Almas Zaidi · Mohammad Saghir Khan
Javed Musarrat *Editors*

Microbes for Legume Improvement

Second Edition

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Editors
Almas Zaidi
Dept of Agricultural Microbiology
Faculty of Agricultural Sciences
Aligarh Muslim University
Aligarh
India

Mohammad Saghir Khan
Dept of Agricultural Microbiology
Faculty of Agricultural Sciences
Aligarh Muslim University
Aligarh
India

Javed Musarrat
Dept of Agricultural Microbiology
Faculty of Agricultural Sciences
Aligarh Muslim University
Aligarh
India

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Preface

Globally, farming communities are finding it difficult to fulfill food demands of human populations due largely to declining crop production. The crop production is dwindling due to declining cultivable lands, fluctuating environments, and excessive usage of chemical fertilizers in order to optimize crop yields. Apart from these, the nutrient pool of soil is deteriorating rapidly, which further intensifies agricultural problems. Due to these, there is a pressing need to find solutions to expensive and environmentally disruptive problems. To solve these problems, soil microbiota have been found as inexpensive and environmentally sustainable options as organic fertilizers in providing adequate nutrients to growing crops including legumes. Legumes grown in many countries improve soil quality by increasing soil organic matter and soil structure and porosity, recycling nutrients, decreasing soil pH, diversifying the rhizosphere microbes, and decreasing disease incidence. The application of rhizobial inoculants and other free-living/associative plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi in legume production has been found extremely useful.

Microbes for Legume Improvement (second edition) written by qualified teachers and scientists presents exceptional, recent, and wide-ranging information on the use of beneficial soil microbiota in legume production across different production systems. The revised edition presents the current status on the taxonomy of bacteria able to establish nitrogen-fixing legume symbiosis. Recent developments in the active biomolecules involved in rhizobia-legume symbiosis are highlighted. The importance of flavonoids and nod factors in legume-microbe interactions and their role in legume improvement is dealt separately. The advances made in recent times on the role of ethylene and bacterial ACC deaminase in legume-*Rhizobium* interactions are also included in this second edition. The latest developments in the field of some novel rhizobial exopolysaccharides and their role in legume-rhizobia symbiosis and environmental monitoring in legume improvement are discussed separately. The rhizobial diversity for tropical pulses and forage and tree legumes in Brazil is discussed separately. The book further describes the potential of rhizobia as plant growth-promoting rhizobacteria for enhancing the production of legumes in different agronomic regions. The deficiency of phosphorus restricts the legume production severely. To address and resolve such problems, meaningful and extensive information on the role of phosphate-solubilizing bacteria in the improvement of legumes is highlighted. The mycorrhizosphere interactions involving mycorrhizal establishment, mycorrhizal management for improving legume productivity, and interactive influence of mycorrhiza on legume development are described. The role of associative plant growth-promoting rhizobacteria especially *Azospirillum* used either alone or as mixture with other PGPR in increasing the productivity of legumes is highlighted. Also, endophytic microbes affecting legume performance are included in this second edition. This book further provides some novel microbial strategies and proposes alternative solution, which if properly applied could help to boost the overall performance of legumes growing under various stressed environments including salt, drought, and heavy metal-polluted soils. Also, this book gives information on how rhizobia abate metal toxicity and consequently enhance legume production in metal-contaminated soil, when used as metal-tolerant inoculants. The information and strategies described in this second edition are very useful which may serve as an important and updated reference material. This revised edition provides an elaborate overview for persons interested in legume research. This revised edition will, therefore, be of great practical interest to research scientists, postgraduate students, bioscience professionals, decision-makers, and farmers who aim to apply microbes for enhancing legume production. It is also likely to serve as a precious resource for agronomists, soil microbiologists, soil scientists, biologists, and biotechnologists involved in legume research.

We are extremely grateful to our well-qualified and internationally renowned colleague authors from different countries for providing their important, authoritative, and cutting-edge scientific information to upgrade this book. All chapters presented in this revised edition have the latest information with well-placed tables and figures and most recent references. The timely help and generous support extended by our loyal and trusted research scholars in revising this book are commendable. We are indeed very thankful to our family members for their unconditional and constant support, who, in their own ways, motivated us to complete this herculean task. We must also appreciate the honest efforts of the book publishing team in responding to all our queries very promptly and without any delay. Finally, if any one finds any mistake, factual or otherwise, or printing errors in this book, they may inform us so that the same is corrected and improved in subsequent print/edition.

Aligarh, UP, India Almas Zaidi
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Editors and Contributors

About the Editors

Almas Zaidi, M.Sc., Ph.D. received her M.Sc., Ph.D. (agricultural microbiology) from Aligarh Muslim University, Aligarh, India, and is currently serving as assistant professor at the Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India. Dr. Zaidi has been teaching microbiology at postgraduate level for more than 12 years and has research experience of 16 years. She has published more than 50 research papers, book chapters, and review articles in journals of national and international repute. Dr. Zaidi has edited seven books published by leading publishers. Her main focus of research is to address problems related with rhizo-microbiology, microbiology, environmental microbiology, and biofertilizer technology.

Mohammad Saghir Khan, Ph.D. is a professor at the Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India. Dr. Khan received his M.Sc. from Aligarh Muslim University, Aligarh, India, and Ph.D. (microbiology) from Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India. He has been teaching microbiology to postgraduate students for the last 20 years and has research experience of 24 years. In addition to his teaching, Dr. Khan is engaged in guiding students for their doctoral degree in microbiology. He has published over 100 scientific papers including original research articles, review articles, and book chapters in various national and international publication media. Dr. Khan has also edited nine books published by the leading publishers. Dr. Khan is deeply involved in research activities focusing mainly on rhizobiology, microbiology, environmental microbiology especially heavy metal-microbe-legume interaction, bioremediation, pesticide-PGPR-plant interaction, biofertilizers, and rhizo-immunology.

Javed Musarrat, M.Sc., Ph.D. (Biochem.) former chairman of the Department of Agricultural Microbiology and ex-dean of the Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, India, is presently working as a vice-chancellor of the Baba Ghulam Shah Badshah University, Rajouri, Jammu and Kashmir, India. He has been teaching biochemistry, microbiology, and molecular biology to postgraduate students for the last 27 years and has research experience of about 33 years. He has published more than 175 national and international scientific publications. Dr. Musarrat has edited five books published by the leading publishers. He is associated with several scientific bodies such as DBT, CSIR, UGC, ICAR, UPCST, and CCRUM in various capacities. His major area of interest includes the molecular microbiology, microbial ecology, nanotechnology, and genetic toxicology.

Contributors

Bilal Ahmad Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, UP, India

Zulfiqar Ahmad Department of Environmental Sciences, PMAS Arid Agriculture University, Rawalpindi, Pakistan

Ademir Sérgio Ferreira Araújo Centro de Ciências Agrárias, Universidade Federal do Piauí, Campus da Soccopo, Teresina, PI, Brazil

Rosario Azcón Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación, Experimental del Zaidín, Granada, Spain

Concepción Azcón-Aguilar Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación, Experimental del Zaidín, Granada, Spain

Muhammad Zahir Aziz Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

José-Miguel Barea Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación, Experimental del Zaidín, Granada, Spain

Anelise Beneduzi Department of Genetics, Institute of Biosciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

Raktim Bhattacharya Microbiology Laboratory, Department of Life Sciences, Presidency University, Kolkata, West Bengal, India

Rabindranath Bhattacharyya Microbiology Laboratory, Department of Life Sciences, Presidency University, Kolkata, West Bengal, India

Madhurima Chatterjee Microbiology Laboratory, Department of Life Sciences, Presidency University, Kolkata, West Bengal, India

Sandip Das Microbiology Laboratory, Department of Life Sciences, Presidency University, Kolkata, West Bengal, India

Abhijit Dey Microbiology Laboratory, Department of Life Sciences, Presidency University, Kolkata, West Bengal, India

Erdal Elkoca Department of Agronomy, Faculty of Agriculture, Ataturk University, Erzurum, Turkey

Ahmet Eşitken Horticulture and Viticulture Department, Faculty of Agriculture, Selcuk University, Konya, Turkey

Felipe José Cury Fracetto Departamento de Agronomia, Dom Manoel de Medeiros, Universidade Federal Rural de Pernambuco, Recife, PE, Brazil

Giselle Gomes Monteiro Fracetto Departamento de Agronomia, Dom Manoel de Medeiros, Universidade Federal Rural de Pernambuco, Recife, PE, Brazil

Adeneide Candido Galdino Departamento de Agronomia, Universidade Federal Rural de Pernambuco, Recife, PE, Brazil

Paula García-Fraile Microbiology Institute, Academy of Science of the Czech Republic, Prague, Czech Republic

Adriana Giongo Institute of Petroleum and Natural Resources, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil

Adem Güneş Agricultural Faculty, Soil and Plant Nutrition Science, Erciyes University, Kayseri, Turkey

M. Baqir Hussain Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

Department of Soil Science, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan

Muhammad Imran Department of Soil Science, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan

M.Rüştü Karaman Vocational High School of Medical and Aromatic Plants, Afyon Kocatepe University, Afyonkarahisar, Turkey

Azeem Khalid Department of Environmental Sciences, PMAS Arid Agriculture University, Rawalpindi, Pakistan

Mohammad Saghir Khan Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, UP, India

Dominika Kidaj Department of Genetics and Microbiology, Maria Curie-Skłodowska University, Lublin, Poland

Nurgül Kitir Engineering Faculty, Genetics and Bioengineering Department, Yeditepe University, Istanbul, Turkey

Mario Andrade Lira Junior Departamento de Agronomia, Dom Manoel de Medeiros, Universidade Federal Rural de Pernambuco, Recife, PE, Brazil

Bruno Brito Lisboa Laboratory of Agricultural Chemistry, Fundação Estadual de Pesquisa Agropecuária, Porto Alegre, Brazil

Shahid Mahmood Department of Environmental Sciences, PMAS Arid Agriculture University, Rawalpindi, Pakistan

Tariq Mahmood Department of Environmental Sciences, PMAS Arid Agriculture University, Rawalpindi, Pakistan

Pilar Martínez-Hidalgo Departamento de Microbiología y Genética and Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), Universidad de Salamanca, Salamanca, Spain

Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA, USA

Eustoquio Martínez-Molina Departamento de Microbiología y Genética, Edificio Departamental de Biología, Doctores de la Reina s/n, Universidad de Salamanca, Salamanca, Spain

Pedro F. Mateos Departamento de Microbiología y Genética and Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), Universidad de Salamanca, Salamanca, Spain

Ijaz Mehboob District Fertility Lab, Kasur, Pakistan

Esther Menéndez Departamento de Microbiología y Genética and Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), Universidad de Salamanca, Salamanca, Spain

Negar Ebrahim Pour Mokhtari Islahiye Vocational School, Organic Farming Department, Gaziantep University, Gaziantep, Turkey

Diriba Muleta Institute of Biotechnology, College of Natural Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Muhammad Naveed Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

Emrah Nikerel Engineering Faculty, Genetics and Bioengineering Department, Yeditepe University, Istanbul, Turkey

Rafaela Simão Abrahão Nóbrega Universidade Federal do Recôncavo da Bahia, Centro de Ciências Agrárias, Ambientais e Biológicas, Cruz das Almas, BA, Brazil

Luciane Maria Pereira Passaglia Department of Genetics, Institute of Biosciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

Alvaro Peix Departamento de Desarrollo Sostenible de Sistemas Agroforestales y Ganaderos, Instituto de Recursos Naturales y Agrobiología (IRNASA-CSIC), Salamanca, Spain

Martha-Helena Ramírez-Bahena Departamento de Microbiología y Genética, Edificio Departamental de Biología, Universidad de Salamanca, Salamanca, Spain

Raúl Rivas Departamento de Microbiología y Genética, Edificio Departamental de Biología, Universidad de Salamanca, Salamanca, Spain

Asfa Rizvi Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, UP, India

Saima Saif Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, UP, India

Mohammad Shahid Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, UP, India

Luis R. Silva Health Sciences Research Center (CICS), Faculty of Health Sciences—University of Beira Interior, Covilhã, Portugal

Krisle Silva Empresa Brasileira de Pesquisa Agropecuária, Embrapa Roraima, Boa Vista, RR, Brazil

Anna Skorupska Maria Curie-Skłodowska University, Department of Genetics and Microbiology, Lublin, Poland

Bahar Soğutmaz Özdemir Engineering Faculty, Genetics and Bioengineering Department, Yeditepe University, Istanbul, Turkey

Leyla Tarhan Engineering Faculty, Genetics and Bioengineering Department, Yeditepe University, Istanbul, Turkey

Şefik Tüfenkçi Department of Agricultural Faculty, Biosystems Engineering, Yüzüncüyıl University, Van, Turkey

Metin Turan Engineering Faculty, Genetics and Bioengineering Department, Yeditepe University, Istanbul, Turkey

Ceren Ünek Engineering Faculty, Genetics and Bioengineering Department, Yeditepe University, Istanbul, Turkey

Deniz Uras Engineering Faculty, Genetics and Bioengineering Department, Yeditepe University, Istanbul, Turkey

Luciano Kayser Vargas Laboratory of Agricultural Chemistry, Fundação Estadual de Pesquisa Agropecuária, Porto Alegre, Brazil

Encarna Velázquez Departamento de Microbiología y Genética, Edificio Departamental de Biología, Doctores de la Reina s/n, Universidad de Salamanca, Salamanca, Spain

Camila Gazolla Volpiano Department of Genetics, Institute of Biosciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

Jerzy Wielbo Department of Genetics and Microbiology, Maria Curie-Skłodowska University, Lublin, Poland

Muhammad Yaseen Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

Ertan Yildirim Faculty of Agriculture, Department of Horticulture, Ataturk University, Erzurum, Turkey

Zahir Ahmad Zahir Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

Hamdi H. Zahran Faculty of Science, Department of Botany, University of Beni-Suef, Beni-Suef, Egypt

Almas Zaidi Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, UP, India

Current Status of the Taxonomy of Bacteria Able to Establish Nitrogen-Fixing Legume Symbiosis

1

Encarna Velázquez, Paula García-Fraile,
Martha-Helena Ramírez-Bahena, Raúl Rivas,
and Eustoquio Martínez-Molina

Abstract

Bacteria forming nitrogen-fixing symbiosis with legumes, classically named *rhizobia*, currently include more than 100 species distributed in the old genera *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Ensifer* (formerly *Sinorhizobium*), *Mesorhizobium* and *Rhizobium* and in the new genera *Neorhizobium* and *Pararhizobium*. In addition, several new *rhizobia* have been described in the twenty-first century belonging, as the classical rhizobia, to the alpha *Proteobacteria* genera *Aminobacter*, *Devosia*, *Methylobacterium*, *Microvirga*, *Ochrobactrum*, *Phyllobacterium* and *Shinella* and to the beta *Proteobacteria Burkholderia*, *Paraburkholderia* (formerly *Burkholderia*) and *Cupriavidus*. These species carry symbiotic genes encoding for nodulation and nitrogen fixation which are located on plasmids or symbiotic islands. These genes determine the host range and confer rhizobia the ability to fix nitrogen in the legume nodules. Depending on the harboured nodulation genes, several symbiovars have recently been described in the classical *rhizobia* genera. In this chapter, we review the different groups of bacteria able of forming symbiosis with legumes and their classification based on core genes (genera and species) as well as on auxiliary ones (symbiovars).

E. Velázquez (✉) • M.-H. Ramírez-Bahena • R. Rivas • E. Martínez-Molina
Departamento de Microbiología y Genética and Instituto Hispanoluso de Investigaciones Agrarias (CIALE), Edificio Departamental de Biología, Doctores de la Reina s/n, Universidad de Salamanca, 37007 Salamanca, Spain
e-mail: evp@usal.es

P. García-Fraile
Microbiology Institute, Academy of Science of the Czech Republic,
Vídeňská 1083, Praha 4-Krč, 14220 Prague, Czech Republic

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1.1 Introduction

Bacteria able to induce the formation of nitrogen-fixing nodules on the root systems of legumes were discovered during nineteenth century. The first isolate of this bacterial group was obtained from a nodule suspension of *Vicia* and was named as *Bacillus radicicola* (Beijerinck 1888). Later on, it was renamed as *Rhizobium leguminosarum* (Frank 1889). Since then, the bacteria able to produce nodules on legume roots were called *rhizobia* and have been included in the genus *Rhizobium*, which initially contained only six species; all of them were able to nodulate legumes. These species were defined primarily on the basis of the legumes they nodulated and the cross-inoculation concepts given by Baldwin and Fred (1929). Physiological characteristics were not used with taxonomic purposes in rhizobia until year 1982 when the genus *Bradyrhizobium* was created to include the species from genus *Rhizobium* that presented slow growth in culture media (Jordan 1982). Nevertheless, the symbiotic criteria continued to be used in both genera for species definition during several years.

The taxonomic changes in this group of bacteria started when Woese placed the nodule bacteria into the alpha subdivision of *Proteobacteria* using 16S rRNA gene sequence analysis (Woese et al. 1984). Following 16S rRNA gene sequencing, the number of rhizobial genera and species began to increase which further led to several relevant changes in the rhizobial classification, most of them were, however, not accepted by the rhizobiologists, because they implied a change in the previous rhizobial concept. For example, the reclassification of *Agrobacterium* and *Sinorhizobium* in *Rhizobium* and *Ensifer*, respectively, (Young et al. 2001; Young 2003) implied that some pathogenic bacteria were included into genus *Rhizobium*, which until year 2000 only contained legume endosymbionts, and *Sinorhizobium*, also a classical rhizobial genus, disappeared after its inclusion into the non-rhizobial genus *Ensifer*. The discomfort of rhizobiologists with this situation was evident, and recently, Mousavi et al. (2015) recovered the old names *Agrobacterium* and *Allorhizobium*. Nevertheless, they could avoid to include pathogenic strains in classic rhizobial genera, since the former species *Agrobacterium vitis* (Ophel and Kerr 1990) belonged to the genus *Allorhizobium*. In the case of *Ensifer*, the genus name has been imposed in taxonomic journals for the recent descriptions of new species, but some old species have been recently reclassified, such as *Ensifer morelensis* and *Ensifer americanus* (Wang et al. 2013c), and one species, *Sinorhizobium chiapanecum*, is still pending for reclassification into the genus *Ensifer* (Rincón-Rosales et al. 2009). Nevertheless, this species should be reclassified into genus *Ensifer* since it is phylogenetically related to *Ensifer mexicanus* (Rincón-Rosales et al. 2009).

Recent advances in the gene sequencing have further allowed the analysis of several genes and even complete genomes. There are evidences that classic rhizobial families now encompass genera that have not been isolated from legume nodules, such as *Ciceribacter* (Kathiravan et al. 2013) and *Pseudorhizobium* (Kimes et al. 2015) from family *Rhizobiaceae* and *Tardiphaga* (de Meyer et al. 2012) and *Metalliresistens* (Noisangiam et al. 2010) from family *Bradyrhizobiaceae* (*Nitrobacteriaceae*). It is however, possible that some of these genera, in the future, will include nodulating species, as happened with the genus *Shinella*, initially found in environmental sources (An et al. 2006). Currently, *Shinella* contains the species *Shinella kummerowiae* able to form nodules in the legume *Kummerowia stipulata* (Lin et al. 2008). Conversely, classic rhizobial genera now also contain many species isolated from sources other than legumes. Some of them coming from non-rhizobial genera, such as *Blastobacter denitrificans* and *Agromonas oligotrophica* that were transferred to genus *Bradyrhizobium* (van Berkum et al. 2006; Ramírez-Bahena et al. 2013a) and *Blastobacter aggregatus* that was first transferred to genus *Rhizobium* (Kaur et al. 2011) and later to the new genus *Pararhizobium* (Mousavi et al. 2015). Nevertheless, the most relevant advancement in the present century was the finding that bacteria that do not belong to classic rhizobial genera are able to induce nodules in legumes. Most of these bacteria belong to genera from the alpha subdivision of Proteobacteria, while some of them belong to the beta subdivision of *Proteobacteria* (Velázquez et al. 2010a; Peix et al. 2015b). The first beta *Proteobacteria* isolated from legume nodules were included into the genera, *Burkholderia* and *Ralstonia*, able to nodulate *Mimosa* (Moulin et al. 2001; Chen et al. 2003a), but they are currently included in the genera *Paraburkholderia* (Sawana et al. 2014) and *Cupriavidus* (Vandamme and Coenye 2004). Although some studies have shown that in some American and Asian countries *Mimosa* is preferably nodulated by alpha *Proteobacteria* (Gehlot et al. 2013; Bontemps et al. 2016), beta *Proteobacteria* are widespread in nodules of legumes from several tribes in different countries and continents (Barrett and Parker 2005; Lemaire et al. 2015). For this reason, the number of new species of this Class of bacteria that are able to nodulate legumes has considerably increased in the last years, although only in the genus *Burkholderia* (*Paraburkholderia*).

In parallel, the development of sequencing techniques also allowed the analysis of symbiotic genes that have been used to define biovars, which are currently named symbiovars (Rogel et al. 2011). The old biovars were defined on the basis of nodulation assays in different legumes (Jordan 1984), but currently the symbiovars are defined on the basis of the nodulation gene analysis and particularly on that of *nodC* gene (Rogel et al. 2011; Peix et al. 2015b). As occurred in the case of genera and species, the number of symbiovars is increasing, but many lineages found in classical rhizobial genera are still undescribed. Taking also into account that symbiovars have not been described to date in non-rhizobial genera and that there are many legumes whose endosymbionts are still poorly studied, it is predicted that there could be a significant increase in the number of symbiovars in the near future. In this chapter, an attempt is made to present a recent update on species in the classic and new genera of rhizobia and symbiovars within the classic rhizobial genera.

1.2 The Classic Rhizobia

In the beginning, the classification of rhizobial strains in different species was based on the legume they effectively nodulated. Following this criterion, in the same year, three fast-growing species, *Rhizobium phaseoli*, *R. trifolii* and *R. meliloti*, nodulating *Phaseolus*, *Trifolium* and *Melilotus*, respectively, were described by Dangeard (1926), and the slow-growing species *Rhizobium japonicum*, nodulating *Glycine*, was described by Buchanan (1926). Five years later, a second slow-growing species *Rhizobium lupini*, nodulating *Lupinus*, was added to the genus *Rhizobium* (Eckhardt et al. 1931). These species were recorded in *Bergey's Manual of Determinative Bacteriology* published in year 1974 (Jordan and Allen 1974) and were later included in the validation lists of Skerman et al. (1980) published in the International Journal of Systematic Bacteriology (IJSB), which is the official journal for prokaryotes systematic, currently named International Journal of Systematic and Evolutionary Microbiology (IJSEM). Therefore, in 1980, the genus *Rhizobium* contained five species, and it was included in the family *Rhizobiaceae*, which presently also contains several old genera (Conn 1938). One of these genera was *Alcaligenes*, which in 1938 had several species, later reclassified as *Agrobacterium* (Conn 1942). When the Bergey's Manual was published in 1984, the Family *Rhizobiaceae* contained the two old genera *Rhizobium* and *Agrobacterium* and two new ones named *Bradyrhizobium* and *Phyllobacterium* (Jordan 1984). The genus *Phyllobacterium* was isolated from leaves of plants (Knösel 1984) and was reported first time in the Bergey's Manual in 1984, being validated in the same year in IJSB. The genus *Bradyrhizobium* was described by Jordan 2 years before the Bergey's Manual publication to include the slow-growing rhizobia which was previously placed into genus *Rhizobium*. Nevertheless, of the two species, *R. japonicum* and *R. lupini*, only *R. japonicum* was reclassified into the genus *Bradyrhizobium* and the first species with the name *Bradyrhizobium japonicum*, because it was considered that *R. lupini* mainly differed from this species in its ability to nodulate *Lupinus* and *Ornithopus* (Jordan 1982). Although the proposal of the new genus *Bradyrhizobium* was considered in the IJSB, and since the species *R. lupini* was not formally rejected in this publication, the name *R. lupini* remained valid. In addition to the description of the new genus *Bradyrhizobium*, Jordan (1984) revised the taxonomic status of the species *R. trifolii* and *R. phaseoli*, which were reclassified into the species *R. leguminosarum*. Therefore, in the Bergey's Manual from year 1984, the genus *Rhizobium* contained only four species, two of them already included in the version from year 1974, *R. leguminosarum*, *R. meliloti*, and a third species described in IJSB and named *R. loti* (Jarvis et al. 1982). Nevertheless, since the reclassification of *R. trifolii* and *R. phaseoli* was made outside the IJSB, these two names remained valid as occurred in the case of *R. lupini.*

Despite variation in physiological traits, the differences in growth rate were used to differentiate the genera *Rhizobium* and *Bradyrhizobium*. However, the ability to nodulate different legumes remained the main criterion for differentiating the species within these genera. In this way the *Bradyrhizobium* strains that were not isolated from soybean nodules were named *Bradyrhizobium* sp. placing in parentheses the name of the species they nodulated. At this time in other bacterial groups, the numerical taxonomy developed by Sokal and Sneath (1963) had already been applied for the classification of their members at genus and species levels, including *Agrobacterium*, which belongs to the same family than rhizobial genera (Kersters and de Ley 1984). The scarce importance that rhizobiologists paid to the phenotypic characteristics, the low number of legumes studied and the erroneous link between the species concept and the ability to nodulate legumes were the probable causes of the underestimation of rhizobial biodiversity.

This situation began to change in 1980s when it was discovered that soybean may also be nodulated by fast-growing rhizobia belonging to the new species *R. fredii* (Scholla and Elkan 1984). The description of this species thus revealed that a single legume may be infected by different rhizobial genera. This led to the reclassification of *Rhizobium fredii* into a new genus named *Sinorhizobium* (Chen et al. 1988). Moreover, from this date, the number of phenotypic characteristics included in the description of new taxa was higher, as occurred in the case of *Rhizobium galegae* isolated from *Galega* nodules (Lindström 1989) and *Rhizobium huakuii* isolated from *Astragalus* nodules (Chen et al. 1991). Also, a high number of phenotypic characteristics and several molecular approaches, such as the rRNA-DNA hybridization, were used in the description of a new genus named *Azorhizobium* whose type species *Azorhizobium caulinodans* forms stem nodules on the legume *Sesbania rostrata* (Dreyfus et al. 1988). This study showed that not only the nodules formed in legume roots are induced by bacteria but also those formed in the stems of tropical legumes.

The most relevant change for bacterial taxonomy was Woese's proposal of a new classification of prokaryotes based on their 16S ribosomal gene sequences (Woese and Fox 1977). The findings of Woese converted this gene in an essential tool for bacterial classification and identification, although initially only partial sequences were obtained. According to the further analysis of the 16S rRNA gene, the rhizobia were placed within the alpha subdivision of *Proteobacteria* (Woese et al. 1984). However, the sequencing of this gene was not included in the rhizobial species descriptions until year 1991, when the minimal standards for species description of new rhizobia and *Agrobacterium* were published (Graham et al. 1991). The first species described on the basis of partial sequencing of the 16S rRNA gene was *Rhizobium tropici* (Martínez-Romero et al. 1991). From 1991 onwards, the 16S rRNA gene sequences were included in all descriptions or reclassifications of the different taxa within family *Rhizobiaceae*, and the reclassification of *R. fredii* into genus *Sinorhizobium* was confirmed by the analysis of this gene (Jarvis et al. 1992). Several years later, the existence of two phylogenetic groups within genus *Rhizobium* was evidenced, and a new genus named *Mesorhizobium* with an intermediate growth rate between *Rhizobium* and *Bradyrhizobium* was described (Jarvis et al. 1997). A year later, the new genus *Allorhizobium* was described with a single species named *Allorhizobium undicola* isolated in Senegal from nodules of *Neptunia natans* (de Lajudie et al. 1992). In addition to these new genera, in the 1990s several new rhizobial species were described (Table 1.1), and some species were changed from the old to the new described genera as occurred in the case of the species *R. meliloti*, which was reclassified into the new genus *Sinorhizobium* (de Lajudie et al. 1994) and the species *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium mediterraneum* and *Rhizobium tianshanense* that were transferred to the new genus *Mesorhizobium* (Jarvis et al. 1997).

These reclassifications were based on the 16S rRNA gene sequences whose phylogenetic analyses also led Young et al. (2001) to reclassify the genera *Agrobacterium* and *Allorhizobium* into genus *Rhizobium*, published in the International Journal of Systematic and Evolutionary Microbiology (IJSEM). Many researchers did not

Table 1.1 Species of 'classical' rhizobia isolated from different sources

Species	Isolation source	References
Family *Rhizobiaceae*		
Genus *Rhizobium*		
Species isolated from legume nodules		
R. acidisoli	*Phaseolus vulgaris*	Román-Ponce et al. (2016)
R. aegyptiacum	*Trifolium alexandrinum*	Shamseldin et al. (2016)
R. altiplani	*Mimosa pudica*	Baraúna et al. (2016)
R. anhuiense	*Vicia faba*, *Pisum sativum*	Zhang et al. (2015b)
R. bangladeshense	*Lens culinaris*	Rashid et al. (2015)
R. binae	*Lens culinaris*	Rashid et al. (2015)
R. calliandrae	*Calliandra grandiflora*	Rincón-Rosales et al. (2013)
R. cauense	*Kummerowia stipulacea*	Liu et al. (2012a)
R. ecuadorense	*Phaseolus vulgaris*	Ribeiro et al. (2015)
R. etli	*Phaseolus vulgaris*	Segovia et al. (1993)
R. fabae	*Vicia faba*	Tian et al. (2008)
R. favelukesii	*Medicago sativa*	Torres Tejerizo et al. (2016)
R. freirei	*Phaseolus vulgaris*	Dall'Agnol et al. (2013)
R. gallicum	*Phaseolus vulgaris*	Amarger et al. (1997)
R. grahamii	*Dalea leporina*, *Leucaena leucocephala*, *Clitoria ternatea*	López-López et al. (2012)
R. helanshanense	*Spaherophysa salsula*	Qin et al. (2012)
R. hidalgonense	*Phaseolus vulgaris*	Yan et al. (2017)
R. jaguaris	*Calliandra grandiflora*	Rincón-Rosales et al. (2013)
R. hainanense	*Desmodium* spp., *Stylosanthes guianensis*, *Centrosema pubescens*, *Tephrosia candida*, *Acacia sinuata*, *Arachis hypogaea*, *Zornia diphylla*, *Uraria crinita*, *Macroptilium lathyroides*	Chen et al. (1997)
R. indigoferae	*Indigofera* spp.	Wei et al. (2002)
R. laguerreae	*Vicia* spp.	Saïdi et al. (2014)
R. leguminosarum	*Pisum sativum*	Frank (1889), Ramírez-Bahena et al. (2008)
R. lentis	*Lens culinaris*	Rashid et al. (2015)
R. leucaenae	*Leucaena leucocephala*, *Phaseolus vulgaris*	Ribeiro et al. (2012)
R. loessense	*Astragalus* spp.	Wei et al. (2003)
R. lusitanum	*Phaseolus vulgaris*	Valverde et al. (2006)
R. mayense	*Calliandra grandiflora*	Rincón-Rosales et al. (2013)

Table 1.1 (continued)

Species	Isolation source	References
R. mesoamericanum	*Phaseolus vulgaris*, *Macroptilium atropurpureum*, *Vigna unguiculata*, *Mimosa púdica*	López-López et al. (2012)
R. mesosinicum	*Albizia julibrissin*, *Kummerowia* spp., *Dalbergia* spp.	Lin et al. (2009)
R. miluonense	*Lespedeza chinensis*	Gu et al. (2008)
R. mongolense	*Medicago ruthenica*	van Berkum et al. (1998)
R. multihospitium	*Lotus* spp., *Alhagi* spp., *Astragalus* spp., *Halimodendron halodendron*, *Oxytropis* spp., *Robinia pseudoacacia*, *Sophora alopecuroides*, *Caragana jubata*, *Lathyrus odoratus*, *Vicia hirsuta*	Han et al. (2008b)
R. pakistanensis	*Arachis hypogaea*	Khalid et al. (2015)
R. paranaense	*Phaseolus vulgaris*	Dall'Agnol et al. (2014)
R. phaseoli	*Phaseolus vulgaris*	Dangeard (1926), Ramírez-Bahena et al. (2008)
R. pisi	*Pisum sativum*	Ramírez-Bahena et al. (2008)
R. pongamiae	*Pongamia pinnata*	Kesari et al. (2013)
R. puerariae	*Pueraria candollei*	Boonsnongcheep et al. (2015)
R. sophorae	*Sophora flavescens*	Jiao et al. (2015b)
R. sophoriradicis	*Sophora flavescens*	Jiao et al. (2015b)
R. sullae	*Hedysarum coronarium*	Squartini et al. (2002)
R. tibeticum	*Trigonella archiducis-nicolai*	Hou et al. (2009)
R. tropici	*Phaseolus vulgaris*, *Leucaena leucocephala*	Martínez-Romero et al. (1991)
R. tubonense	*Oxytropis glabra*	Zhang et al. (2011a)
R. vallis	*Phaseolus vulgaris*, *Mimosa pudica*, *Indigofera spicata*	Wang et al. (2011)
R. vignae[c]	*Vigna radiata*, *Desmodium microphyllum*, *Astragalus* spp.	Ren et al. (2011a)
R. yanglingense	*Coronilla varia*, *Gueldenstaedtia multiflora*, *Amphicarpaea trisperma*	Tan et al. (2001)
Species isolated from other sources		
R. alamii	Rhizosphere of *Arabidopsis thaliana*	Berge et al. (2009)
R. albus	Lake water	Li et al. (2017)
R. alvei	Freshwater river	Sheu et al. (2015c)
R. azooxidifex	Soil	Behrendt et al. (2016)
R. capsici	Root tumour on *Capsicum annuum* plants	Lin et al. (2015)

(continued)

Table 1.1 (continued)

Species	Isolation source	References
R. cellulosilyticum	Sawdust of *Populus alba*	García-Fraile et al. (2007)
R. daejeonense	Cyanide treatment bioreactor	Quan et al. (2005)
R. endolithicum	Beach sand	Parag et al. (2013)
R. endophyticum	Seeds of *Phaseolus vulgaris*	López-López *et al.* (2010)
R. flavum	Soil	Gu et al. (2014)
R. gei	*Geum aleppicum* stem	Shi et al. (2016)
R. halophytocola	Roots of *Rosa rugosa*	Bibi et al. (2012)
R. halotolerans	Chloroethylene-contaminated soil	Diange and Lee (2013)
R. helianthi	Rhizosphere of sunflower	Wei et al. (2015)
R. ipomoeae	Water convolvulus field	Sheu et al. (2016)
R. lemnae	*Lemna aequinoctialis*	Kittiwongwattana and Thawai (2014)
R. marinum	Seawater	Liu et al. (2015)
R. metallidurans	Seedlings of *Anthyllis vulneraria*	Grison et al. (2015)
R. naphtalenivorans	Sediment of a polychlorinated-dioxin-transforming microcosm	Kaiya et al. (2012)
R. oryzicola	Rice roots	Zhang et al. (2015a)
R. oryziradicis	Rice roots	Zhao et al. (2016)
R. petrolearium[a]	Oil-contaminated soil	Zhang et al. (2012c)
R. phenanthrenilyticum[a]	Petroleum residue treatment system	Wen et al. (2011)
R. populi	*Populus euphratica*	Rozahon et al. (2014)
R. pusense	Rhizosphere of *Cicer arietinum*	Panday et al. (2011)
R. qilianshanense	*Oxytropis ochrocephala*	Xu et al. (2013)
R. rhizoryzae	*Oryza sativa* roots	Zhang et al. (2014a)
R. rosettiformans	Hexachlorocyclohexane dumpsite	Kaur et al. (2011)
R. selenitireducens	Bioreactor	Hunter et al. (2007)
R. smilacinae	*Smilacina japonica*	Zhang et al. (2014c)
R. soli	Soil	Yoon et al. (2010)
R. straminoryzae	Rice straw	Lin et al. (2014)
R. subbaraonis	Beach sand	Ramana et al. (2013)
R. tarimense	Soil	Turdahon et al. (2013)
R. yantingense	Mineral water	Chen et al. (2015)
Genus *Ensifer* (formerly *Sinorhizobium*)		
Species isolated from legume nodules		
E. alkalisoli	*Sesbania cannabina*	Li et al. (2016b)
E. americanus	*Acacia* spp.	Toledo et al. (2003), Wang et al. (2013c), Oren and Garrity (2015a)

Table 1.1 (continued)

Species	Isolation source	References
E. arboris	*Acacia senegal*, *Prosopis chilensis*	Nick et al. (1999), Young (2003)
E. fredii	*Glycine max*	Scholla and Elkan (1984), Jarvis et al. (1992), Young (2003)
E. garamanticus	*Argyrolobium uniflorum*, *Medicago sativa*	Merabet et al. (2010)
E. glycinis	*Glycine max*, *Astragalus mongholicus*	Yan et al. (2016)
E. kostiensis	*Acacia senegal*, *Prosopis chilensis*	Nick et al. (1999), Young (2003)
E. kummerowiae	*Kummerowia stipulacea*	Wei et al. (2002), Young (2003)
E. medicae	*Medicago truncatula*	Rome et al. (1996), Young (2003)
E. meliloti	*Medicago sativa*	Dangeard (1926), de Lajudie et al. (1994), Young (2003)
E. mexicanus	*Acacia angustissima*	Lloret et al. (2007)
E. numidicus	*Argyrolobium uniflorum*, *Lotus creticus*	Merabet et al. (2010)
E. psoraleae	*Psoralea corylifolia*, *Sesbania cannabina*	Wang et al. (2013c)
E. saheli	*Sesbania* spp.	de Lajudie et al. (1994), Young (2003)
E. sesbaniae	*Sesbania cannabina*, *Medicago lupulina*	Wang et al. (2013c)
E. sojae	*Glycine max*	Li et al. (2011)
E. terangae	*Acacia* spp., *Sesbania* spp.	de Lajudie et al. (1994), Young (2003)
Species isolated from other sources		
E. adhaerens	Soil	Casida (1982)
E. morelensis	Associated to nodules *Leucaena leucocephala*	Wang et al. (2002), Wang et al. (2013c), Oren and Garrity (2015a)
Genus *Neorhizobium*		
Species isolated from legume nodules		
N. alkalisoli	*Caragana intermedia*	Lu et al. (2009a), Mousavi et al. (2014)
N. galegae	*Galega officinalis*	Lindström (1989), Mousavi et al. (2014)
N. huautlense	*Sesbania herbacea*	Wang et al. (1998), Mousavi et al. (2014)

(continued)

Table 1.1 (continued)

Species	Isolation source	References
Genus *Allorhizobium*		
Species isolated from legume nodules		
A. taibaishanense	*Kummerowia striata*	Yao et al. (2012), Mousavi et al. (2015)
A. undicola	*Neptunia natans*	de Lajudie *et al.* (1998), Young et al. (2001), Mousavi et al. (2015)
Species isolated from other sources		
A. borbori	Activated sludge	Zhang et al. (2011b), Mousavi et al. (2015)
A. oryzae	Roots of *Oryza alta*	Peng et al. (2008), Mousavi et al. (2015)
A. paknamense	*Lemna aequinoctialis* tissues	Kittiwongwattana and Thawai (2013), Mousavi et al. (2015)
A. pseudoryzae	Rhizosphere of *Oryza sativa*	Zhang et al. (2011c)¸ Mousavi et al. (2015)
A. vitis	tumours on *Vitis vinífera*	Ophel and Kerr (1990), Young et al. (2001), Mousavi et al. (2015)
Genus *Pararhizobium*		
Species isolated from legume nodules		
P. giardinii	*Phaseolus vulgaris*	Amarger et al. (1997), Mousavi et al. (2015)
P. herbae	*Astragalus membranaceus*, *Oxytropis cashemiriana*	Ren et al. (2011b), Mousavi et al. (2015)
P. sphaerophysae	*Sphaerophysa salsula*	Xu et al. (2011), Mousavi et al. (2015)
Species isolated from other sources		
P. capsulatum	Surface lake water	Kaur et al. (2011), Mousavi et al. (2015)
P. polonicum	Galls of *Prunus* rootstocks	Puławska et al. (2016)
Genus *Sinorhizobium*[d]		
Species isolated from legume nodules		
S. abri	*Abrus precatorius*	Ogasawara et al. (2003)
S. chiapanecum	*Acaciella angustissima*	Rincón-Rosales et al. (2009)
S. indiaense	*Sesbania rostrata*	Ogasawara et al. (2003)
Family *Phyllobacteriaceae*		
Genus *Mesorhizobium*		
Species isolated from legume nodules		
M. abyssinicae	*Acacia abyssinica*	Degefu et al. (2013)
M. acaciae	*Acacia melanoxylon*	Zhu et al. (2015)

Table 1.1 (continued)

Species	Isolation source	References
M. albiziae	*Albizia*	Wang et al. (2007)
M. alhagi	*Alhagi sparsifolia*	Chen et al. (2010)
M. amorphae	*Amorpha fruticosa*	Wang et al. (1999b)
M. australicum	*Biserrula pelecinus*	Nandasena et al. (2009)
M. calcicola	*Sophora* spp.	De Meyer et al. (2015)
M. camelthorni	*Alhagi sparsifolia*	Chen et al. (2011)
M. cantuariense	*Sophora microphylla*	De Meyer et al. (2015)
M. caraganae	*Caragana*	Guan et al. (2008)
M. chacoense	*Prosopis*	Velázquez et al. (2001a)
M. ciceri	*Cicer arietinum*	Nour et al. (1994), Jarvis et al. (1997)
M. erdmanii	*Lotus* spp.	Martínez-Hidalgo et al. (2015b)
M. gobiense	*Astragalus filicaulis*, *Lotus* spp., *Oxytropis glabra*	Han et al. (2008a)
M. hawassense	*Sesbania sesban*	Degefu et al. (2013)
M. huakuii	*Astragalus sinicus*	Chen et al. (1991), Jarvis et al. (1997)
M. japonicum	*Lotus corniculatus*	Martínez-Hidalgo et al. (2016)
M. jarvisii	*Lotus corniculatus*	Martínez-Hidalgo et al. (2015b)
M. kowhaii	*Sophora* spp.	De Meyer et al. (2016)
M. loti	*Lotus corniculatus*	Jarvis et al. (1997), Martínez-Hidalgo et al. (2015b)
M. mediterraneum	*Cicer arietinum*	Nour et al. (1995), Jarvis et al. (1997)
M. metallidurans	*Anthyllis vulneraria*	Vidal et al. (2009)
M. muleiense	*Cicer arietinum*	Zhang et al. (2012a)
M. newzealandense	*Sophora* spp.	De Meyer et al. (2016)
M. olivaresii	*Lotus corniculatus*	Lorite et al. (2016)
M. opportunistum	*Biserrula pelecinus*	Nandasena et al. (2009)
M. plurifarium	*Acacia* spp., *Prosopis juliflora*, *Chamaecrista ensiformis*, *Leucaena* spp.	de Lajudie et al. (1998)
M. qingshengii	*Astragalus sinicus*	Zheng et al. (2013)
M. robiniae	*Robinia pseudoacacia*	Zhou et al. (2010)
M. sangaii	*Astragalus* spp.	Zhou et al. (2013)
M. septentrionale	*Astragalus adsurgens*	Gao et al. (2004)
M. shangrilense	*Caragana* spp.	Lu et al. (2009b)
M. shonense	*Acacia abyssinica*	Degefu et al. (2013)

(continued)

Table 1.1 (continued)

Species	Isolation source	References
M. silamurunense	*Astragalus* spp.	Zhao et al. (2012)
M. sophorae	*Sophora* spp.	De Meyer et al. (2016)
M. tamadayense	*Anagyris latifolia*, *Lotus berthelotii*	Ramírez-Bahena et al. (2012)
M. tarimense	*Lotus frondosus*	Han et al. (2008a)
M. temperatum	*Astragalus adsurgens*	Gao et al. (2004)
M. tianshanense	*Glycyrrhiza* spp., *Sophora alopecuroides*, *Halimodendron holodendron*, *Caragana polourensis*, *Swainsona salsula*, *Glycine* spp.	Chen et al. (1995), Jarvis et al. (1997)
M. waimense	*Sophora longicarinata*	De Meyer et al. (2015)
M. waitakense	*Sophora* spp.	De Meyer et al. (2016)
Species isolated from other sources		
M. sediminum	Deep-sea sediment	Yuan et al. (2016)
M. soli	Rhizosphere of *Robinia pseudoacacia*	Nguyen et al. (2015)
M. thiogangeticum	Rhizosphere of *Clitoria ternatea*	Ghosh and Roy (2006)
Family *Nitrobacteriaceae* (*Bradyrhizobiaceae*)		
Genus *Bradyrhizobium*		
Species isolated from legume nodules		
B. americanum	*Centrosema macrocarpum*	Ramírez Bahena et al. (2016)
B. arachidis	*Arachis hypogaea*	Wang et al. (2013b)
B. canariense	*Chamaecytisus proliferus*	Vinuesa et al. (2005)
B. centrosemae	*Centrosema molle*	Ramírez Bahena et al. (2016)
B. cytisi	*Cytisus villosus*	Chahboune et al. (2011)
B. daqingense	*Glycine max*	Wang et al. (2013a)
B. diazoefficiens	*Glycine max*	Delamuta et al. (2013)
B. elkanii	*Glycine max*	Kuykendall et al. (1992)
B. embrapense	*Neonotonia wightii*, *Desmodium heterocarpon*	Delamuta et al. (2015)
B. erythrophlei	*Erythrophleum fordii*	Yao et al. (2015)
B. ferriligni	*Erythrophleum fordii*	Yao et al. (2015)
B. ganzhouense	*Acacia melanoxylon*	Lu et al. (2014)
B. guangdongense	*Arachis hypogaea*	Li et al. (2015)
B. guangxiense	*Arachis hypogaea*	Li et al. (2015)
B. huanghuaihaiense	*Glycine max*	Zhang et al. (2012a)
B. icense	*Phaseolus lunatus*	Durán et al. (2014a)
B. ingae	*Inga laurina*	da Silva et al. (2014)
B. japonicum	*Glycine max*	Buchanan (1926), Jordan (1982)

Table 1.1 (continued)

Species	Isolation source	References
B. jicamae	*Pachyrhizus erosus*	Ramírez-Bahena et al. (2009)
B. kavangense	*Vigna subterranea*, *Arachis hypogaea*	Grönemeyer et al. (2015b)
B. lablabi	*Lablab purpureus*, *Arachis hypogaea*	Chang et al. (2011)
B. lupini	*Lupinus* spp.	Eckhardt et al. (1931), Peix et al. (2015a)
B. liaoningense	*Glycine max*	Xu et al. (1995)
B. manausense	*Vigna unguiculata*	Silva et al. (2014)
B. neotropicale	*Centrolobium paraense*	Zilli et al. (2014)
B. ottawaense	*Glycine max*	Yu et al. (2014)
B. pachyrhizi	*Pachyrhizus erosus*	Ramírez-Bahena et al. (2009)
B. paxllaeri	*Phaseolus lunatus*	Durán et al. (2014a)
B. retamae	*Retama* spp.	Guerrouj et al. (2013)
B. rifense	*Cytisus villosus*	Chahboune et al. (2012)
B. subterraneum	*Vigna subterranea*, *Arachis hypogaea*	Grönemeyer et al. (2015a)
B. stylosanthis	*Stylosanthes*	Delamuta et al. (2016)
B. tropiciagri	*Neonotonia wightii*, *Desmodium heterocarpon*	Delamuta et al. (2015)
B. valentinum	*Lupinus mariae-josephae*	Durán et al. (2014b)
B. vignae	*Vigna subterranea*, *Arachis hypogaea*	Grönemeyer et al. (2016)
B. viridifuturi	*Centrosema pubescens*	Helene et al. (2015)
B. yuanmingense	*Lespedeza cuneata*	Yao et al. (2002)
Species isolated from other sources		
B. betae	Root tumours on *Beta vulgaris*	Rivas et al. (2004)
B. denitrificans[b]	Water	Hirsch and Müller (1985), van Berkum et al. (2006)
B. iriomotense	Root tumours on *Entada koshunensis*	Islam et al. (2008)
B. oligotrophicum	Rice paddy soil	Ohta and Hattori (1983), Ramírez-Bahena et al. (2013a)
Family *Hyphomicrobiaceae*		
Genus *Azorhizobium*		
Species isolated from legume nodules		
A. dobereinerae	*Sesbania virgata*	Souza Moreira et al. (2006)
A. caulinodans	*Sesbania rostrata*	Dreyfus et al. (1988)

(continued)

Table 1.1 (continued)

Species	Isolation source	References
Species isolated from other sources		
A. oxalatiphilum	Macerated petioles of *Rumex* sp.	Lang et al. (2013)

[a]These species are synonyms since they have identical core gene sequences

[b]This species is able to fix nitrogen in *Aeschynomene* nodules according to van Berkum and Eardly (2002)

[c]This species was not officially rejected by Mousavi et al. (2014)

[d]The genus *Sinorhizobium* has been reclassified into genus *Ensifer*, but the species *S. abri*, *S. chiapanecum* and *S. indiaense* have still been not reclassified

accept the reclassification of the genus *Agrobacterium* into genus *Rhizobium* (Farrand et al. 2003), which was only justified in the case of the species *Agrobacterium rhizogenes* closely related to the species *R. tropici* (Yanagi and Yamasato 1993; Young et al. 2001; Velázquez et al. 2005, 2010b). Also, the reclassification of *Sinorhizobium*, a classical rhizobial genus, into genus *Ensifer* (Casida 1982) was performed according to the decision of the Judicial Commission of the International Committee on Systematic of Prokaryotes (2008) after several requests for an opinion sent to IJSEM (Willems et al. 2003; Young 2003). Despite that these two reclassifications were controversial, they were finally accepted because they were officially published and were based on the 16S rRNA gene analysis, which until year 2001 was the only gene analysed in rhizobia.

The taxonomic relevance of the 16S rRNA gene was also pointed out after the publication of the Bergey's Manual published in year 2005 in which the genera from the family *Rhizobiaceae* were dispersed into several new families within the new order *Rhizobiales* (Kuykendall et al. 2005) whose name is illegitimate because the order *Hyphomicrobiales* has preference (this order includes the family *Hyphomicrobiaceae* encompassing the rhizobial genus *Azorhizobium*). The rhizobia were included in the old families *Rhizobiaceae* and *Hyphomicrobiaceae* and in two new ones named *Bradyrhizobiaceae* and *Phyllobacteriaceae*. In the Bergey's Manual of 2005, the family *Rhizobiaceae* included the rhizobial genera *Rhizobium*, *Allorhizobium* and *Sinorhizobium*, because the reclassification of this last genus into genus *Ensifer* was not considered. The old family *Hyphomicrobiaceae* included the genus *Azorhizobium*, the only legume nodulating genus within this family. The new family *Phyllobacteriaceae* was proposed in Bergey's Manual (Mergaert and Swings 2005) and later validated (Validation list No. 107 2006) and contained the rhizobial genera *Phyllobacterium* and *Mesorhizobium*. The genus *Bradyrhizobium* was included in the new family *Bradyrhizobiaceae* (Garrity et al. 2005) whose name is also illegitimate since *Nitrobacteraceae*, a previously described family, includes the genus *Nitrobacter*, closely related to *Bradyrhizobium.* After the sequencing of the 16S rRNA gene, which currently remain as the basic for the classification and identification of rhizobia, the most important contribution to the taxonomy of this group of bacteria was the sequencing of several housekeeping genes. Two of these genes, *recA* and *atpD*, were firstly analysed in rhizobia by Gaunt et al.

(2001), and currently they are the most analysed housekeeping genes in rhizobia. Nevertheless, other genes such as *glnII*, *rpoB*, *dnaK* or *gyrB* have commonly been analysed in the recently described species of rhizobia, and the analysis of multilocus sequences (MLSA or MLST) including three or more housekeeping genes has supported the description of new genera and species, the reclassification of several genera and the recovery of old genera. For example, the new genera *Neorhizobium* (Mousavi et al. 2014) and *Pararhizobium* (Mousavi et al. 2015) have been described, and the old genera *Agrobacterium* and *Allorhizobium* have been recovered (Mousavi et al. 2015). The analysis of several housekeeping genes had also allowed the confirmation of *R. phaseoli* as a valid species (Ramírez-Bahena et al. 2008) and the reclassification of the old valid species *R. lupini* into the genus *Bradyrhizobium* as *Bradyrhizobium lupini* (Peix et al. 2015a). Moreover, since at least three housekeeping genes are commonly analysed, the descriptions of all recently described species within the classic rhizobial genera are based in multilocus sequence analysis.

The reliability of the analysis of housekeeping genes exceeds that of the 16S rRNA gene for rhizobial species differentiation which was pointed out during the description of *Rhizobium lusitanum*, the first species in which the phylogenetic analyses of two housekeeping genes, *recA* and *atpD*, were included (Valverde et al. 2006). Even several new rhizobial species have identical 16S rRNA genes and only differ in their housekeeping genes. This has been found in *Bradyrhizobium icense* and *Bradyrhizobium paxllaeri* which had identical 16S rRNA genes (Durán et al. 2014a) and in *Mesorhizobium acaciae* which had 16S rRNA gene identical to that of *Mesorhizobium plurifarium* (Zhu et al. 2015). Nevertheless, the higher number of valid species which have identical 16S rRNA genes belongs to genus *Rhizobium* and particularly to the *R. leguminosarum-Rhizobium etli* phylogenetic group. Species such as *R. laguerreae* (Saïdi et al. 2014), *R. sophorae* (Jiao et al. 2015b), *R. anhuiense* (Zhang et al. 2015b), *R. acidisoli* (Román-Ponce et al. 2016) and *R. leguminosarum* (Ramírez-Bahena et al. 2008) have identical 16S rRNA genes. Also, the 16S rRNA genes of *R. binae* and *R. bangladeshense* are identical (Rashid et al. 2015), while *R. ecuadorense* (Ribeiro et al. 2015) has a 16S rRNA gene identical to that of *R. pisi* (Ramírez-Bahena et al. 2008).

The most recent innovation in rhizobial taxonomy has been the sequencing of complete genomes. The complete genome sequence is currently available for the type strains of several old and recent rhizobial species and has already been used to describe new rhizobial species isolated from lentil, such as *R. lentis*, *R. binae* and *R. bangladeshense* (Rashid et al. 2015), and from soybean, such as *Ensifer glycinis* (Yan et al. 2016). Moreover, many strains isolated from nodules of different legumes in different ecosystems are in project for genome analysis which will lead to important changes in the taxonomy of nodule bacteria. Currently, the classic rhizobial species are included in the genera *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Ensifer* (formerly *Sinorhizobium*), *Mesorhizobium*, *Neorhizobium*, *Pararhizobium* and *Rhizobium* (Table 1.1). Nevertheless, the complete list of valid species of rhizobia is constantly updated and recorded in the List of Prokaryotic Names with Standing in Nomenclature (http:// www.bacterio.cict.fr).

1.3 Symbiovars and Legume Promiscuity in Rhizobia

Since the first decade of the past century, several studies on root nodule bacteria to establish nitrogen-fixing symbiosis with legumes were conducted. The findings of such studies resulted in the definition of cross-inoculation groups (Baldwin and Fred 1929), and both legumes and endosymbionts were divided in restrictive and promiscuous (Wilson 1939). In the 1970s, it was discovered that nodulation determinants are codified in plasmids, and in many cases it was autoconjugative, for example, in the genus *Rhizobium* (Zurkowski and Lorkiewic 1979). In the 1980s, symbiosis-specific genes were found located in megaplasmids in *R. meliloti* (Rosenberg et al. 1981), and the nodulation (*nod*) and nitrogen fixation (*nif*) genes were sequenced in several species of rhizobia (Fuhrmann and Hennecke 1984; Schofield and Watson 1986; Debellé and Sharma 1986; Norel and Elmerich 1987; Goethals et al. 1989). From the 1990s onwards, the functions of the symbiotic genes were widely revealed, and it was established that the *nodABC* genes are determinants of the host range (Relic et al. 1994; Roche et al. 1996; Perret et al. 2000). Finally, in the first years of twenty-first century, it has been reported that in *Bradyrhizobium* and several *Mesorhizobium* species these genes are integrated in the chromosome (Göttfert et al. 2001; Sullivan et al. 2002) and that in some photosynthetic bradyrhizobia, the nodulation genes are absent (Giraud et al. 2007). The symbiotic genes, also named 'auxiliary' or 'accessory' genes, particularly the *nodA*, *nodC* and *nifH* genes, have been included in MLST analyses comparing their phylogenies with those obtained after the 'core' gene analyses (Wei et al. 2009; Diouf et al. 2010; Mierzwa et al. 2010; Wdowiak-Wróbel and Małek 2010; Degefu et al. 2011; Lorite et al. 2012; Bakhoum et al. 2015; Gnat et al. 2015; Mousavi et al. 2016; Bontemps et al. 2016). The symbiotic genes are also very useful for biogeography studies (Stepkowski et al. 2007; Steenkamp et al. 2008; Lu et al. 2009c; Wei et al. 2009; Zhang et al. 2014a; Ji et al. 2015; Li et al. 2016a, b), and the *nodC* gene is the most used gene in recent studies (Ramírez-Bahena et al. 2013b; Bejarano et al. 2014; Díaz-Alcántara et al. 2014; Horn et al. 2014; Verástegui-Valdés et al. 2014; Ji et al. 2015; Wang et al. 2016). Also, some symbiotic genes are still included in most descriptions of rhizobial species, although they are not useful in taxonomy because of their ability to be transferred in nature (Finan 2002) from plasmids to islands (Nakatsukasa et al. 2008), from bacteria to plants (Broothaerts et al. 2005) and among bacteria (Rogel et al. 2001). Nevertheless, they are essential for definition of symbiotic biovarieties, initially named biovars (Jordan 1984) and currently named symbiovars (Rogel et al. 2011).

The first biovars proposed in rhizobia were those from the species *R. leguminosarum* with the names viciae, trifolii and phaseoli, defined on the basis of their host specificity (Jordan 1984). According to this proposal, the two former species *R. trifolii* and *R. phaseoli* were included within *R. leguminosarum* as biovars. This is a clear example of an erroneous use of the symbiotic abilities in taxonomy, because a revision of the taxonomic status of these three old species concluded that *R. trifolii* is a later subjective synonym of *R. leguminosarum*, but *R. phaseoli* is a valid species (Ramírez-Bahena et al. 2008). The reiteration of *nifH* genes was later