

CURRENT RHIZOBIUM TAXONOMY

Abstract

Classification of rhizobia knows constant change. Recent studies have shown the existence of a great diversity among nitrogen-fixing bacteria isolated from different legumes. Currently, 16 genus, including 98 species of rhizobia have been described. The genus *Rhizobium*, *Sinorhizobium*, *Allorhizobium*, *Mezorhizobium*, *Phyllobacterium*, *Ochrobacterium*, *Azorhizobium*, *Methylobacterium*, *Bradyrhizobium*, *Blastobacter*, *Devosia* (Class of α -proteobacteria), Burkholderia and Ralstonia (Class of β -proteobacteria) and some γ -proteobacteria [1], form the set of the bacteria known as legume's symbionts. There is certainly much to discover, since only 0.5% of known legumes were until now identified specifically for symbiotic relationship [2]. The discovery of new symbionts associated with legumes is expected to create deep changes in the taxonomy of the Rhizobiaceae. A literature review of the currently recognized classification of Rhizobia is presented in this paper.

Keywords: Rhizobia, Legume, Proteobacteria, Taxonomy, Classification.

1. Introduction

Rhizobia are soil bacteria able to form nodules and establish a symbiosis with the roots or the stems of leguminous plants. During the process of symbiosis, rhizobia reduce atmospheric nitrogen into a form directly assimilated by plants (ammonium).

The ability of rhizobia to fix nitrogen significantly reduced the use of chemical fertilizers in agriculture. In fact, nearly 90% of the nitrogen plant needs are well satisfied and the soil is enriched with nitrogen that will be used by subsequent crops, with no need to add chemical fertilizers. In general, this symbiosis has several advantages including improved agricultural productivity, maintenance and restoration of soil fertility, safety of expensive manures in currency and in energy and limitation of groundwater's pollution by nitrates; playing therefore a significant ecological and economical function.

31 The study of the rhizobial diversity is a valuable biological resource and attempts to find
32 bacterial strains with interesting features to maximize the legumes productivity [3]. Such
33 studies allow not only to find new strains of rhizobia, but they also support research efforts
34 aiming at choosing and selecting efficient combinations of Rhizobium - Legume associations.

35 Because of their ecological and economical importance, the diversity and taxonomy of these
36 microorganisms have been extensively studied over the last thirty years. From one genus
37 including four species in 1981, the classification now includes at least 16 genus comprising
38 more than 98 species and this number continue to increase [1].

39 Zakhia et al. (2004) proposed the term Bacteria Nodulating Legumes (BNL) to avoid
40 confusion between the general term of Rhizobium and genus name. Currently, all BNL
41 described until now belong to the Proteobacteria class. The majority of them belong to genus
42 of the α -Proteobacteria class, called *Rhizobium*, *Mesorhizobium*, *Sinorhizobium*,
43 *Allorhizobium*, *Bradyrhizobium*, *Azorhizobium*. In addition, new types were found in the α -
44 Proteobacteria class, namely *Methylobacterium*, *Devosia*, *Blastobacter*, *Ensifer*,
45 *Ochrobactrum* and *Phyllobacterium* [5].

46 The BNL were also found in the β -Proteobacteria class, such as *Burkholderia*, *Ralstonia* [6]
47 and *Herbaspirillum* [7; 8; 9; 10]. Besides, Benhizia et al., (2004) reported the association
48 between legumes of the genus *Hedysarum* and strains belonging to the γ -Proteobacteria class,
49 namely *Pantoea agglomerans*, *Enterobacter Kobei*, *Enterobacter cloacae*, *Leclercia*
50 *adecarboxylata*, *Vulneris coli* and *Pseudomonas* sp. although their nodulation capacity is not
51 yet clearly demonstrated [11].

52 More than 19.700 legume species grow around the world, but only a few microsymbionts
53 have been studied [12]. The increase in advanced study of legume's new species in different
54 geographical regions opens new perspectives to isolate and to characterize more species of
55 *Rhizobium*.

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57 2. Phylogeny of rhizobia

58 The comparison of data obtained from bacterial strains or species with different molecular
59 approaches allows the identification of groups of taxa. Different taxa obtained can be
60 compared and used for tracing lines of descent commonly recognized under the name of
61 phylogenetic tree (Figure 1). Thus, the phylogeny can be defined as the evolutionary history

62 that reveals the existing relationships between a group of organisms and an ancestor located at
63 a higher taxonomic level.

64 Nowadays, use of 16S rDNA sequence is the main tool in the study of microbial phylogeny.
65 However, new molecular techniques have been recently adopted. The most commonly used
66 techniques are: FT-IR technique (Fourier-transform infrared spectroscopy), which allows to
67 study the diversity of bacteria at an intraspecific level [13], the technique of typing by PCR
68 target (target PCR fingerprinting) [14] and especially the technique of DNA chips commonly
69 known as DNA microarrays. In this last technique, the conception of the biochips enabled to
70 detect simultaneously thousands of sequences or even to cover the entire genome of an
71 organism [15]. By the same technique, Rüberg et al. (2003) have analyzed the entire genome
72 of *Sinorhizobium meliloti* [16]. All these techniques can reveal more information about the
73 diversity of rhizobia and can therefore provide more data available for their phylogenic study.

74 Thus, modern bacterial taxonomy is based on the integration of all phenotypic, genotypic and
75 phylogenetic data for a more stable classification [17, 18]. The following paragraphs will
76 discuss the current classification of rhizobia with this new approach.

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78 **3. Polyphasic taxonomy**

79 Originally based on phenotypic criteria, the application of polyphasic terminology has been
80 reviewed by the revolution of different molecular techniques of which mainly the PCR or
81 Polymerase Chain Reaction [19]. Thus, the standard principles were proposed in 1991, for the
82 description of new genus or species on the basis of numerical analysis which is the synthesis
83 of the results of different phenotypic and molecular tests [20]. This new definition of the
84 polyphasic approach allowed a proper systematic study of major bacterial groups, an
85 assessment of taxonomic resolution of different techniques of diversity study [18] (Figure 2)
86 and an establishment of comparable databases between different applied microbiology
87 laboratories [21].

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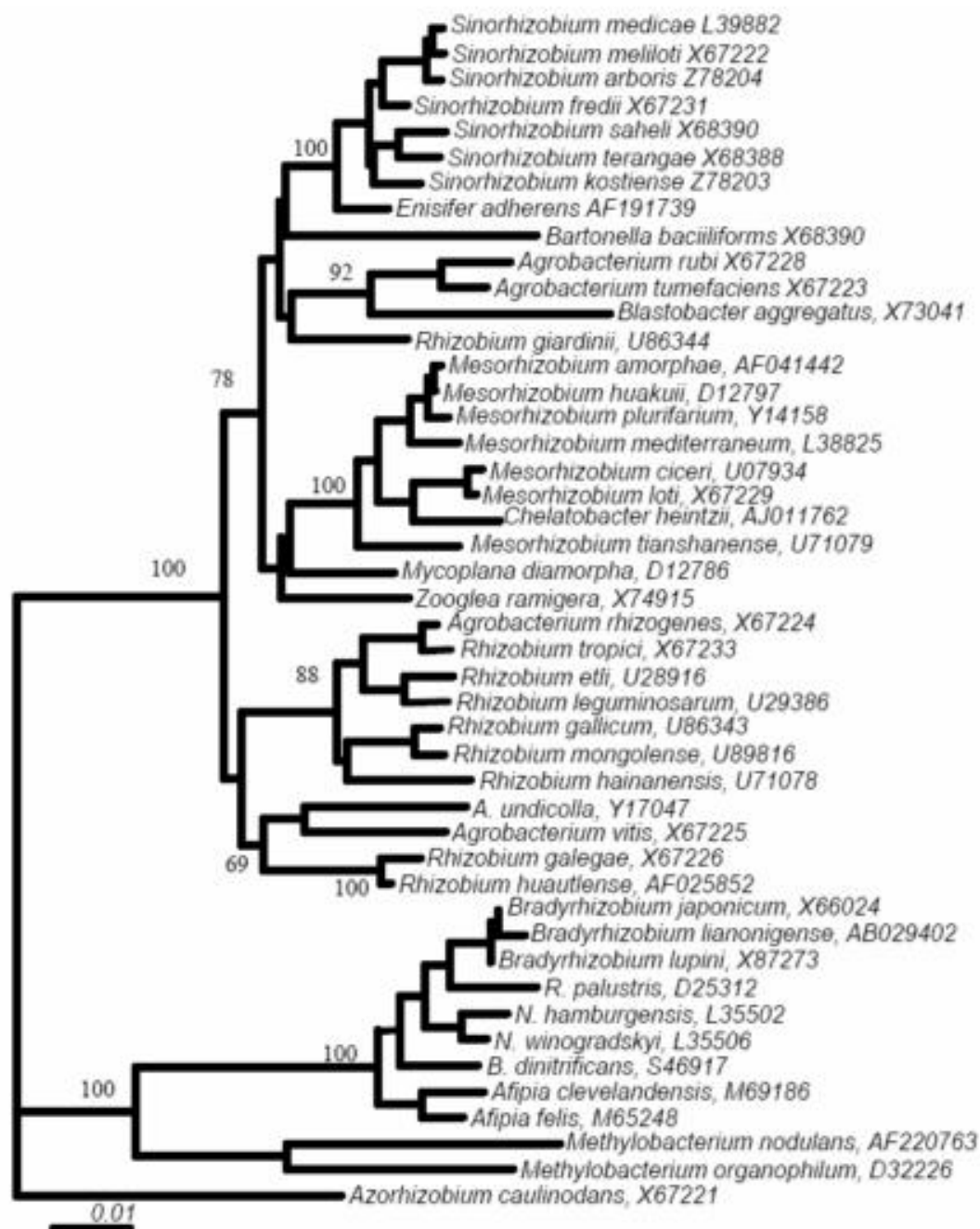


Figure 1: Phylogenetic tree of rhizobia and related species built by the neighbor-joining method from the complete sequence of 16S rDNA [22].

	Famille	Genre	Espèce	Souche
Marquage aux sondes d'ADN				
Séquençage de l'ADN				
Analyse phénotypique (classique, API, etc.)				
Séquençage de l'ARN				
Hybridation ADN : ARNr				
Analyse chimiotaxonomique (polyamines, quinones, etc.)				
FAME				
Hybridation ADN : ADN				
PCR/ARNr				
% en G+C				
RFLP – ARDRA				
RAPD – DAF – APPCR				
AFLP				
Rep-PCR				
Analyse des protéines cellulaires totales				
Polymorphisme des isoenzymes				
Phage typing				
MLEE				
Sérologie				

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95 **Figure 2:** Taxonomic resolution of the various techniques used to study the diversity of

96 rhizobia [18; 23]

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98 **4. Current classification of rhizobia**

99 The first classifications were based on cross-inoculation tests between rhizobia and host
100 plants for their identification. The host plant was not the only criteria taken into account for
101 the classification of rhizobia which species were classified into two genus: the genus
102 *Rhizobium* corresponding to strains with fast growth and genus *Bradyrhizobium* for slow-
103 growing strains, based on their generation time and rate of growth on culture medium [24].

104 However, discordant observations between the notion of bacterial growth speed and the host
105 range showed a lot of doubt on the validity of this classification. This makes place otherwise
106 to comparative methods such as the serology, the coefficient of Chargaff, RNA / DNA or
107 DNA / DNA hybridization analysis of plasmids etc... This period marked the beginning of a
108 new taxonomy based on the comparison of results from different phenotypic and biochemical
109 analysis for the identification of symbiotic bacteria [25]. Since then, the isolation of rhizobia
110 from an increasing number of plant species in the world and their characterization by modern
111 polyphasic taxonomy has led to the description of other new genera and species.

112 The current literature describes 98 species grouped in 11 genus belonging to the subclass α -
113 Proteobacteria and 3 genus belonging to the order of Burkholderiales in subclass β -
114 Proteobacteria [17; 16; 26; 27], one genus belonging to the order Enterobacteriales in the
115 subclass γ -Proteobacteria, and finally, one genus of the order Pseudomonadales in the same
116 subclass [11] (Table 1).

117 In summary, on the basis of the DNA ribosomal 16S sequence, the legume's symbionts
118 belong to three main distinct phylogenetic subclasses (Figure 3).

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Table 1: Current classification of different species of rhizobia [28]

Genus	Species	Host plant	References
Class :Alphaproteobacteria			
Order :Rhizobiales			
Family :Rhizobiaceae			
<u>Genus: Rhizobium</u>			
	<i>R. leguminosarum</i>		[29]
	<i>biovar viciae</i>	<i>Pisum, viciae, Lens, Lathyrus</i>	[29; 30]
	<i>biovar trifolii</i>	<i>Trifolium pratense</i>	[29; 30]
	<i>biovar phaseoli</i>	<i>Phaseolus vulgaris</i>	[29; 30]
	<i>R. galegae</i>	<i>Galega, Leucaena</i>	[31]
	<i>biovar officinalis</i>	<i>Galega orientalis</i>	
	<i>biovar orientalis</i>	<i>Galega officinalis</i>	
	<i>R. tropici</i> type IIA et IIB	<i>Phaseolus, Medicago, Macroptilium</i>	[32]
	<i>R. etli</i>	<i>Phaseolus,</i>	[33]
	<i>biovar mimosae</i>	<i>Mimosa affinis</i>	[34]
	<i>R. gallicum</i>	<i>Phaseolus vulgaris</i>	[35]
	<i>biovar phaseoli</i>	<i>Phaseolus vulgaris</i>	
	<i>biovar gallicum</i>		
	<i>R. giardinii</i>	<i>Phaseolus vulgaris</i>	[35]
	<i>biovar phaseoli</i>		
	<i>biovar giardinii</i>		
	<i>R. hainanensis</i>	<i>Desmodium sinuatum, Centrosema, etc.</i>	[36]
	<i>R. huautlense</i>	<i>Sesbania herbacea</i>	[37]
	<i>R. mongolense</i>	<i>Medicago ruthenica, Phaseolus</i>	[38]
	<i>R. yanglingense</i>	-	[39]
	<i>R. larrymoorei</i>	<i>Ficus benjamina</i>	[40]
	<i>R. indigoferae</i>	<i>Indigofera spp.</i>	[41]
	<i>R. sullae</i>	<i>Hedysarum</i>	[42]
	<i>R. loessense</i>	<i>Astragalus, Lespedeza</i>	[43]
	<i>R. cellulosilyticum</i>	<i>Populus alba</i>	[44]
	<i>R. miluonense</i>	<i>Lespedeza</i>	[45]
	<i>R. multihospitium</i>		[46]
	<i>R. oryzae</i>	<i>Oryza alta</i>	[47]
	<i>R. pisi</i>	<i>Pisum sativum</i>	[48]
	<i>R. mesosinicum</i>	<i>Albizia, Kummerowia Dalbergia</i>	[49]
	<i>R. alamii</i>	<i>Arabidopsis thaliana</i>	[50]
	<i>R. alkalisolii</i>	<i>Caragana intermedia</i>	[51]
	<i>R. tibeticum</i>	<i>Trigonella archiducis-nicolai</i>	[52]
	<i>R. tubonense</i>	<i>Oxytropis glabra</i>	[2]
	<i>R. halophytocola</i>		[52]
<u>Genus: Sinorhizobium</u>			
	<i>S. meliloti</i>	<i>Medicago, Melilotus, Trigonella</i>	[53]

<i>S. fredii</i>		
<i>biovar fredii</i>	<i>Glycine, Vigna, Cajanus</i>	[54]
<i>biovar siensis</i>	<i>Glycine</i>	[36]
<i>S. saheli</i>	<i>Acacia, Prosopis, Neptunia, Leucaena</i>	[55]
<i>S. terangae</i>		[55]
<i>biovar acaciae</i>	<i>Acacia</i>	[56]
<i>biovar sesbania</i>	<i>Sesbania</i>	[57]
<i>S. medicae</i>	<i>Medicago truncatula, Melilotus</i>	[58]
<i>S. arboris</i>	<i>Acacia, Prosopis</i>	[59]
<i>S. kostiense</i>	<i>Acacia, Prosopis</i>	[59]
<i>S. xingianense</i>	<i>Glycine max</i>	[60]
<i>S. morelense</i>	<i>Leucaena leucocephala</i>	[61]
<i>S. kummerowiae</i>	<i>Kummerowiae stipulaceae</i>	[41]
<i>S. americanum</i>	<i>Acacia</i>	[62]
<i>E. mexicanus</i>	<i>Acacia angustissima</i>	[63]
<i>E. numidicus</i>		[64]
<u>Genus: Allorhizobium</u>		
<i>A. undicola</i>	<i>Neptunia natans</i>	[65]
Family : Phyllobacteriaceae		
<u>Genus : Mesorhizobium</u>		
<i>M. loti</i>	<i>Lotus, Cicer, Anthyllis, Astragalus, etc.</i>	[66]
<i>M. huakuui</i>	<i>Astragalus sinicus</i>	[67]
<i>M. ciceri</i>	<i>Cicer arietinum</i>	[68]
<i>M. tianshanense</i>	<i>Glycyrrhiza pallidiflora</i>	[60]
<i>M. mediterraneum</i>	<i>Cicer arietinum</i>	[69]
<i>M. plurifarium</i>	<i>Acacia, Chamaecrista, Leucaena, Prosopis,</i>	[70]
<i>M. amorphae</i>	<i>Amorpha fruticosa</i>	[71]
<i>M. chacoense</i>	<i>Prosopis alba</i>	[72]
<i>M. sesptentrionale</i>	<i>Astragalus adsurgens</i>	[73]
<i>M. temperatum</i>	<i>Astragalus adsurgens</i>	[73]
<i>M. thiogangeticum</i>	<i>soil (India, Clitoria ternatea)</i>	[73]
<i>M. albiziae</i>	<i>Albizia kalkora</i>	[73]
<i>M. caraganae</i>	<i>Caragana spp.</i>	[61]
<i>M. gobiense</i>		[46]
<i>M. tarimense</i>		[46]
<i>M. australicum</i>	<i>Biserrula pelecinus L.</i>	[74]
<i>M. opportunistum</i>	<i>Biserrula pelecinus L.</i>	[74]
<i>M. metallidurans</i>	<i>Anthyllis vulneraria</i>	[75]
<i>M. alhagi</i>	<i>Alhagi</i>	[76]
<i>M. camelthorni</i>	<i>Alhagi sparsifolia.</i>	[76]
<u>Genus : Phyllobacterium</u>		
<i>P. trifolii</i>	<i>Trifolium pratense</i>	[61]
<i>P. ifriqiyense</i>		[77]
<i>P. leguminum</i>		[77]
Family : Methylobacteriaceae		
<u>Genus : Methylobacterium</u>		

<i>M. nodulans</i>	<i>Crotalaria spp</i>	[78]
Family : Brucellaceae		
<u>Genus : Ochrobactrum sp.</u>		
<i>Ochrobactrum sp.</i>	<i>Acacia mangium</i>	[79; 80]
<i>Ochrobactrum lupini</i>	<i>Lupinus albus</i>	
Family: Hyphomicrobiaceae		
<u>Genus: Azorhizobium</u>		
<i>A. caulinodans</i>	<i>Sesbania rostrata</i>	[81]
<i>A. johannense</i>	<i>Sesbania virgata</i>	[81]
<i>Azorhizobium sp</i>	<i>Sesbania rostrata</i>	[81]
<u>Genus: Devosia</u>		
<i>Devosia neptuniae</i>	<i>Neptunia natans</i>	[82]
Family : Bradyrhizobiaceae		
<u>Genus : Bradyrhizobium</u>		
<i>Br. Japonicum</i>	<i>Glycine max, Glycine soja</i>	[30 ; 83]
<i>Br. Elkanii</i>	<i>Glycine max</i>	[84]
<i>Br. Liaoningensis</i>	<i>Glycine max</i>	[85]
<i>Br. Yuanmingense</i>	<i>Lespedeza</i>	[86]
<i>Br. Betae</i>	<i>Betae vulgaris</i>	[87]
<i>Br. Canariense</i>	<i>Genisteae et Loteae</i>	[88]
<i>Bradyrhizobium sp.</i>	<i>Acacia, Aeschynomene</i>	
<i>Br. iriomotense</i>	<i>Entada koshunensis</i>	[89]
<i>Br. jicamae</i>	<i>Pachyrhizus erosus</i>	[48]
<i>Br. lablabi</i>	<i>Lablab purpureus</i>	[89]
<i>Br. huanghuaihaiense</i>	<i>soybean</i>	[90]
<u>Genus : Blastobacter</u>		
<i>B. denitrificans</i>	<i>Aeschynomene</i>	[88]
Class : Beta Proeobacteria		
Order : Burkholderiales		
Family: Burkholderiaceae		
<u>Genus : Burkholderia</u>		[91]
<i>Burkholderia sp</i>	<i>Machaerium lunatum,</i>	
<i>B. caribensis</i>	<i>Aspalatus</i>	
<i>B. cepacia</i>	<i>Alysicarpus glumaceus</i>	[6]
<i>B.tuberum</i>	<i>Aspalatus carnosa</i>	
<i>B. phymatum</i>	<i>Aspalatus carnosa</i>	
<i>B.nodosa</i>	<i>Mimosa bimucronata</i>	[9]
<i>B. sabiae</i>	<i>Mimosa caesalpiniiifolia</i>	[9]
<u>Genus : Wautersia(Ralstonia)</u>		
<i>W. taiwanensis</i>	<i>Aspalatus carnosa</i>	
	<i>Mimosa spp</i>	[18]
<u>Genus : Herbaspirillum</u>		
<i>H. lusitanum</i>	<i>Hedysarum carnosum,</i>	
	<i>H. Spinosissimum,</i>	[26]
Class: Gamma-Proteobacteria		
Order : Enterobacteriales		
<i>Pantoea agglomerans</i>	<i>Hedysarum carnosum,</i>	[11]
<i>Enterobacter Kobei</i>	<i>Hedysarum carnosum</i>	[11]

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4.1. Subclass of α -Proteobacteria

4.1.1. *Rhizobium* /*Agrobacterium* branch

This branch is subdivided in four sub branches in the genus: *Rhizobium* [29], *Sinorhizobium* [36; 55], *Mesorhizobium* [93] and *Allorhizobium* [70]. These genus are grouped with:

- ✓ *Agrobacterium*, non-symbiotic bacterial genus involved in pathogenic interactions with plants;
- ✓ Bacteria ameliorative plant growth (PGPR: Plant growth-promoting rhizobacteria) as *Azospirillum*,
- ✓ Human and animal pathogens including *Brucella*, *Ochrobactrum*, *Bartonella* or soil bacteria as *Mycoplana*.

Despite the phenotypic differences, phylogenetic proximity of the 16S rDNA between the genus *Rhizobium*, *Agrobacterium* and *Allorhizobium*, allowed us to suggest their grouping in the genus *Rhizobium* [93].

The first sub-branch corresponds to genus *Rhizobium* and includes *R. leguminosarum*, *R. tropici* [32], *R. etli* [94], *R. gallicum* [35], *R. mongolense* [38] and *Agrobacterium biovar 2*. The members of this genus are short Gram-negative rods, of a 0.5 to 0.9 μm width and a 1.2 to 3 μm length, often with an uncolored region due to the presence of polymer β -hydroxybutyrate (PHB). Rhizobia do not form endospores but are motile with polar or peritrichous flagella (4 to 6), aerobic, chemo-organotrophs. They become pleomorphic under adverse conditions [95].

The second sub-branch corresponds to genus *Sinorhizobium* and includes the species *S. fredii* and *S. xinjiangensis* [36], *S. meliloti*, *S. teranga* and *S. sahelense* [55; 96], *S. medicae* [58], *S. kostiense* and *S. Arboris* [59]. Rods of this genus are 0.5 to 0.9 μm wide and 1.2 to 3 μm length, motile by polar flagellum or some peritrichous flagella, Gram-negative, aerobic accumulating PHB. Optimal growth of this genus is at 25-30°C (10-35°C) and at pH 6-8 (5-10.5) and tolerates 10 g/l of NaCl [28].

The third sub-branch corresponds to Type *Mesorhizobium* [93], which contains *M. loti* [66], *M. huakuii* [67], *M. ciceri* [68], *M. mediterraneum* [69], *M. tianshanense* [60], *M. plurifarum* [70] and *M. amorphae* [97]. They are Gram-negative rods, motile by polar or subpolar flagellum, aerobic, accumulating PHB. They assimilate glucose, rhamnose, and sucrose with acid production [28].

The fourth sub-branch includes *Agrobacterium biovar 1*, *Agrobacterium rubi*, *Agrobacterium vitis*, also *R. galegae* [31], *R. giardinii* [35], *R. huautlense* [37] and *Allorhizobium undicola* [98]. The members of this genus are rods of 0.6-1 x 1.5-3 μm , motile by one to six

peritrichous flagella, strict aerobic, chemo-organotrophic but not hydrolytic. They produce acid from sugars and abundant mucus of polysaccharide sugars [28].

In 2001, Young et al. had revised taxonomic data and showed that the discriminatory phenotypic characteristics to distinguish between these types are not convincing, and that the phylogenetic relationships between genera deduced from the comparative analysis of 16S rDNA sequences differ depending on the chosen algorithm and more especially on the chosen sequences. Therefore, despite phenotypic differences, these authors suggested to combine the three genera *Rhizobium*, *Agrobacterium* and *Allorhizobium* in a single genre: *Rhizobium*, according to their phylogenetic proximity of the 16S rDNA.

4.1.2. Azorhizobium branch

This branch regroups only one genus and only one species of symbiotic bacterium: *Azorhizobium caulinodans*. They are short rods very similar to *Rhizobium*, which present a polar and peritrichous ciliation but produce an alkaline reaction on glucose as *Bradyrhizobium*. Only this sugar is used. Strains also degrade fatty acids, organic acid and alcohols and are the only ones to use alcohols. Their growth is intermediate between the two previous genus, Generation time = 7-9 h. No strain is denitrifying. They grow up to 43 °C. They have only the dihydrolase arginine and lysine decarboxylase. They do not use mannitol, conventional substrate of the other two genera, but lactate gives good growth yields. They seem more sensitive to antibiotics [28]. In addition, it doesn't only form nodules on root parts but also on aerial parts of *Sesbania rostrata* [81]. A second species *Azorhizobium johannae* was proposed [82]. However, this species is characterized by a low level of DNA / DNA hybridization compared to the typic strain of the genus.

The analysis of the 16S rDNA sequences showed that the typic strain is mixed with species of the genus *Xanthobacter* and *Aquabacter*. The combination of these last two genus with *Azorhizobium* was investigated without being suggested because of the great dissimilarity of phenotypic traits [99; 100].

4.1.3. Bradyrhizobium branch

It has been defined to include all slow-growing rhizobia [30]. These are rods with a single polar or subpolar flagellum and a generation time of 10 - 12 h. The colonies diameter do not exceed 1 mm in agar + mannitol medium. These symbiotic bacteria use many sugars and organic acids but prefer pentoses, with production of polysaccharidic mucus. Some species can grow as chimolithotrophic in the presence of H₂, CO₂ and low content in O₂ due to the

presence of an hydrogenase. Some strains can fix N₂ in vitro. It's more resistant to antibiotics than *Rhizobium* [28].

This branch has included, for a long time, a single species: *Bradyrhizobium japonicum* that regrouped all strains nodulating the soybean (*Glycine max*). Very heterogeneous, it was then divided into three groups (I, Ia and II), based on homologies obtained by DNA/DNA hybridization [101]. A new species: *Bradyrhizobium elkanii* was created in the Group II, which differs from the species *Bradyrhizobium japonicum* [84]. For other very slow growing strains, isolated from nodules of soybean *Glycine max* and *Glycine soya* in China, *Bradyrhizobium liaoningense* species has been proposed [85]. The *Bradyrhizobium* genus includes strains for which the taxonomic position is not clearly defined at the species level. The discrepancy between *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* is clear. It is more important than the divergence between *Bradyrhizobium* and non-symbiotic species as: *Afipia* (animal bacterial pathogens), *Nitrobacter* (nitrifying bacteria in the soil), *Rhodopseudomonas palustris* (photosynthetic bacteria) and *Blastobacter denitrificans* [91].

In recent years, the characterization of different strains of *Bradyrhizobium* by comparing several molecular techniques revealed the existence of 11 different genotypes, three of which correspond to known strains, while 8 genotypes are very distinct.

A new species *B. yuanmingene* has recently been isolated from the genus *Lespedeza* [86; 102]. In the same year, a strain of *Bradyrhizobium* nodulating a wild plant of the genus *Phaseolus* has been reported [103]. Other recognized species of this group are *Bradyrhizobium betae* [87] and *Bradyrhizobium canariense* [88].

4.1.4. Methylobacterium branch

The *Methylobacterium* genus [104] defines a group of 20 species, including *M. extorquens*, *M. mesophilicum*, *M. chloromethanicum* and *M. dichloromethanicum* [105]. Bacteria of this genus have the ability to grow on single-carbon compounds such as formate, formaldehyde, methylamine and methanol as only source of carbon and energy. These are chemo-organotrophic and optional methylotrophic, able to use multi-carbon compounds for growth. The cells of this genus are rod shaped (0.8 - 1.0 x 1.0 to 3.0 µm), most frequently isolated and occasionally in rosette. They are often branched or pleomorphic, especially for cultures in late stationary phase. They are strictly aerobic bacteria, Gram-negative, oxydase and catalase positive and have a typical pink pigmentation due to the presence of carotenoids [106]. *Methylobacterium* strains are commonly called "pink-pigmented facultative Methylophs" (PPFM). However, at least one case, *M. nodulans* is not pigmented [80]. They are mesophilic

and optimal temperature is between 25 and 30°C. All these strains are motile with a single polar flagellum. Often, cells contain inclusions of poly-β-hydroxybutyrate and sometimes inclusions of polyphosphates [107].

The *Methylobacterium* sp. strains are unable to oxidize the methane and doesn't possess any methane mono-oxygenase, except two cases. *Methylobacterium organophilum* XX was originally described as an optional methanotrophic, however, this feature seems to have been lost since. It was suggested that this was encoded by a plasmid that was lost because the strain has not been maintained under selective pressure with methane as a carbon source [108]. Otherwise, *M. populi*, a new methylotrophic bacterium that uses methane was isolated [109].

4.2. Subclass of β-Proteobacteria:

4.2.1. Burkholderia branch :

The *Burkholderia* genus has been proposed in 1992 by Yabuuchi and al. Its definition was based primarily on genomic considerations and on cellular lipid's composition. Regarding the main phenotypic characteristics, they are right bacilli, Gram negative, usually accumulating granules of poly-beta-hydroxybutyrate, motile with one or more polar flagella (*Burkholderia mallei* is however lacking flagella and stationary), strictly aerobic, catalase positive, oxidase variable depending on the species, can grow using as sole carbon source glucose, glycerol, inositol, galactose, sorbitol, mannose (unlike *Ralstonia* sp. that do not use this sugar) and mannitol. The different species of the genus can be differentiated by the results of auxanogrammes (137 substrates were used in the study of Gillis et al., 1995) [111].

This genus contains 39 species including rhizospheric bacteria, human pathogenic bacteria, as well as nitrogen fixing bacteria as *Burkholderia vietnamiensis* and *Burkholderia kururiensis*. Nevertheless, the genus also includes important species to the ecology of environment. In particular *Burkholderia xenovorans* (formerly known as *Pseudomonas cepacia*, then *Burkholderia cepacia* and *Burkholderia fungorum*) was studied for its faculties of deterioration of the organochlorine links in pesticides and polychlorobiphenyl (PCB).

Moreover, some species of *Burkholderia* have been studied for their ability to rehabilitate the chemical brownfields soil, while other species of the same genus present an interest for cultures due to their rizhospheric roles. By their resistance to antibiotics and the mortality they cause, the species *Burkholderia mallei* and *Burkholderia pseudomallei* can be classified as potential biological weapons.

Following the discoveries of Moulin et al. (2001) [6] and Chen et al. (2003) [7] showing the ability of bacteria from the subclass β-Proteobacteria (*Burkholderia* and *Ralstonia*) to induce

nodulation in legumes, bacteria of the subclass γ -Proteobacteria (*Pantoea agglomerans*, *Enterobacter Koei*, *Enterobacter cloacae*, *Leclercia adecarboxylata*, *Escherichia vulneris*, *Pseudomonas* sp.) were also isolated from legume nodules [11]. Based on these findings, it is not excluded to find other bacteria able to nodulate legumes, even outside the Proteobacteria phylum.

5. Conclusion

From one genus and 4 species in 1981, the rhizobia taxonomic studies have led to a total of 16 genera and 98 recognized species. This is in constant progress due to various studies in the world. Progress in taxonomy is also due to increasing numbers of effective techniques available in the characterization of bacteria, more accessible to laboratories.

So far only 23% of the total number of legumes species (between 16 500 and 19 700) were characterized for their microsymbionts. The majority (88%) of the studied legumes proved to be able of forming nodules. Specifically, tropical rhizobia are still poorly documented compared to what can be expected based on recent data, which show a great diversity in China, Brazil, Senegal, Sudan, Morocco.

In 2001, Sy et al. [79] reported that some rhizobia isolated from *Crotalaria* sp. in Senegal were phylogenetically separated from all known rhizobia. They are a new species of the genus *Methylobacterium*, for which the name of *M. nodulans* was proposed. These finding suggests that several other new groups of symbiotic leguminous plant's bacteria (genera, species) may emerge in the future.

6. References

1. Weir BS. NZ rhizobia. Submitted by Bevan Weir on 27 April, 2013. Available: <http://www.rhizobia.co.nz/>
2. El Akhal MR. Diversité des rhizobiums nodulant l'arachide (*Arachis hypogaea* L.) au Maroc et l'effet de la salinité sur le couple symbiotique. Thèse de doctorat de l'Université Abdelmalek Essaadi, Faculté des Sciences et Techniques- Tanger, Maroc; 2008. French.
3. Dai J, Liu X and Wang Y. Genetic diversity and phylogeny of rhizobia isolated from *Caragana microphylla* growing in desert soil in Ningxia, China. Genet. Mol. Res. 2012; 11 (3): 2683-2693.

- 301 4. Zakhia F, Jeder H, Domergue O, Willems A, Cleyet-Marel JC, Gillis M, Dreyfus B, and de
302 Lajudie P. Characterisation of wild legume nodulating bacteria (LNB) in the infra-arid zone
303 of Tunisia. Syst. Appl. Microbiol. 2004; pp 380-395.
- 304 5. N'zoué A, Domergue O, Moulin L, Avarre JC and de Lajudie P. Tropical Legume
305 Nodulating Bacteria. Mol Biol of Tropical Plants. 2006 ; 105-141.
- 306 6. Moulin L, Munive A, Dreyfus B, and Boivin-Masson C. Nodulation of legumes by
307 members of the β -sb class of Proteobacteria. Nature. N° 41. 2001; 948-950.
- 308 7. Chen WM, Moulin L, Bontemps C, Vandamme P, Bena G, Boivin-Masson C. Legume
309 symbiotic nitrogen fixation by beta-proteobacteria is widespread in nature. J Bact. 2003; 185,
310 7266-7272.
- 311 8. Willems A. The taxonomy of rhizobia: an overview. Plant Soil. 2006; 287: 3-14.
- 312 9. Chen WF, Guan SH, Zhao CT, Yan XR, et al. Different *Mesorhizobium* species associated
313 with *Caragana carry* similar symbiotic genes and have common host ranges. FEMS
314 Microbiol Lett. 2008; 283: 203-209.
- 315 10. Muresu R, Polone E, Sulas L, Baldan B, et al. Coexistence of predominantly non
316 culturable rhizobia with diverse, endophytic bacterial taxa within nodules of wild legumes.
317 FEMS Microbiol Ecol. 2008; 63: 383-400.
- 318 11. Benhizia Y, Goudjil H, Benguedouar A, Rosella M, Giacomini A, Squartini A. Gamma
319 proteobacteria can nodulate legumes of the genus *Hedysarum*. System Appl Microbial. 2004;
320 27: 462-468.
- 321 12. Allen EK. The Leguminosae, A Source Book of Characteristics, Uses, and Nodulation.
322 University of Wisconsin Press. 1981.
- 323 13. Oberreuter HJ, Chrzinski and S. Scherer. Intraspecific diversity of *Brevibacterium linens*,
324 *Corynebacterium glutamium* and *Rhodococcus erythropolis* based on partial 16S rDNA
325 sequence analysis and Fourier-transform infrared (FT-IR) spectroscopy. Microbiology. 2002;
326 148:1523-1532.
- 327 14. Perret X, and WJ Broughton. Rapid identification of *Rhizobium* strains by targeted PCR
328 fingerprinting. Plant & Soil. 2002; 204: 21-34.

- 329 15. Shalon D, Smith SJ and Brown PO. A DNA microarray system for analyzing complex
330 DNA samples using two-color fluorescent probe hybridization. *Genome Res.* 1996; 6: 639-
331 645.
- 332 16. Rüberg S, Tian ZX, Krol E, Linke B, Meyer F, Wang Y, Pühler A, Weidner S and Becker
333 A. Construction and validation of a *Sinorhizobium meliloti* whole genome DNA microarray:
334 genome-wide profiling of osmoadaptive gene expression. *J. Biotechnol.* 2003, 106, 2556.
- 335 17. Zakhia F, De Lajudie P. Taxonomy of rhizobia. *Agronomie.* 2001; 21: 569-576.
- 336 18. Vandamme P, Pot B, Gillis M, De Vos P, Kersters K and Swings J. Polyphasic taxonomy,
337 a consensus approach to bacterial systematics. *Microbiol. Rev.* 1996; 60: 407-438.
- 338 19. Mullis KB, Faloona FA. Specific synthesis of DNA I vitro via a polymerase catalysed
339 chain reaction. *Methods Enzymol.* 1987; 155: 335-350.
- 340 20. Graham PH, Sadowsky MJ, Kersters HH, Barnet YM, Bradley RS, Cooper JE, De Ley
341 DJ, Jarvis BDW, Roslycky EB, Strijdom BW and Young JPW. Proposed minimal standards
342 for the description of new genera and species of root-and stem-nodulating bacteria. *Int. J.*
343 *Syst. Bacteriol.* 1991; 41: 582-587.
- 344 21. Gillis MP, Vandamme P, De Vos JY, Swings Kersters K. Polyphasic taxonomy. 43-48. In
345 *Bergey's Manual of Systematic Bacteriology*, 2nd Edition, Volume I. Boone DR, Castenholz
346 RW, Garrity GM (eds.) 2001.
- 347 22. Terefework Z, Nick G, Suomalainen S, Paulin L and Lindström K. Phylogeny of
348 *Rhizobium galegae* with respect to other rhizobia and agrobacteria. *Int. J. Syst. Bacteriol.*
349 1998; 48: 349-356.
- 350 23. De Bruijn FJ, Mc Spadden-Gadener ME, Millcamp B, Rademaker JL, Ragatz W, Schultz
351 D, Struffi ML and Stoltzfus J. Molecular approaches in microbial ecology to assess genomic
352 diversity and stress induced gene expression in plantassociated diazotrophs. In *Nitrogen*
353 *Fixation: fundamentals and applications*, pp. 571-576. Edited by Elmerich C, Kondorosi A
354 and Newton WE. Kluwer. The Netherlands. 1997.
- 355 24. Vincent JM. *A Manual for the Practical Study of Root Nodule Bacteria.* IBP handbook,
356 no. 15. Blackwell Scientific Publications, Ltd., Oxford, England. 1970.

- 357 25. Saoudi M. Les bactéries nodulant les légumineuses (B.N.L) : Caractérisation des bactéries
358 associées aux nodules de la légumineuse *Astragalus armatus*. Mémoire de Magister.
359 Université Mentouri de Constantine. Faculté des Sciences de la Nature et de la Vie. Algérie.
360 2008. French.
- 361 26. Chen WM, Laevens S, Lee TM, Coenye T, De Vos P, Mergeay M, Vandamme P.
362 *Ralstonia taiwanensis* sp. nov., isolated from root nodules of *Mimosa* species and sputum of
363 a cystic fibrosis patient. P. Int. J. Syst. Evol. Microbiol. 2001; 51:1729-35.
- 364 27. Nogom A, Nakagawa Y, Sawada H, Tsukahara J, wakabayashi S, Uchiumi T, Nuntagij A,
365 kotepong S, Suzuki A, Higashi S and Abe M. A novel symbiotic nitrogen-fixing member of
366 the *Ochrobacterium* clade isolated from root nodules of *Acacia mangium*. J. gen. Appl.
367 Microbiol. 2004; 50, 1727.
- 368 28. Garcia JL, Cayol JL et Roger P. Taxonomie des Procaryotes. Rhizobiales,
369 Ordre VI des Alpha-proteobacteria, Classe I des Proteobactéries,
370 Phylum BXIII du Domaine bacteria. Mis à jour en Mai 2012.
371 http://garciajeanlouis9051.perso.neuf.fr/aaBXIII1_O6.html#Carbophilus.
- 372 29. Frank B. Über die Pilzsymbiose der Leguminosen. Ber. Dtsc. Bot. Ges. 1889; 7: 332-346.
- 373 30. Jordan DC. Transfer of *Rhizobium japonicum* Buchanan 1980 to *Bradyrhizobium* gen.
374 nov., a genus of slow growing root nodule bacteria from leguminous plants, Int. J. Syst.
375 Bacteriol. 1982; 32: 136-139.
- 376 31. Lindström K. *Rhizobium galegae*, a new species of legume root bacteria. Int. J. Syst.
377 Bacteriol. 1989; 39: 365-367.
- 378 32. Martinez-Romero E, Segovia L, Mercante FM, Franco AA, Graham P and Pardo MA.
379 *Rhizobium tropici*, a novel species nodulating *Phaseolus vulgaris* L. Beans and *Leucaena* sp.
380 Trees. Int. J. Syst. Bacteriol. 1991; 41: 417-426.
- 381 33. Segovia L, Young JPW and Martinez-Romero. Reclassification of American *Rhizobium*
382 *leguminosarum* biovar phaseoli type I strains as *Rhizobium etli* sp. nov. Int. J. Syst. Bacteriol.
383 1993; 43: 374-377.
- 384 34. Wang LX, Antonio Rogel M, los Santos AG, Martnez-Romero J, Cevallos MA and
385 Martinez-Romero. *Rhizobium etli* bv. *mimosae*, a novel biovar isolated from *Mimosa affinis*.
386 Int. J. Syst. Evol. Microbiol. 1999b; 49: 1475-1491.

35. Amarger N, Macheret V and Laguerre G. *Rhizobium gallicum* sp. nov. and *Rhizobium giardinii* sp. nov. from *Phaseolus vulgaris* nodules. Int. J. Syst. Bacteriol. 1997; 47: 996-1006.
36. Chen WX, Yan GH, Li JL. Numerical taxonomic study of fast-growing soybean rhizobia and a proposal that *Rhizobium fredii* be assigned to *Sinorhizobium* gen. nov., Int. J. Syst. Bacteriol. 1988; 38: 392-397.
37. Wang ET, Van Berkum Beyene PD, Sui XH, Dorado O, Chen WX and Martinez-Romero E. *Rhizobium huautlense* sp. nov., a symbiont of *Sesbania herbacea* which has a close phylogenetic relationships with *Rhizobium galegae*. Int. J. Syst. Evol. Microbiol. 1998; 51: 1023-1026.
38. Van Berkum P, Beyene D, Bao G, Campbell TA and Eardly BD. *Rhizobium mongolense* sp. nov. in one of three rhizobial genotypes identified which nodulate and form nitrogen fixing symbioses with *Medicago ruthenica* [(L.) Ledebour]. Int. J. Syst. Evol. Microbiol. 1998; 48: 13-22.
39. Tan ZY, Kan FL, Peng GX, Wang ET, Reinhold-Hurek B and Chen WX. *Rhizobium yanglingense* sp. nov., isolated from arid and semi-arid regions in China. Int. J. Syst. Evol. Microbiol. 2001; 51, 909-914.
40. Bouzar HD. and Jones JB. *Agrobacterium Larrymoorei* sp. nov., a pathogen isolated from aerial tumors of *Ficus benjamina*. Int. J. Syst. Evol. Microbiol. 2001; 51: 1023-1026.
41. Wei GH, Wang ET, Tan ZY, Zhu ME and Chen WX. *Rhizobium indigoferae* sp. nov. and *Sinorhizobium kummerowiae* sp. nov., respectively isolated from *Indigofera spp.* and *Kummerowia stipulacea*. Int. J. Syst. Evol. Microbiol. 2002; 52: 2231-2239.
42. Squartini A, Struffi P, Döring H, Seleka-Pobell S, Tola E, Giacomini A, Vendramin E, Velásquez E, Mateos PF, Martinez-Molina E, Dazzo FB, Casella S and Nuti MP. *Rhizobium sullae* sp. nov. (formerly “*Rhizobium hedysari*”), the rot nodule microsymbiont of *Hedysarum coronarium* L. Int. J. Syst. Evol. Microbiol. 2002; 52: 1267-1276.
43. Wei GH, Tan ZY, Zhu ME, Wang ET, Han SZ and Chen WX. 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. 2003; 53: 1575-1583.

- 417 44. Garcia-Fraile P, Rivas R, Willems A, Peix A, Martens M, Martinez-M, Eustoquio M,
418 Pedro F, Velazquez E. *Rhizobium cellulosilyticum* sp. nov., isolated from sawdust of *Populus*
419 *alba*. Int J Syst Evol Microbiol. 2007; 57: 844-848.
- 420 45. Gu CT, Wang ET, Tian CF, Han TX, Chen WF, Sui XH and Chen WX. *Rhizobium*
421 *miluonense* sp. nov., a symbiotic bacterium isolated from *Lespedeza* root nodules. Int J Syst
422 Evol Microbiol. 2008; 58: 1364-1368.
- 423 46. Han TX, Han LL, Wu LJ, Chen WF, Sui XH, Gu JG, Wang ET and Chen WX.
424 *Mesorhizobium gobiense* sp. nov. and *Mesorhizobium tarimense* sp. nov., isolated from wild
425 legumes growing in desert soils of Xinjiang, China. Int J Syst Evol Microbiol. 2008; 58(11):
426 2610-2618.
- 427 47. Peng G, Yuan Q, Li H, Zhang W, Tan Z. *Rhizobium oryzae* sp. nov., isolated from the
428 wild rice *Oryza alta*. Int J Syst Evol Microbiol. 2008; 58: 2158-2163.
- 429 48. Ramirez-Bahena MH, Garcia-Fraile P, Peix A, Valverde A, Rivas R, Igual Jose MM,
430 Pedro F, Martinez-Molina E, Velazquez E. Revision of the taxonomic status of the species
431 *Rhizobium leguminosarum* (Frank 1879) Frank 1889AL, *Rhizobium phaseoli* Dangeard
432 1926AL and *Rhizobium trifolii* Dangeard 1926AL. *R. trifolii* is a later synonym of *R.*
433 *leguminosarum*. Reclassification of the strain *R. leguminosarum* DSM 30132 (=NCIMB
434 11478) as *Rhizobium pisi* sp. nov. Int J Syst Evol Microbiol. 2008; 58: 2484-2490.
- 435 49. Lin DX, Wang ET, Tang HH, Tian XH, Yu Rong G, Su H, Chen WX. *Shinella*
436 *kummerowiae* sp. nov., a symbiotic bacterium isolated from root nodules of the herbal legume
437 *Kummerowia stipulacea*. Int J Syst Evol Microbiol. 2008; 58: 1409-1413.
- 438 50. Berge O, Lodhi A, Brandelet G, Santaella C, Roncato MA, Christen R, Heulin T,
439 Achouak W. *Rhizobium alamii* sp. nov., an exopolysaccharide-producing species isolated
440 from legume and non-legume rhizospheres. Int J Syst Evol Microbiol. 2009; 59: 367-372.
- 441 51. Lu YL, Chen W., Han LL, Wang ET, Zhang XX, Chen WX, Su HZ. *Mesorhizobium*
442 *shangrilense* sp. nov., isolated from root nodules of *Caragana* spp. Int J Syst Evol Microbiol.
443 2009; 59: 3012-3018.
- 444 52. Hou BC, Wang ET, Li Y, Jr J, Rui Z, Chen WF, Gao YD, Ren J, Chen WX. *Rhizobium*
445 *tibeticum* sp. nov., a symbiotic bacterium isolated from *Trigonella archiducis-nicolai* (Sirj.)
446 Vassilcz. Int J Syst Evol Microbiol. 2009; 59: 3051-3057.

- 447 53. Dangeard PA. Recherches sur les tubercules radicaux des légumineuses. Le botaniste,
448 Ser.16. 270 p. Paris. 1926. French.
- 449 54. Scholla MH and Elkan GH. *Rhizobium fredii* sp. nov., a fast growing species that
450 effectively nodulates soybean. Int. J. System. Bact. 1984; 34: 484-486.
- 451 55. de Lajudie P, Willems A, Pot B et al. Polyphasic taxonomy of rhizobia: emendation of the
452 genus *Sinorhizobium* and description of *Sinorhizobium meliloti* comb. nov., *Sinorhizobium*
453 *saheli* sp. nov., and *Sinorhizobium teranga* sp. nov. Int. j. Syst. Bacteriol. 1994; 44, 715-733.
- 454 56. Young JM, Kuykendall LD, Martinez-Romero E, Kerr A, Sawada H. A revision of
455 *Rhizobium* Frank 1889, with an emended description of the genus, and the inclusion of all
456 species of *Agrobacterium* Conn 1942 and *Allorhizobium undicola* de Lajudie et al. 1998 as
457 new combinations: *Rhizobium radiobacter*, *R. rhizogenes*, *R. rubi*, *R. undicola* and *R. vitis*,
458 Int. J. Syst. Evol. Microbiol. 2001; 51:89-103.
- 459 57. Lorquin J, Lortet G, Ferro M, Mear N, Dreyfus B, Prome JC and Boivin C. Nod factors
460 from *Sinorhizobium saheli* and *S. teranga* bv. *sesbaniae* are both arabinosylated and
461 fucosylated, a structural feature specific to *Sesbania rostrata* symbionts. Mol. Plant-Microbe
462 Interact. 1997b; 10: 879-890.
- 463 58. Rome S, Fernandez MP, Brunel B, Normand P and Cleyet-Marel JC. *Sinorhizobium*
464 *medicae* sp. nov., isolated from annual *Medicago* spp. Int. J. Syst. Evol. Microbiol. 1996; 46:
465 972-980.
- 466 59. Nick G, de Lajudie P, Eardly BD, Suomalainen S, Plin L, Zhang X, Gillis M and
467 Lindstrom K. *Sinorhizobium arboris* sp. Nov. and *Sinorhizobium kostiense* sp. Nov. isolated
468 from leguminous trees in Sudan and Kenya. Int. J. Syst. Bacteriol. 1999; 49: 1359-1368.
- 469 60. Chen WX, Wang ET, Wang SY, Ly YB, Chen XQ, Li Y. Characteristics of *Rhizobium*
470 *tianshanense* sp. nov., a moderately and slowly growing root nodule bacterium isolated from
471 an acid saline environment in Xingjian, People's Republic of China, Int. J. Syst. Bacteriol.
472 1995; 45:153–159.
- 473 61. Wang FQ, Wang ET, Liu J, Chen Q et al. *Mesorhizobium albiziae* sp. nov., a novel
474 bacterium that nodulates *Albizia kalkora* in a subtropical region of China. Int. J. Syst. Evol.
475 Microbiol. 2007; 57: 1192-1199.

- 476 62. Toledo I, Lloret L and Martinez-Romero E. *Sinorhizobium americanus* sp. nov., a new
477 *Sinorhizobium* species nodulating native *Acacia* spp. in Mexico. Syst. Appl. Microbiol. 2003;
478 26: 54-64.
- 479 63. Lloret L, Ormeno-Orrillo E, Rincon R, Martinez-Romero J, Rogel-Hernandez MA,
480 Martinez-Romero E. *Ensifer mexicanus* sp. nov. a new species nodulating *Acacia*
481 *angustissima* (Mill.) Kuntze in Mexico. Syst Appl Microbiol. 2007.
- 482 64. Merabet C, Martens M, Mahdhi M, Zakhia F, Sy A, Le Roux C, Domergue O, Coopman
483 R, Bekki A, Mars M, Willems A, de Lajudie P. Multilocus sequence analysis of root nodule
484 isolates from *Lotus arabicus* (Senegal), *Lotus creticus*, *Argyrolobium uniflorum* and
485 *Medicago sativa* (Tunisia) and description of *Ensifer numidicus* sp. nov. and *Ensifer*
486 *garamanticus* sp. nov. Int J Syst Evol Microbiol. 2010 ; 60: 664-674 .
- 487 65. De Lajudie P, Laurent-Fulele E, Willems A, Torck U, Coopman R, Collins MD, Kersters
488 K, and Gillis M. *Allorhizobium undicola* gen. Nov. sp. nov., nitrogen-fixing bacteria that
489 efficiently nodulate *Neptunia natans* in Senegal. Int.J. Syst.Bacteriol. 1998a; 48,:1277-1290.
- 490 66. Jarvis BDW, Pankhurst CE, and Pastel JJ. *Rhizobium loti*, a new species of legume root
491 nodule bacteria. Int. J. Syst. Bacteriol. 1982; 32: 378-380.
- 492 67. Chen H, Richardson AE, Cartner E, Diordjevic MA, Roughley RJ and Rolfe BG.
493 Construction of an acid-tolerant *Rhizobium leguminosarum* biovar *trifolii* strain with
494 enhanced capacity for nitrogen fixation. Appl. Environ. Microbiol. 1991; 57: 2005-2011.
- 495 68. Nour SM, Fernandez MP, Normand P and Cleyet-Marel JC. *Rhizobium ciceri* sp. nov.,
496 consisting of strains that nodulate chickpea (*Cicer arietinum* L.). Int. J. Syst. Bacteriol. 1994a;
497 44: 511-522.
- 498 69. Nour SM, Cleyet-Marel JC, Normand P, Fernandez MP. Genomic heterogeneity of strains
499 nodulating chickpeas (*Cicer arietinum* L.) and description of *Rhizobium mediterraneum* sp.
500 nov., Int. J. Syst. Bacteriol. 1995; 45: 640-648.
- 501 70. De Lajudie P, Willems A, Nick G, Moreira A, Molouba F, Hoste B, Torck U, Neyra M,
502 Collins MD, Lindstrom K, Dreyfus B and Gillis M. Characterisation of tropical tree rhizobia
503 and description of *Mesorhizobium plurifarum* sp. Nov. Int.J.Syst. Bacteriol. 1998b; 48: 369-
504 382.

- 505 71. Wang ET, Van Berkum P, Sui XH, Beyene D, Chen WX and Martinez-Romero E.
506 Diversity of rhizobia associated with *Amorpha fruticosa* isolated from Chinese soils and
507 description of *Mesorhizobium amorpha* sp. nov. Int J Syst Evol Microbiol. 1999a; 49: 51-65.
- 508 72. Velásquez, E, Igua JM.I, Williams Murez A, Mateos PF, Abril A, Toro N, Normand P,
509 Cervantes E, Gillis G and Martnez-Romero E. *Mesorhizobium Chocoense* sp. nov., a novel
510 species that nodulates *Prosopis alba* in the Chaco Arido region (Argentina). Int. J. Syst.
511 Bacteriol. 2001; 51: 1011-1021.
- 512 73. Gao JL, Turner SL, Kan FL, Wang ET, Tan ZY, Qiu YH, Gu J, Terefework Z, Young
513 JPW, Lindström K and Chen WX. *Mesorhizobium septentrionale* sp. nov. and *Mesorhizobium*
514 *temperatum* sp. nov., isolated from *Astragalus adsurgens* growing in the northern regions of
515 China. Int. J. Syst. Evol. Microbiol. 2004; 54: 2003-2012.
- 516 74. Nandasena, Kemanthi G, O'Hara, Graham W, Tiwari RP, Willems A, Howieson JG.
517 *Mesorhizobium australicum* sp. nov. and *Mesorhizobium opportunistum* sp. nov., isolated
518 from *Biserrula pelecinus* L. in Australia Int J Syst Evol Microbiol. 2009; 59: 2140-2147.
- 519 75. Vidal C, Chantreuil C, Berge OM, Lucette E, Jose BG, Brunel B, Cleyet-Marel JC.
520 *Mesorhizobium metallidurans* sp. nov., a metal-resistant symbiont of *Anthyllis vulneraria*
521 growing on metalicolous soil in Languedoc, France. Int J Syst Evol Microbiol. 2009; 59:
522 850-855.
- 523 76. Chen WM, Zhu WF, Bontemps C, Young J, Peter W, Wei GH. *Mesorhizobium*
524 *camelthorni* sp. nov., isolated from *Alhagi sparsifolia*. Int J Syst Evol Microbiol. 2011 ; 61:
525 574-579.
- 526 77. Mantelin S, Le Saux MF, Zakhia F, Bena G, Bonneau S, Jeder H, de Lajudie P and
527 Cleyet-Marel JC. Emended description of the genus *Phyllobacterium* and description of four
528 novel species associated with plant roots: *Phyllobacterium bourgognense* sp. nov.,
529 *Phyllobacterium ifriqiyense* sp. nov., *Phyllobacterium leguminum* sp. nov. and
530 *Phyllobacterium brassicacearum* sp. nov. Int J Syst Evol Microbiol. 2006; 56 (4): 827.
- 531 78. Valverde A, Velazquez E, Fernandez-Santos F, Vizcaino N, Rivas R, Gillis M, Mateos P
532 F, Martinez-Molina E, Igual JM and Willems. *Phyllobacterium trifolii* sp. nov. nodulating
533 *Trifolium* and *Lupinus* in Spanish soils. Int. J. Syst. Evol. Microbiol. 2005; 55: 1985-1989.

- 534 79. Sy A, Giraud E, Jourand P, Garcia N, Willems A, de Lajudie, Prin Y, Neyra M, Gillis M,
535 Boivin-Masson C and Dreyfus B. Methylophilic Methylobacterium bacteria nodulate and fix
536 nitrogen in symbioses with legumes. J. Bacteriol. 2001; 183: 214-220.
- 537 80. Jourand P, Giraud E, Bena G, Sy A, Willems A, Gillis M, Dreyfus B & de Lajudie P.
538 *Methylobacterium nodulans* sp. nov., for a group of aerobic, facultatively methylophilic,
539 legume rootnodule-forming and nitrogen-fixing bacteria. International Journal of Systematic
540 and Evolutionary Microbiology. 2004; 54: 2269-2273.
- 541 81. Dreyfus B, Garcia JL, and Gillis M. Characterization of *Azorhizobium caulinodans* gen.
542 Nov., sp. nov., a stem-nodulating nitrogen-fixing bacterium isolated from *Sesbania rostrata*.
543 Int. J. Syst. Bacteriol. 1988; 38: 89-98.
- 544 82. Rinaudo G, Orenge S, Fernandez MP, Meugnier and Bardin R. DNA homologies among
545 members of the genus *Azorhizobium* and other stem- and root-nodulating bacteria isolated
546 from the tropical legume *Sesbania rostrata*. Int. J. Syst. Bacteriol. 1991; 41: 114-120.
- 547 83. Jordan DC. Family III. Rhizobiaceae, p.234-242. In N. R. Krieg and J. G. Holt (ed.),
548 Bergey's Manual of Systematic Bacteriology, vol.1. The Williams & Wilkins Co., Baltimore.
549 1984.
- 550 84. Kuykendall LD, Saxena B, Devine TE and Udell SE. Genetic diversity in *bradyrhizobium*
551 *japonicum* Jordan 1982 and a proposal for *bradyrhizobium elkanii* sp nov. Can. J. Microbiol.
552 1992; 38: 501-505.
- 553 85. Xu LM, Ge C, Cui Z, Li and Fan H. *Bradyrhizobium liaoningense* sp. nov. isolated from
554 the root nodules of soybeans. Int. J. Syst. Bacteriol. 1995; 45: 706-711.
- 555 86. Yao ZY, Kan FL, Wang ET, Wei GH and Chen WX. Characterization of rhizobia that
556 nodulate legume species of the genus *Lespedeza* and description of *Bradyrhizobium*
557 *yuanmingense* sp. nov. Int. J. Syst. Bacteriol. 2002; 52: 2219-2230.
- 558 87. Rivas R, Willems A, Palomo JL, Garcia-Benavides P, Mateos PF, Martinez-Molina E,
559 Gillis M. and Velazquez E. "*Bradyrhizobium betae* sp. nov. isolated from roots of *Beta*
560 *vulgaris* affected by tumor-like deformations." Int. J. Syst. Evol. Microbiol. 2004; 54: 1271-
561 1275.
- 562

88. Vinuesa P, Leon-Barrios M, Silva C, Willems A, Jarabo-Lorenzo A, Perez Galdona R, Werner D and Martinez-Romero E. *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. 2005; 55: 569-575.
89. Islam MS, Kawasaki H, Muramatsu Y, Nakagawa Y and Seki T. "*Bradyrhizobium iriomotense* sp. nov., isolated from a tumor-like root of the legume *Entada koshunensis* from Iriomote Island in Japan." Biosci. Biotechnol. Biochem. 2008; 72:1416-1429.
90. Zhang JJ, Liu TY., Chen WF, Wang ET, Sui XH, Zhang XX, Li Y, Li Y, Chen WX. *Mesorhizobium muleiense* sp. nov., nodulating with *Cicer arietinum* L. in Xinjiang, China. Int J Sy. 2012.
91. Van Berkum P, Eardly BD. The aquatic budding bacterium *Blastobacter denitrificans* is a nitrogenfixing symbiont of *Aeschynomene indica*. Appl and Env Microbiol. 2002; 68 : 1132-1136.
92. Boivin MC, Bontemps C, Geoffroy G, Gris LC, Talini L. Détection et typage du gène à l'aide de biopuces à ADN : perspectives pour l'étude de la diversité et de l'écologie moléculaire des rhizobia. Les Actes du BRG. 2006 ; 6 : 97-110. French.
93. Jarvis BDW, van Berkum P, Chen WX, Nour S, Fernandez MP, CleyetMarrel JC and Gillis M. Transfert of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium mediterraneum*, and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. Int. J. Syst. Bacteriol. 1997 ; 47: 895-898.
94. So RB, Ladha JK and Young JP. Photosynthetic symbionts of *Aeschynomene spp.* form a cluster with bradyrhizobia on the basis of fatty acid and rRNA analyses. Int J Syst Bacterio. 1994; 144: 392-403.
95. Prescott LM, Harley JP, Klein DA, Claire-Michèle BC, Dusart J. Microbiologie, chapitre 22. Les bactéries. Les protéobactérie., Edition. De Boeck Université. p.492. 2007. French.
96. Trüper HG, de Clari L. Taxonomic note: necessary correction of specific epithets formed as substantives (nouns) in "apposition", Int. J. Syst. Bacteriol. 1997; 47:908-909.

- 592 97. Wang ET, van Berkum P, Sui XH, Beyene D, Chen WX, Martinez-Romero E. Diversity
593 of rhizobia associated with *Amorpha fruticosa* isolated from chinese soils and description of
594 *Mesorhizobium amorphae* sp. nov., Int. J. Syst. Bacteriol. 1999; 49: 51-65.
- 595 98. de Lajudie P, Fulele-Laurent E, Willems A, Torck U, Coopman R, Collins MD, Kersters
596 K, Dreyfus BL, Gillis M. Description of *Allorhizobium undicola* gen. nov. sp. nov. for
597 nitrogen-fixing bacteria efficiently nodulating *Neptunia natans* in Senegal, Int. J. Syst.
598 Bacteriol. 1998; 48: 1277-1290.
- 599 99. Trinick MJ. Relationships amongst the fast-growing rhizobia of *Lablab purpureus*,
600 *Leucaena leucocephala*, *Mimosa spp.*, *Acacia farnesiana* and *Sesbania grandiflora* and their
601 affinities with other rhizobial groups. J. Appl. Bact. 1980; 49: 39-53.
- 602 100. Kuykendall LD. Genus *Azorhizobium* of the family *Hypomicrobiaceae*. Bergey's Manual
603 of Systematic Bacteriology, 2nd Edition, 2nd Volume. 2004a.
- 604 101. Hollis AB, Kloos WE, and Elkan GH. Hybridization studies of *rhizobium japonicum* and
605 related Rhizobiaceae. J. Gen. Microbiol. 1981; 123: 215-222.
- 606 102. Euzéby JP and Findall BJ. Status of strains that contravene rules 27(3) and 30 of the
607 Bacteriological Code. Request for an Opinion. Int. J. Syst. Evol. Microbiol. 2004; 54:, 293-
608 301.
- 609 103. Parker MA. Bradyrhizobia from wild *Phaseolus*, *Desmodium* and *Macroptilium* species
610 in northern Mexico. Appl. Env. Microbiol. 2002; 68 : 2044-2048.
- 611 104. Patt TE, Cole GE, Hanson RS. *Methylobacterium*, a new genus of facultatively
612 methylotrophic bacteria. International Journal of Systematic Bacteriology. 1976 ; 26: 226-
613 229.
- 614 105. Kato Y, Asahara M, Arai D, Goto K & Yokota A. Reclassification of *Methylobacterium*
615 *chloromethanicum* and *Methylobacterium dichloromethanicum* as later subjective synonyms
616 of *Methylobacterium extorquens* and of *Methylobacterium lusitanum* as a later subjective
617 synonym of *Methylobacterium rhodesianum*. Journal of General and Applied Microbiology.
618 2005; 51: 287-299.

- 619 106. van Dien SJ, Marx CJ, O'Brien BN & Lidstrom ME. Genetic characterization of the
620 carotenoid biosynthetic pathway in *Methylobacterium extorquens* AM1 and isolation of a
621 colorless mutant. *Applied and Environmental Microbiology*. 2003; 69: 7563-7566.
- 622 107. Hourcade E. Dégradation du dichlorométhane et adaptation à la production
623 intracellulaire d'acide chez *Méthylobacterium*. Thèse de Doctorat. Université Louis Pasteur,
624 Strasbourg I. 2007. French.
- 625 108. Green PN. The genus *Methylobacterium*, p. 2342-2349. In A. Balows, Trüper, M.
626 Dworkin, W. Harder, and KH. Schleifer (eds), *The Prokaryotes*, vol. 3. Springer-Verlag, New
627 York. 1992.
- 628 109. Van Aken B, Peres CM, Doty SL, Yoon JM. & Schnoor JL. *Methylobacterium populi* sp
629 nov., a novel aerobic, pink-pigmented, facultatively methylotrophic, methane-utilizing
630 bacterium isolated from poplar trees (*Populus deltoides* x *nigra* DN34). *International Journal*
631 *of Systematic and Evolutionary Microbiology*. 2004; 54: 1191-1196.
- 632 110. Yabuuchi E, Kosako Y, Oyaizu H, Yano I, Hotta H, Hashimoto Y., Ezaki T, Arakawa
633 M. Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus
634 *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia*
635 *cepacia* (Palleroni and Holmes, 1981) com. nov. *Microbiology and Immunology*. 1992; 36,
636 1251-1275.
- 637 111. Gillis M, Van TV, Bardin R, Goor M, Hebbbar P, Willems A, Segers P, Kersters K,
638 Heulin T and Fernandez MP. Polyphasic taxonomy in the genus *Burkholderia* leading to an
639 emended description of the genus and proposition of *Burkholderia vietnamiensis* sp. nov. for
640 N₂-fixing isolates from rice in Vietnam. *Int. J. Syst. Bacteriol*. 1995; 45: 274-289.