenes*) and avirulent (*A. radiobacter*) strains, includes type strain of *A. rhizogenes*
A. rhizogenes (hairy roots)	Biovar 3: tumorigenic on *Vitis* (*A. tumefaciens* and *A. vitis*)
A. rubi (*Rubiaceae*)	*A. rubi*
A. vitis (*Vitis*)	
A. larrymoorei (*Ficus*)	

[a]The official classification includes the species that are in the Approved List of Bacterial Names (Skerman et al., 1980) or were subsequently published in International Journal of Systematic Bacteriology (now International Journal of Systematic and Evolutionary Microbiology). All *Agrobacterium* species are transferred to *Rhizobium* in the proposal of Young et al. (2001).
[b]The polyphasic classification is a consensus classification based on different methods as presented in Bergey's Manual of Systematic Bacteriology (Kersters and De Ley, 1984).

would have required biovar 1 to be named *A. tumefaciens* and biovar 2 *A. rhizogenes*. These names would then apply to strains with and without the phytopathological properties their name implies. This was regarded as unacceptable at the time and thus the official species classification and the polyphasic biovar system have been used in parallel for many years. This situation is clearly unsatisfactory and in 1993 Sawada et al. (1993) proposed that biovar 1 be named *A. radiobacter* and biovar 2 *A. rhizogenes*. This was thought to go against Opinion 33 of the Judicial Commission (Bouzar, 1994) and was not widely adopted.

From the 16S rDNA phylogeny (Figure 1), it is clear that *Rhizobium* and *Agrobacterium* are highly related and their species are interwoven. In particular, biovar 2 groups with the majority of *Rhizobium* species. Biovar 1 consists of several smaller groups representing different genospecies. *A. rubi* and *A. larrymoorei* are closely related to these biovar 1 genospecies. *A. vitis* is close to *Allorhizobium*. *Rhizobium giardinii* is the most peripheral of the whole group. Young et al. (2001) proposed the transfer all these taxa to *Rhizobium*. They proposed to unite *A. radiobacter* and *A. tumefaciens* in *R. radiobacter*, which thus represents biovar 1, while *A. rhizogenes* becomes *R. rhizogenes* and represents biovar 2. *A. rubi* and *A. vitis* are transferred to *Rhizobium* as distinct species and also *A. larrymoorei* is transferred as *R. larrymoorei* (Young, 2004). This proposal solves the species matching the biovars and the placement of *A. rhizogenes* in *Rhizobium* is clearly justified, but it is not widely accepted (Farrand et al., 2003) and several problems remain to be addressed:

(1) Many strains of *Agrobacterium* species have in the past been named on the basis of phytopathological effects they cause on plants and are listed in culture collection catalogues as such, often without their biovar status being known. It is not clear which *Rhizobium* species these should be classified as. For example, an *A. tumefaciens* strain may belong to biovar 1, 2 or 3 and depending on this should be classified as *R. radiobacter*, *R. rhizogenes* or *R. vitis*, respectively.
(2) The biovar 1 genospecies are ignored in the new scheme.
(3) The incomplete phenotypic differentiation of these genospecies.
(4) The proposed enlarged genus *Rhizobium* would be a large, widely defined and phylogenetically deep genus (Figure 1).
(5) The species *Blastobacter capsulatus*, *Blastobacter aggregatus* and *Zooglea ramigera*, that group with the *Agrobacterium–Rhizobium* phylogenetic cluster (Figure 1) and therefore would group in the proposed large genus *Rhizobium*, should be taken into account.

When considering the 16S rDNA phylogeny of part of the alpha-Proteobacteria (Figure 1), it is obvious that the new genus *Rhizobium* is rather large and represents a phylogenetically more divers (deeper) group then the other genera in its phylogenetic vicinity. However, to present an alternative proposal, additional data are essential. The current proposal was based mostly on 16S rDNA data. It is clear that data from other genes can provide useful insights to unravel relationships in these groups. With the new complete genome sequence data that are now becoming available, it may soon become possible to make a more comprehensive comparison and arrive at a suitable classification. Meanwhile, microbiologists should be aware that all validly published names can be used: it is the scientific community that decides on the value of any new proposal by either using it or, alternatively, by using a previously validly published classification.

Other nitrogen-fixing legume symbionts

Recently, a number of isolates have been reported from legume nodules, capable of nitrogen fixation but phylogenetically located outside the traditional groups of rhizobia in the alpha-Proteobacteria. New lines that contain nitrogen-fixing legume symbionts include *Methylobacterium*, *Devosia*, *Ochrobactrum* and *Phyllobacterium* in the alpha-Proteobacteria and *Burkholderia*, *Ralstonia* and *Cupriavidus* in the beta-Proteobacteria.

In *Burkholderia*, a genus that contains over 20 species of plant pathogens, soil and plant-associated bacteria and clinical isolates, the following symbiotic, nitrogen fixing strains have been identified: (1) two strains from *Mimosa* were found to belong to *B. caribensis*, a species that was first described for soil isolates from Martinique; (2) one strain from *Alysicarpus* was found to belong to *B. cepacia* genomovar VI (now *B. dolosa* [Vermis et al., 2004], a group previously only found in cystic fibrosis patients; (3) one strain from *Machaerium* was identified as a new species for which the name *B. phymatum* has been proposed and (4) one strain from *Aspalathus* was identified as a second new species for which the name *B. tuberum* was proposed (Moulin et al., 2001; Vandamme et al., 2003).

In *Ralstonia*, like *Burkholderia* a genus of plant pathogenic or plant associated, soil and clinical organisms, *Ralstonia taiwanensis* was proposed for strains from *Mimosa* species in Taiwan (Chen et al., 2001).

In the alpha-Proteobacteria, *Devosia neptuniae* was proposed for strains from *Neptunia natans* from India (Rivas et al., 2003) and *Methylobacterium nodulans* for strains from *Crotalaria* (Jourand et al., 2004; Sy et al., 2001). *Ochrobactrum lupinus* was described for nodule isolates from *Lupinus* sp. (Trujillo et al., 2005) and *Phyllobacterium lupinii* for isolates nodulating *Trifolium* and *Lupinus* (Valverde et al., 2005).

All these new nodulating bacteria are phylogenetically (16S rDNA) distinct from the rhizobia, but do carry nod genes similar to those of rhizobia. These genes encode for Nod factors, signal molecules in the bacterium–legume communication that accompanies nodulation. It is in fact from studies of nod gene diversity that some discoveries of new nodulating strains outside the rhizobia have originated. The nod genes were most probably obtained by these new nitrogen-fixing legume symbionts through lateral-gene transfer (Moulin et al., 2001; Sy et al., 2001). Most of the new nodulating bacteria belong to genera that have at least some plant-associated species and that are therefore likely to have the molecular strategies to overcome plant defenses. Recent reports confirm that it is quite likely that more such bacteria, capable of effective nodulation will be discovered outside the traditional rhizobia (Barret and Parker, 2006; Rasolomampianina et al., 2005; Zakhia et al., 2006).

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References

Alazard D 1985 Stem and root nodulation in *Aeschynomene* sp. Appl. Evironm. Microbiol. 50, 732–734.

Amarger N, Macheret V and Laguerre G 1997 *Rhizobium gallicum* sp. nov. and *Rhizobium giardinii* sp. nov., from *Phaseolus vulgaris*. Int. J. Syst. Bacteriol. 47, 996–1006.

Balkwill D L 2005 Genus VI. *Ensifer* Cassida 1982, 343^{VP}. *In* Bergey's Manual of Systematic Bacteriology 2nd ed. Vol. 2, part C. Ed. G M Garrity. pp. 354–358. Springer, New York.

Barrett C F and Parker M A 2006 Coexistence of *Burkholderia*, *Cupriavidus*, and *Rhizobium* sp nodule bacteria on two *Mimosa* spp. in Costa Rica. Appl. Environ. Microbiol. 72, 1198–1206.

Beijerinck M W 1888 Cultur des *Bacillus radicola* aus den Knöllchen. Bot. Ztg. 46, 740–750.

Bouzar H 1994 Request for a Judicial Opinion concerning the type species of *Agrobacterium*. Int. J. Syst. Bacteriol. 44, 373–374.

Bouzar H and Jones J B 2001 *Agrobacterium larrymoorei* sp. nov., a pathogen isolated from aerial tumours of *Ficus benjamini*. Int. J. Syst. Evol. Microbiol. 51, 1023–1026.

Casida L E Jr 1982 *Ensifer adhaerens*, gen. nov., sp. nov.: A bacterial predator of bacteria in soil. Int. J. Syst. Bacteriol. 32, 339–345.

Chantreuil C, Giraud E, Prin Y, Lorquin J, Bâ A, Gillis M, de Lajudie P and Dreyfus B 2000 Photosynthetic bradyrhizobia are natural endophytes of the African wild rice *Oryza breviligulata*. Appl. Environ. Microbiol. 66, 5437–5447.

Chen W-M, Laevens S, Lee T-M, Coenye T, De Vos P, Mergeay M and Vandamme P 2001 *Ralstonia taiwanensis* sp. nov., isolated from root nodules of *Mimosa* species and sputum of cystic fibrosis patients. Int. J. Syst. Evol. Microbiol. 51, 1729–1735.

Chen W X, Li G S, Qi Y L, Wang E T, Yuan H L and Li J L 1991 *Rhizobium huakuii* sp. nov. isolated from the root nodules of *Astragalus sinicus*. Int. J. Syst. Bacteriol. 41, 275–280.

Chen W-X, Tan Z-Y, Gao J-L, Li Y and Wang E T 1997 *Rhizobium hainanense* sp. nov., isolated from tropical legumes. Int. J. Syst. Bacteriol. 47, 870–873.

Chen W, Wang E, Wang S, Li Y, Chen X and Li Y 1995 Characterization of *Rhizobium tianshanense* sp. nov., a moderately and slow growing root nodule bacterium isolated from an arid saline environment in Xinjiang, People's Republic of China. Int. J. Syst. Bacteriol. 45, 153–159.

Chen W X, Yan G H and Li J L 1988 Numerical taxonomic study of fast-growing soybean rhizobia and proposal that *Rhizobium fredii* be assigned to *Sinorhizobium* gen. nov. Int. J. Syst. Bacteriol. 38, 392–397.

Conn H J 1942 Validity of the genus *Alcaligenes*. J. Bacteriol. 44, 353–360.

Dangeard P A 1926 Recherches sur les turbercles radicaux des Légumineuses. Botaniste (Paris) 16, 1–275.

Date R A and Decker A M 1965 Minimal antigenic constitution of 28 strains of *Rhizobium japonicum*. Can. J. Microbiol. 11, 1–8.

de Lajudie P, Laurent-Fulele E, Willems A, Torck U, Coopman R, Collins M D, Kersters K, Dreyfus B and Gillis M 1998a *Allorhizobium undicola* gen. nov., sp. nov., nitrogen-fixing bacteria that efficiently nodulate *Neptunia natans* in Senegal. Int. J. Syst. Bacteriol. 48, 1277–1290.

de Lajudie P, Willems A, Nick G, Moreira F, Molouba F, Hoste B, Torck U, Neyra M, Collins M D, Lindström K, Dreyfus B and Gillis M 1998b Characterization of tropical tree rhizobia and description of *Mesorhizobium plurifarium* sp. nov. Int. J. Syst. Bacteriol. 48, 369–382.

de Lajudie P, Willems A, Pot B, Dewettinck D, Maestrojuan G, Neyra M, Collins M D, Dreyfus B, Kersters K and Gillis M 1994 Polyphasic taxonomy of rhizobia: Emendation of the genus *Sinorhizobium* and description of *Sinorhizobium meliloti* comb. nov., *Sinorhizobium saheli* sp. nov., and *Sinorhizobium teranga* sp. nov. Int. J. Syst. Bacteriol. 44, 715–733.

De Ley J 1974 Phylogeny of Prokaryotes. Taxon 23, 291–300.

De Ley J and Rassel A 1965 DNA base composition, flagellation and taxonomy of the genus *Rhizobium*. J. Gen. Microbiol. 41, 85–91.

Dreyfus B, Garcia J L and Gillis M 1988 Characterization of *Azorhizobium caulinodans* gen. nov., sp. nov., a stem-nodulating nitrogen-fixing bacterium isolated from *Sesbania rostrata*. Int. J. Syst. Bacteriol. 38, 89–98.

Elkan G H and Bunn C R 1992 Chapter 107. The rhizobia. *In* The Prokaryotes. A handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications. Eds. A Balows, H G Trüper, M Dworkin, W Harder and K-H Schleifer. pp. 2197–2213. 2nd edition, Vol. III Springer-Verlag, New York.

Euzéby J P 1998 Taxonomic note: Necessary correction of specific and subspecific epithets according to Rules 12c and 13b of the International Code of Nomenclature of Bacteria (1990 Revision). Int. J. Syst. Bacteriol. 48, 1073–1075.

Evans W R, Fleischman D E, Calvert H E, Pyati R V, Alter G M and Subba Rao N S 1990 Bacteriochlorophyll and photosynthetic reaction centers in *Rhizobium* strain BTAi1. Appl. Environ. Microbiol. 56, 3445–3449.

Farrand S K, van Berkum P B and Oger P 2003 *Agrobacterium* is a definable member of the family *Rhizobiaceae*. Int. J. Syst. Evol. Microbiol. 53, 1681–1687.

Frank B, 1879 Ueber die Parasiten in den Wurzelanschwillungender Papilionaceen Ber. Dtsch. Bot. Ges. 37, 376–387 and 394–399.

Frank B 1889 Ueber die Pilzsymbiose der Leguminosen. Ber. Deut. Bot. Ges. 7, 332–346.

Fred E B, Baldwin I L and McCoy E 1932 Root Nodule Bacteria and Leguminous Plants. University of Wisconsin Studies in Science, number 5. University of Wisconsin Press, Madison.

Gao J-L, Turner S L, Kan F L, Wang E T, Tan Z Y, Qiu Y H, Gu1 J, Terefework Z, Young J P W, Lindström K and Chen W X 2004 *Mesorhizobium septentrionale* sp. nov. and *Mesorhizobium temperatum* sp. nov., isolated from *Astragalus adsurgens* growing in the northern regions of China. Int. J. Syst. Evol. Microbiol. 54, 2003–2012.

Ghosh W and Roy P 2006 *Mesorhizobium thiogangeticum* sp. nov., a novel sulfur-oxidizing chemolithoautotroph from rhizosphere soil of an Indian tropical leguminous plant. Int. J. Syst. Evol. Microbiol. 56, 91–97.

Graham P H 1963 Antigenic affinities of the root-nodule bacteria of legumes. Antonie van Leeuwenhoek J. Microbiol. Serol. 29, 281–291.

Graham P H 1964 The application of computer techniques to the taxonomy of the root-nodule bacteria of legumes. J. Gen. Microbiol. 35, 511–517.

Graham P H, Sadowsky M J, Keyser H H, Barnet Y M, Bradley R S, Cooper J E, De Ley J, Jarvis B D W, Roslycky E B, Stijdom B W and Young J P W 1991 Proposed minimal standards for the description of new genera and species of root- and stem-nodulating bacteria. Int. J. Syst. Bacteriol. 41, 582–587.

Jarvis B D W, Downer H L and Young J P W 1992 Phylogeny of fast-growing soybean-nodulating rhizobia supports synonymy of *Sinorhizobium* and *Rhizobium* and assignment to *Rhizobium fredii*. Int. J. Syst. Bacteriol. 42, 93–96.

Jarvis B D W, Pankhurst C E and Patel J J 1982 *Rhizobium loti*, a new species of legume root nodule bacteria. Int. J. Syst. Bacteriol. 32, 378–380.

Jarvis B D W, van Berkum P, Chen W X, Nour S M, Fernandez M P, Cleyet-Marel J C and Gillis M 1997 Transfer of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium mediterraneum*, and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. Int. J. Syst. Bacteriol. 47, 895–898.

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.

Jordan D C 1984 Family III. Rhizobiaceae Conn 1938. *In* Bergey's Manual of Systematic Bacteriology. Eds. N Krieg and R G Holt. pp. 234–235. 1st edition, Vol. 1 The Williams & Wilkins Co, Baltimore.

Jourand P, Giraud E, Béna G, Sy A, Willems A, Gillis M, Dreyfus B and de Lajudie P 2004 *Methylobacterium nodulans* sp. nov., for a group of aerobic, facultatively methylotrophic, legume root-nodule-forming and nitrogen-fixing bacteria. Int. J. Syst. Evol. Microbiol. 54, 2269–2273.

Judicial Commission 1970 Opinion 33. Conservation of the generic name *Agrobacterium* Conn 1942. Int. J. Syst. Bacteriol 20, 10.

Kersters K and De Ley J 1984 Genus III. *Agrobacterium* Cohn 1942. *In* Bergey's Manual of Systematic Bacteriology. Eds. N Krieg and R G Holt. pp. 244–254. 1st edition, Vol. 1 The Williams & Wilkins Co, Baltimore.

Kuykendall L D, Saxena B, Devine T E and Udell S E 1992 Genetic diversity in *Bradyrhizobium japonicum* Jordan 1982 and a proposal for *Bradyrhizobium elkanii* sp. nov. Can. J. Microbiol. 38, 501–505.

Kuykendall L D, Young J M, Martínez-Romero E, Kerr A and Sawada H 2005 Genus I. Rhizobium Frank 1889, 338^{AL}. *In* Bergey's Manual of Systematic Bacteriology, 2nd ed., Vol. 2, part C. Ed. G M Garrity. pp. 325–340. Springer, New York.

Lautrop H 1967 *Agrobacterium* spp. isolated from clinical specimens. Acta Pathol. Microbiol. Scand. 187, 63–64.

Lindström K 1989 *Rhizobium galegae*, a new species of legume root nodule bacteria. Int. J. Syst. Bacteriol. 39, 365–367.

Martínez-Romero E, Segovia L, Martins Mercante F, Franco A A, Graham P and Pardo M A 1991 *Rhizobium tropici*, a novel species nodulating *Phaseolus vulgaris* L. Beans and *Leucaena* sp. trees. Int. J. Syst. Bacteriol. 41, 417–426.

Moffet M L and Colwell R R 1968 Adansonian analysis of the *Rhizobiaceae*. J. Gen. Microbiol. 51, 245–266.

Molouba F, Lorquin J, Willems A, Hoste B, Giraud E, Dreyfus B, Gillis M, de Lajudie P and Masson-Boivin C 1999 Photosynthetic bradyrhizobia from *Aeschynomene* spp. are specific to stem-nodulated species and form a separate 16S ribosomal DNA restriction fragment length polymorphism group. Appl. Environ. Microbiol. 65, 3084–3094.

Moulin L, Munive A, Dreyfus B and Boivin-Masson C 2001 Nodulation of legumes by members of the β-subclass of Proteobacteria. Nature 411, 948–950.

Nick G, de Lajudie P, Eardly B D, Suomalainen S, Paulin L, Zhang X, Gillis M and Lindström K 1999 *Sinorhizobium arboris* sp. nov. and *Sinorhizobium kostiense* sp. nov., isolated from leguminous trees in Sudan and Kenya. Int. J. Syst. Bacteriol. 49, 1359–1368.

Nour S M, Cleyet-Marel J-C, Normand P and Fernandez M P 1995 Genomic heterogeneity of strains nodulating chickpeas (*Cicer arietinum* L.) and description of *Rhizobium mediterreaneum* sp. nov. Int. J. Syst. Bacteriol. 45, 640–648.

Nour S M, Fernandez M P, Normand P and Cleyet-Marel J-C 1994 *Rhizobium ciceri* sp. nov., consisting of strains that nodulate chickpeas (*Cicer arietinum* L.). Int. J. Syst. Bacteriol. 44, 511–522.

Ophel K and Kerr A 1990 *Agrobacterium vitis* sp. nov. for strains of *Agrobacterium* biovar 3 from grapevines. Int. J. Syst. Bacteriol. 40, 236–241.

Peng G X, Tan Z Y, Wang E T, Reinhold-Hurek B, Chen W F and Chen W X 2002 Identification of isolates from soybean nodules in Xinjiang region as *Sinorhizobium xinjiangense* and genetic differentiation of *S. xinjiangense* from *Sinorhizobium fredii*. Int. J. Syst. Evol. Microbiol. 52, 457–462.

Popoff M Y, Kersters K, Kiredjian M, Miras I and Coynault C 1984 Position taxonomique de souches de *Agrobacterium* d'origine hospitalière. Ann. Microbiol. (Inst. Pasteur) 135A, 427–442.

Quan Z-X, Bae H-S, Baek J-H, Chen W-F, Im W-T and Lee S-T 2005 *Rhizobium daejeonense* sp. nov. isolated from a cyanide treatment bioreactor. Int. J. Syst. Evol. Microbiol. 55, 2543–2549.

Rasolomampianina R, Bailly X, Fetiarison R, Rabevohitra R, Bena G, Ramaroson L, Raherimandimby M, Moulin L, De Lajudie P, Dreyfus B and Avarre J C 2005 Nitrogen-fixing nodules from rose wood legume trees (*Dalbergia* spp.) endemic to Madagascar host seven different genera belonging to alpha- and beta-Proteobacteria. Mol. Ecol. 14, 4135–4146.

Riley P S and Weaver R E 1977 Comparison of thirty-seven strains of Vd-3 bacteria with *Agrobacterium radiobacter*: Morphological and physiological observations. J. Clin. Microbiol. 5, 172–177.

Rivas R, Willems A, Palomo J L, García-Benavides P, Mateos P F, Martínez-Molina E, Gillis M and Velázquez E 2004 *Bradyrhizobium betae* sp. nov., isolated from roots of *Beta vulgaris* affected by tumour-like deformations. Int. J. Syst. Evol. Microbiol. 54, 1271–1275.

Rivas R, Willems A, Subba-Rao N, Mateos P F, Dazzo F B, Martínez-Molina E, Gillis M and Velàzquez E 2003 Description of *Devosia neptunia* sp. nov. that nodulates and fixes nitrogen in symbiosis with *Neptunia natans*, an aquatic legume from India. Syst. Appl. Microbiol. 26, 47–53.

Rome S, Fernandez M P, Brunel B, Normand P and Cleyet-Marel J-C 1996 *Sinorhizobium medicae* sp. nov., isolated from annual *Medicago* spp. Int. J. Syst. Bacteriol. 46, 972–980.

Sawada H, Ieki H, Oyaizu H and Matsumoto S 1993 Proposal for rejection of *Agrobacterium tumefaciens* and revised descriptions for the genus *Agrobacterium* and for *Agrobacterium radiobacter* and *Agrobacterium rhizogenes*. Int. J. Syst. Bacteriol. 43, 694–702.

Scholla M H and Elkan G H 1984 *Rhizobium fredii* sp. nov., a fast-growing species that effectively nodulates soybean. Int. J. Syst. Bacteriol. 34, 484–486.

Schroeter J 1886 Schizomycetes. *In* Kryptogamenflora von Sclesien, Bd. 3, Heft 3, Pilze. Ed. Cohn. pp. 1–814. J U Kern's Verlag, Breslau.

Segovia L, Young J P W and Martínez-Romero E 1993 Reclassification of American *Rhizobium leguminosarum* biovar *phaseoli* type I strains as *Rhizobium etli*. Int. J. Syst. Bacteriol. 43, 374–377.

Skerman V B D, McGowan V and Sneath P H A 1980 Approved lists of bacterial names. Int. J. Syst. Bacteriol. 30, 225–420.

Sprent J I 1995 Legume trees and shrubs in the tropics: N_2 fixation in prespective. Soil Biol. Biochem. 27, 401–407.

Squartini A, Struffi P, Döring H, Selenska-Pobell S, Tola E, Giacomini A, Vendramin E, Velàzquez E, Mateos P F, Martínez-Molina E, Dazzo F B, Casella S and Nuti M P 2002 *Rhizobium sullae* sp. nov. (formerly '*Rhizobium hedysari*'), the root-nodule microsymbiont of *Hedysarum coronarium* L. Int. J. Syst. Evol. Microbiol. 52, 1267–1276.

Starr M P and Weiss J E 1943 Growth of phytopathogenic bacteria in a synthetic asparagine medium. Phytopathology 33, 314–318.

Sy A, Giraud E, Jourand P, Garcia N, Willems A, de Lajudie P, Prin Y, Neyra M, Gillis M, Boivin-Masson C and Dreyfus B

2001 Methylotrophic *Methylobacterium* bacteria nodulate and fix nitrogen in symbiosis with legumes. J. Bacteriol. 183, 214–220.

Tan Z Y, Kan F L, Peng G X, Wang E T, Reinholdt-Hurek B and Chen W X 2001 *Rhizobium yanglingense* sp. nov., isolated from arid and semi-arid regions in China. Int. J. Syst. Evol. Microbiol. 51, 909–914.

'tMannetje L 1967 A re-examination of the taxonomy of the genus *Rhizobium* and related genera using numerical analysis. Antonie van Leeuwenhoek J. Microbiol. Serol. 33, 477–491.

Toledo I, Lloret L and Martínez-Romero E 2003 *Sinorhizobium americanus* sp. nov., a new *Sinorhizobium* species nodulating native *Acacia* spp. in Mexico. Syst. Appl. Microbiol. 26, 54–64.

Trujillo M E, Willems A, Abril A, Planchuelo A-M, Rivas R, Ludeña D, Mateos P F, Martínez-Molina E and Velázquez E 2005 Nodulation of *Lupinus* by strains of *Ochrobactrum lupini* sp. nov. Appl. Environ. Microbiol. 71, 1318–1327.

Trüper H G and De'Clari L 1997 Taxonomic note: necessary correction of specific epithets formed as substantives (nouns) "in apposition". Int. J. Syst. Bacteriol. 47, 908–909.

Valverde A, Velázquez E, Fernández-Santos F, Vizcaíno N, Rivas R, Gillis M, Mateos P F, Martínez-Molina E, Igual J M and Willems 2005 *Phyllobacterium trifolii* sp. nov. nodulating *Trifolium* and *Lupinus* in Spanish soils. Int. J. Syst. Evol. Microbiol. 55, 1985–1989.

van Berkum P, Beyene D, Bao G, Campbell T A and Eardly B D 1998 *Rhizobium mongolense* sp. nov. is one of three rhizobial genotypes identified which nodulate and form nitrogen-fixing symbioses with *Medicago ruthenica* [(L.) Ledebour]. Int. J. Syst. Bacteriol. 48, 13–22.

van Berkum P and Eardly B D 2002 The aquatic budding bacterium *Blastobacter denitrificans* is a nitrogen-fixing symbiont of *Aeschynomene indica*. Appl. Environ. Microbiol. 68, 1132–1136.

van Berkum P, Leibold J M and Eardly B D 2006 Proposal for combining *Bradyrhizobium* spp. (*Aeschynomene indica*) with *Blastobacter denitrificans* and to transfer *Blastobacter denitrificans* (Hirsch and Muller, 1985) to the genus *Bradyrhizobium* as *Bradyrhizobium denitrificans* (comb. nov.). Syst. Appl Microbiol 29, 207–215.

Vandamme P, Goris J, Chen W-M, De Vos P and Willems A 2003 *Burkholderia tuberum* sp. nov. and *Burkholderia phymatum* sp. nov., nodulate the roots of tropical legumes. Syst. Appl. Microbiol. 25, 507–512.

Vandamme P, Pot B, Gillis M, De Vos P, Kersters K and Swings J 1996 Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol. Rev. 60, 407–438.

Velázquez E, Igual J M, Willems A, Fernández M P, Muñoz E, Mateos P F, Abril A, Toro N, Normand P, Cervantes E, Gillis M and Martínez-Molina E 2001 Description of *Mesorhizobium chacoense* sp. nov., a novel species that nodulates *Prosopis alba* in the Chaco Arido region (Argentina). Int. J. Syst. Evol. Microbiol. 51, 1011–1021.

Vermis K, Coenye T, LiPuma J J, Mahenthiralingam E, Nelis H J and Vandamme P 2004 Proposal to accommodate *Burkholderia cepacia* genomovar VI as *Burkholderia dolosa* sp. nov. Int. J. Syst. Evol. Microbiol. 54, 689–691.

Vincent J M and Humphrey B A 1970 Taxonomically significant group antigens in *Rhizobium*. J. Gen. Microbiol. 63, 379–382.

Vinuesa P, León-Barrios M, Silva C, Willems A, Jarabo-Lorenzo A, Pérez-Galdona R, Werner D and Martínez-Romero E 2005 *Bradyrhizobium canariense* sp. nov., an acid-tolerant endosymbiont that nodulates endemic genistoid legumes (*Papilionoideae*: *Genisteae*) from the Canary Islands, along with *Bradyrhizobium japonicum* bv. *genistearum*, *Bradyrhizobium* genospecies alpha and *Bradyrhizobium* genospecies beta. Int. J. Syst. Evol. Microbiol. 55, 569–575.

Wang E T, van Berkum P, Beyene D, Sui X H, Dorado O, Chen W X and Martínez-Romero E 1998 *Rhizobium huautlense* sp. nov., a symbiont of *Sesbania herbacea* that has a close phylogenetic relationship with *Rhizobium galegae*. Int. J. Syst. Bacteriol. 48, 687–699.

Wang E, Tan Z Y, Willems A, Fernández-López M, Reinhold-Hurek B and Martínez-Romero E 2002 *Sinorhizobium morelense*, sp. nov. a *Leucaena leucocephala*-associated bacterium that is highly resistant to multiple antibiotics. Int. J. Syst. Evol. Microbiol. 52, 1687–1693.

Wang E T, van Berkum P, Sui X H, Beyene D, Chen W X and Martínez-Romero E 1999 Diversity of rhizobia associated with *Amorpha fruticosa* isolated from Chinese soils and descritpion of *Mesorhizobium amorphae* sp. nov. Int. J. Syst. Bacteriol. 49, 51–65.

Wei G H, Tan Z Y, Zhu M E, Wang E T, Han S Z and Chen W X 2003 Characterization of rhizobia isolated from legume species within the genera *Astragalus* and *Lespedeza* grown in the Loess Plateau of China and description of *Rhizobium loessense* sp. nov. Int. J. Syst. Evol. Microbiol. 53, 1575–1583.

Wei G H, Wang E T, Tan Z Y, Zhu M E and Chen W X 2002 *Rhizobium* indigoferae sp. nov. and *Sinorhizobium kummerowiae* sp. nov., respectively isolated from *Indigofera* spp. and *Kummerowia stipulaceae*. Int. J. Syst. Evol. Microbiol. 52, 2231–2239.

Willems A, Coopman R and Gillis M 2001a Phylogenetic and DNA: DNA hybridization analyses of *Bradyrhizobium* species. Int. J. Syst. Evol. Microbiol. 51, 111–117.

Willems A, Coopman R and Gillis M 2001b Comparison of sequence analysis of 16S–23S spacer regions, AFLP analysis and DNA–DNA hybridizations in *Bradyrhizobium*. Int. J. Syst. Evol. Microbiol. 51, 623–632.

Willems A, Doignon-Bourcier F, Goris J, Coopman R, de Lajudie P, De Vos P and Gillis M 2001c DNA–DNA hybridization study of *Bradyrhizobium* strains. Int. J. Syst. Evol. Microbiol. 51, 1315–1322.

Willems A, Fernández-López M, Muñoz-Adelantado E, Goris J, De Vos P, Martínez-Romero E, Toro N and Gillis M 2003 Description of New *Ensifer* strains from nodules and proposal to transfer *Ensifer adhaerens* Cassida 1982 to *Sinorhizobium* as *Sinorhizobium adhaerens* comb. nov. Request for an Opinion. Int. J. Syst. Evol. Microbiol. 53, 1207–1217.

Wilson J K 1944 Over five hundred reasons for abandoning the cross inoculation groups of legumes. Soil Sci. 58, 61–69.

Xu M L, Ge C, Cui Z, Li J and Fan H 1995 *Bradyrhizobium liaoningense* sp. nov., isolated from the root nodules of soybeans. Int. J. Syst. Bacteriol. 45, 706–711.

Yao Z Y, Kan F L, Wang E T, Wei G H and Chen W X 2002 Characterization of rhizobia that nodulate legume species within the genus Lespedeza and description of *Bradyrhizobium yuanmingense* sp. nov. Int. J. Syst. Evol. Microbiol. 52, 2219–2230.

Young J M 2003 The genus name *Ensifer* Casida 1982 takes priority over *Sinorhizobium* Chen et al. 1988, and *Sinorhizobium morelense* Wang et al. 2002 is a later synonym of *Ensifer adhaerens* Casida 1982. Is the combination '*Sinorhizobium adhaerens*' (Casida 1982) Willems et al. 2003 legitimate? Request for an Opinion. Int. J. Syst. Evol. Microbiol. 53, 2107–2110.

Young J M 2004 Renaming of *Agrobacterium larrymoorei* Bouzar and Jones 2001 as *Rhizobium larrymoorei* (Bouzar and Jones 2001) comb. nov. Int. J. Syst. Evol. Microbiol. 54, 149.

Young J M, Kuykendall L D, Martínez-Romero E, Kerr A and Sawada H 2001 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*. Int. J. Syst. Evol. Microbiol. 51, 89–103.

Young J M, Pennycook S R and Watson D R W 2006 Proposal that *Agrobacterium radiobacter* has priority over *Agrobacterium tumefaciens*. Request for an Opinion. Int. J. Syst. Evol. Microbiol. 56, 491–493.

Zakhia F, Jeder H, Willems A, Dreyfus B, de Lajudie P 2006 Diverse bacteria associated with root nodules of spontaneous legumes in Tunisia and first report for nifH-like gene within the genera *Microbacterium* and *Starkeya*. Microb. Ecol. 51, 375–393.