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CHAPTER 5

The biodiversity of beneficial microbe-host mutualism: The case of rhizobia**

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**This contribution is dedicated to the memory of Dr. Peter H. Graham, who through his activities worldwide raised the interest in microbial genresources as the foundation for improved agriculture and food. For a long time he also served as the chair person for the Subcommittee for *Agrobacterium* and *Rhizobium* of the International Committee for the Systematics of Prokaryotes.

Abstract

Symbiotic nitrogen fixation is the main route for sustainable input of nitrogen into ecosystems. Nitrogen fixation in agriculture can be improved by inoculation of legume crops with suitable rhizobia. Knowledge of the biodiversity of rhizobia and of local populations is important for the design of successful inoculation strategies. Soybeans are major nitrogen-fixing crops in many parts of the world. Bradyrhizobial inoculants for soybean are very diverse, yet classification and characterization of strains have long been difficult. Recent genetic characterization methods permit more reliable identification and will improve our knowledge of local populations. Forage legumes form another group of agronomically important legumes. Research and extension policies valorising rhizobial germplasm diversity and preservation, farmers training for proper inoculant use and legal enforcement of commercial inoculant quality have proved a successful approach to promote the use of forage legumes while enhancing biological N_2 fixation. It is worth noting that taxonomically important strains may not necessarily be important reference strains for other uses such as legume inoculation and genomics due to specialization of the different fields. This article points at current knowledge on one hand and and gaps to be filled on the other, for further interaction and improvement of a rhizobial commons.

Key Words: indigenous, diverse, adaptation, promiscuous, rhizobium, bradyrhizobium, inoculant, competition, evolution, Chronos, Kairos

1. The importance of legumes

Symbiotic nitrogen fixation with rhizobia is the most important route for sustainable nitrogen input into agro-ecosystems. The legume-rhizobium symbiosis represents a significant basic model for symbiosis, evolution and differentiation in agriculture for sustainable production and other fields. The signal exchange beween rhizobia and their host plants has been elucidated in great detail, including interacting proteins and encoding genes, and is schematically depicted in Fig. 1. The role of root nodule bacteria for growth promotion through biological nitrogen fixation (BNF) and the carryover of environmental benefits in agro-ecosystems have been reported already in ancient times. The most recent estimates of annual nitrogen fixation inputs by crop legumes, as given in a recent report (Herridge et al., 2008), were 21.45 Tg, and the inputs of pasture and fodder legumes 12-25 Tg. In comparison, the inputs for rice, sugar cane, non-legume cropland and extensive savannas were estimated to be < 23.5 Tg, giving a total input from biological nitrogen fixation (BNF) of 50-70 Tg in agricultural systems. Since symbiotic BNF in legumes is mediated by nodule bacteria, here collectively called rhizobia, these bacteria account for at least half of all biologically fixed nitrogen in agriculture. If we consider the efficiency of the process, crop legumes fix on an average 66% of the nitrogen recovered in the crop, 23-176 kg N ha^{-1}, depending on the plant species, but also on the rhizobia present in the nodules.

Fig. 1.

The rhizobia have during the last decades received much attention, and the number of bacterial species able to form nitrogen-fixing symbioses with legumes has increased almost exponentially. The symbionts occur in several, even distantly related bacterial genera (Supplementary table 1). The description of new species of rhizobia has partly masked the fact that our knowledge of what makes a good symbiont is still very vague.

2. Inoculation as a current and essential practice

Inoculation of legume seeds with rhizobia is perhaps the oldest agro-biotechnological application. Inoculants are generally produced regionally or locally, making use of selected bacterial strains and carrier materials available in that area. For example, in Finland the inoculant producer Elomestari Ltd. provides rhizobium inoculants for clovers, lucerne (alfalfa), fodder galega, lotus, pea, faba bean, vetches and lupins for the Nordic market. They use locally available, neutralised and ground peat, suitable prepared to accomodate rhizobia and give the preparation a long shelflife. Peat-based inoculants are appplied to legume seed as a slurry just before sowing. Other technological approaches are liquid inoculants, pre-coated seed and granules. During the last decades, the technological development has been along thses lines, with emphasis laid on the utilisation of local waste material as carriers on one hand and ease of use on the other (Ben Rehab et al., 2007; Brockwell and Bottomley, 1995; Denton et al., 2009). Inoculation is recommended if the field has no history of legume cultivation, especially if the plant is exotic to a new environment, or if the soil is acidic, saline or otherwise hostile to rhizobia. The Finnish inoculant strains come from Elomestari´s own collection or from the HAMBI collection at the University of Helsinki. They have been deposited by scientists who isolated the strains from the field and tested them for efficiency on selected plants. In the case of the inoculant for lucerne, the strain still in use today was isolated by myself (KL) in 1981 from a nodule in a lucerne field inoculated with a commercial inoculant provided by the Nitragin Company in the U.S.

This is an example of how the microbial commons for rhizobium inoculants evolve. The main purpose of the commons is the exchange of inoculant strains with highly desirable properties, such as good field performance and stability of symbiotic properties in culture. These strains are not necessarily otherwise well documented or used in taxonomy or molecular biology, exceptions being for example the type strains of *Rhizobium galegae,* HAMBI 540^T (Lindström, 1989) and *Sinorhizobium* (syn. *Ensifer*) *arboris* HAMBI 1552^T (Nick et al. 1999), which were both originally identified as good symbiotic partners for their respecitve host legumes in the field.

3. Knowledge from genomes and ecology

3.1. Core and accessory genome compartments

The approach in inoculant development was traditionally the isolation, testing and selection of single strains with desired properties, such as high nitrogen fixation efficiency in symbiosis with selected host plants. In cases where indigenous rhizobia capable of nodulating the host were already present in soil but not very efficient, the capacity of the inoculant to outcompete those strains became important. The selection of strains is still hampered by the fact that even though the genes encoding nodulation and nitrogen fixation in rhizobia are well-known (e.g. Franche et al., 2009), the genetic basis for symbiotic effectiveness, competitiveness and tolerance to environmental stress factors are largely not known. Milestones in unraveling the genetic basis of symbiosis in rhizobia were the cloning of nodulation genes of *S. meliloti* strain 1021 (Long et al., 1982), another the completion of the whole genome sequence of the same strain (Galibert et al., 2001). This important work has not yet had practical field implications.

In an EU funded research project BACDIVERS (2003-2005; N° QLRT-2001-02097) scientists and inoculant manufacturers got together in order to utilize modern phylogenetic and genomic methods for dissection of the genomes of important inoculant strains as well as taxonomic type strains. Focus was on genes involved in stress tolerance, which is an important property for an inoculant strain while desiccating on the seed or later in a hostile soil environment. Comparative genomic hybridizations revealed that *Sinorhizobium* strains from the wild had significantly different genomes from that of the molecular reference strain *Sinorhizobium meliloti* strain 1021, especially among genes involved in recombination and DNA repair. Multilocus sequence analysis was applied and evaluated for taxonomic purposes, especially for the genus *Sinorhizobium,* and found to be a valuable tool (Martens et al., 2007, 2008) for rhizobium classification and speciation studies, giving similar but more detailed information than classical methods, but no inoculant strains were yet inlcuded among the strains studied.

Conceptually, the rhizobial genomes can be divided into compartments. The core genome carries housekeeping genes necessary for proper functioning of the cell. The phylogeny of housekeeping genes indicate speciation and forms a good biological basis for rhizobium taxonomy (Martens et al., 2007, 2008; Vinuesa et al., 2008). The accessory genome on the other hand is responsible for properties such as symbiosis and other kinds of ecological niche adaptation (Young et al. 2006).

Symbiotic nodulation functions are encoded partly by accessory genes found in almost all rhizobia ("common nodulation genes") and partly by genes encoding host specificity. These genes evolved under host constraint and clearly belong to the accessory gene pool (Suominen et al., 2001). By using microarrays, Ruberg et al. (2003) demonstrated how osmotic stress influenced the gene expression in strain 1021. Over one hundred genes were identified showing significant changes in gene expression resulting from the osmotic upshift. Among these genes repression was observed for genes related to motility and chemotaxis, genes encoding amino acid biosynthesis enzymes and genes involved in iron uptake whereas some genes involved in transport of small molecules and genes related to polysaccharide biosynthesis were induced. Jensen et al. (2005) demonstrated the role of trehalose transport in competitiveness of *Sinorhizobium meliloti* strains on *Medicago sativa,* emphasizing the important role of different saccarides in the ecology of rhizobia. These genes, which are involved in adaptation to certain environmetal conditions, might belong to both the core and the accessory gene pools. Further studies should clarify their roles and hopefully assist the development of inoculation strategies.

Biogeographical studies of *S. meliloti* populations revealed that their genetic composition varied in diverse gene centers, such as Siberia, China and Central Asia (Roumiantseva et al., 2002). Several recent studies also addressed biogeography, but did not yet answer questions related to agronomy (e.g. Vinuesa et al. 2008). Thus, genes important for effective, high-yielding nitrogen fixation or responsible for e.g. competitiveness have not yet been properly pinpointed, they obviously vary depending on bacteria, hosts and the environment, and are possibly part of the accessory genome. Further studies into genome composition and evolution in combination with biogeographic approaches are likely to shed more light on the genetics of symbiotic nitrogen fixation in nature.

3.2. Chronos and Kairos evolution of bacterial genomes

Also the evolution of micro-organisms can be viewed conceptually from two different perspectives, which are here termed *'Chronos'* and *'Kairos'* evolution. Both words stem from the old Greek, who recognized two different words for 'time'. Chronos evolution is characterized through its long-term perspective; slowly evolving genes and genomes leading to speciation, when suitable genes are used as molecular clocks or chronometers (Turner and Young, 2000). Evolutionary events can be fixed on a time scale in chronological order. In contrast, Kairos evolution is fast, takes place under special conditions, leads to niche adaptation and cannot be used to measure evolutionary time. Thus, Kairos is the time when big events take place, often in a short measurable time. The hypothesis is that chronos evolution occurs among all DNA sequences (core and accessory genomes) within an organism, while Kairos evolution impacts most effectively the accessory genome. Speciation in rhizobia is mainly Chronos evolution of the core genome, whereas symbiotic interactions with plants and other kinds of niche adaptation are largely determined by accessory genes, subjected to both Chronos and Kairos evolution (for a review, see Dresler-Nurmi et al., 2009). A classical example of Kairos evolution is the study by Sullivan et al. (1995), in which the symbiotic island from an inoculant *Mesorhizobium* strain spread into a native population of non-nodulating rhizobia, which thereby became symbiotic.

4. Microbial commons in the context of rhizobia

4.1. Where to search for inoculant strains

With this brief background information in mind, what is the best place to look for good inoculant strains to be used in agro-biotechnology? Vavilov (1926) proposed that the greatest diversity of agricultural plants was to be found in their gene centers (centers of origin). Thus, the gene centers of the plants would be the places to look for a wide range of diversity among strains. Indeed, we have shown that the diversity of both plants and rhizobia of the species *Galega orientalis* was greater in the gene center of the plant than in other places (Andronov et al. 2003). In this very host specific symbiosis the plant seems to be an important determinant for rhizobial diversity. In other systems the situation might be different. With less specific, suitable symbiotic genes available, local populations representing even diverse species might quickly adapt to newly introduced plant species and varieties via Kairos evolution. The potential for great plasticity has been detected in all rhizobial genomes sequenced (reviewed by MacLean et al., 2007). Work with inoculant strain development on diverse continents have demonstrated the importance of diversity of indigenous rhizobial populations for symbiotic nitrogen fixation on one hand and the success of inoculation on the other.

4.2. Purposes of strain exchange

A main function of the rhizobial microbial commons is type strain deposition in recognised collections with public databases (e.g. ATCC, DSMZ, LMG, HAMBI) and exchange for comparative taxonomic purposes. The taxonomic type strains and representatives of described species mainly stem from collection efforts aiming at sampling the biodiversity of rhizobia from diverse host plants and geographic regions. Such strains are also material for population genetic studies aiming at revealing patterns of Chronos and Kairos evolution though for this purpose many more strains that what is required for species descriptions should be used. Such collections are often kept by individual researchers and not available to the whole scientific community. Obstacles for free exchange of rhizobial collections are nowadays protection of intellectual property rights by restriction of strain exchange based on the Cartagena protocol. Even though molecular markers form the core in species delineations, molecular biological studies and whole genome sequencing do not normally require strain exchange, since scientists work with "in house" model strains. Inoculant strains for agriculture are not regularly deposited in culture collections, but mainly maintained by companies or research institutes. These strains are not often listed in public databases and thus only available locally or via personal contacts. In conclusion, strain exchange for taxonomic purposes is well organised and readily accessible to the scientific community. However, collections of strains with agro-bioechnological interest are mainly regional or local and less accessible for exchange.

5. Biodiversity of soil rhizobia

5.1. Indigenous rhizobia

Indigenous rhizobia are those found naturally in the soil of a given locality. A great diversity occurs in most soils and an enhanced population size occurs where compatible legumes are grown and the soil is fertile (Zengeni et al., 2006). Rhizobia are very diverse at species and strain levels. One soil may contain various species and various strains within a species (Bala et al., 2001), while also similar isolates may be found in distant places (Abaidoo et al., 2007). When the bacteria are not dormant, the accessory genomes of indigenous soil rhizobia probably underago frequent Kairos evolution. A challenge for agriculture is to match rhizobia and legume crops for optimal performance either by having plant genotypes adapted to local rhizobial populations or by inoculation with effective strains adapted to prevailing environmental conditions and with good competitive ability against local, less effective strains.

5.2. Diversity and specificity

The plant family Leguminosae, the members of which rhizobia form symbioses with is very diverse ranging from field grain annual legumes such as soybean to perennial trees such as *Sesbania*. Whilst there may be cross-inoculation of strains compatible with any given legume (Abaidoo et al., 2007), the wide diversity of rhizobia requires more precise matching in the symbioses. This specificity of an association enables maximisation of nitrogen fixation. Subsequently, grain legumes can yield up to about 300 kg ha^{-1} $year^{-1}$ whilst some tree legumes fix as much as 600kg N ha^{-1} per year (Giller, 2001) with well matched symbionts. For example, a wide diversity of soybean nodulating rhizobia has been found in Zimbabwean soils (Musiyiwa et al., 2005; Vance et al., 1988). However, only three out of the 129 isolates obtained from one study had higher nitrogen fixation efficiency than the standard commercial strain MAR 1491 used in the country. This emphasises the possibility of closer matching of strain and crop for improved symbiotic efficiency by careful strain selection.

The occurrence of a wide diversity of strains increases the opportunity of a legume host finding a compatible rhizobium in any particular soil. Some smallholder farmers prefer using promiscuous varieties of soybean as opposed to the higher yielding specific varieties because of the challenges they face in getting access to inoculants (Mpepereki et al., 2000; Musiyiwa et al., 2005). Many developing countries do not have inoculant factories and therefore indigenous rhizobia become an important resource in their natural state.

5.3. Access to indigenous rhizobia

Success stories of the exploitation of indigenous rhizobia by farmers with no access to inoculants have been reported. The International Institute of Tropical Agriculture (IITA) has bred promiscuous varieties through crossing specific American varieties with promiscuous Asian varieties of soybean (Vanlauwe and Giller, 2006). Conversely erratic nodulation and nitrogen fixation in Zimbabwe and Zambia by these crosses have also been reported (Mpepereki et al., 2000; Sanginga, 2003). It was concluded that it may be impossible to develop a promiscuous soybean variety that nodulates in all regions of the Guinean savannah. Additionally, striving for nodulation is not sufficient because some nodulating, non-nitrogen fixing associations occur. Due to the heterogeneous nature of native rhizobia populations it is difficult to access this resource in its natural condition and lack of knowledge regarding the genetics of symbiotic effectiveness hampers development.

Population sizes of indigenous rhizobia compatible with the legume crop of choice are often very low in economically significant soils. Population sizes ranging from 2 to 4200 cells per gram of soil were reported in a study across nine African countries (Abaidoo et al., 2007). The numbers were enhanced by previous cropping of the compatible legumes. About 38% of isolates from this experiment were as effective as USDA 110 when inoculated on soybean, forming a rich source for inoculant development yet emphasizing the need to supplement soil rhizobia for economic production of specific legumes by inoculation.

5.4. Population dynamics

Unfortunately, adaptation of indigenous rhizobial populations to local environments, which is a big advantage for selection of inoculant strains, may pose a challenge to productivity. The adaptability of indigenous rhizobia to their environment results in high levels of saprophytic competence (Zengeni et al., (2006). Sometimes indigenous rhizobia may be found in greater numbers than those of the inoculated strains which are also limited in mobility. This is a challenge that must be overcome by the inoculant strains and it raises the standard of the inoculant required with regards to competitiveness and nitrogen fixation (Tas et al., 1995). Rhizobia may also be an indicator of soil properties.

Indigenous rhizobia are affected by soil acidity, temperature, moisture and other factors and therefore the diversity may be an indicator of the soil condition. Fields which receive consistent fertility management and legume cropping host higher rhizobial numbers and diversity. The application of manure has been noted to increase rhizobial numbers in soil populations (Zengeni et al., 2006).

5.5. indigenous rhizobia as sources for inoculants

All inoculant strains originate in indigenous rhizobial pools. Thus, although indigenous rhizobia may pose challenges of competition to inoculated strains, they are an important resource that must be preserved by integrated soil fertility approaches. Continual isolation and characterisation to identify new isolates offers the opportunity to improve BNF with minor limits geographically to the areas of use. It may be difficult to find an isolate bearing all the required attributes to the desired level. Indigenous rhizobia are a source for genes that may be required for the enhancement of rhizobial performance using molecular biological techniques (Appunu and Dhar, 2008). A prerequisite for this approach is more insight into the genetic makeup of rhizobia and the dynamics of their genomes.

It is important to continually isolate higher nitrogen fixing isolates to be used as inoculant strains from the wide diversity of indigenous rhizobia. There have also been reports of inoculant strains losing their symbiotic properties (Weaver and Wright, 1987). A wide diversity of isolates ensures a sustainable source of replacement strains and may be developed into strains for commercial use (Musiyiwa et al., 2005).

6. *Bradyrhizobium* strains as inoculants for soybean

6.1. Importance of soybean

Soybean is a major agricultural crop worldwide (Table 1). It is used for human food and livestock feed and increasingly additional uses for soybean oil are being developed, including biodiesel and industrial products such as inks, plastics, building materials and lubricants (http://www.ilsoy.org/soybean-uses/new-uses/). These increasing demands together with an increased competition for land with the corn ethanol industry, fuel a constant drive for improved yields (Graham and Vance, 2000; Salvagiotti et al. (2008).

Table 1

6.2. Bradyrhizobia as inoculants

High yielding soybean plants require a lot of nitrogen and it is estimated that BNF can cover 60 to 70% of the nitrogen requirement of the plant (Herridge et al., 2008). Salvageotti et al. (2008) compared studies with a wide range of yield levels and environments and concluded that on average, about 50 to 60% of nitrogen required by soybean plants is provided by BNF. They point to a lack of data to assess the real contribution of varying levels of belowground nitrogen and call for more research to elucidate whether optimised BNF systems can sustain optimal yields with minimal input of additional nitrogen.

The main rhizobial partners of soybean are the slow-growing bradyrhizobia and particularly the species *B. japonicum* (Jordan, 1982), *B. elkanii* (Kuykendall et al., 1992) and *B. liaoningense* (Xu et al., 1995). The latter species is very close to *B. japonicum* (Van Berkum and Fuhrmann, 2000). In addition, the moderately fast-growing *Mesorhizobium tianshanense* (Chen et al., 1995) and the fast-growing *Sinorhizobium* (*Ensifer*) *fredii* (Scholla and Elkan, 1984) and *S. xinjiangense* (Chen et al., 1988) also nodulate soybean and can be as effective as bradyrhizobia in suitable ecological conditions (Albareda et al., 2009). Possible synergistic effects promoting soybean plant growth by bradyrhizobia with other bacteria such as some strains of *Pseudomonas* (Chebotar et al., 2001), *Bacillus thuringiensis* (Mishra et al., 2009) and *Azospirillum brasilense* (Cassan et al., 2009) have also been documented.

Because soils do not always contain a sufficiently large population of suitable rhizobia, inoculation of soybean crops is a widespread practice. When a legume is introduced into an area there is the opportunity to co-introduce rhizobia that are adapted to that environment. Some important environmental constraints include acidity and salt tolerance, drought and high temperature tolerance (Musiyiwa et al., 2005).

Commercial inoculant production is an important agricultural industry (Catroux et al., 2001) and selected bradyrhizobial inocula with superior symbiotic capacities are produced to improve production and grain yields. The success of such introduced strains to establish a symbiotic relationship and persist in a field is dependant on local soil conditions and the presence of competing indigenous rhizobial strains (Botha et al., 2004; Brockwell et al., 1995; Sadowsky, 2000). This competition may comprise effective colonizers that are less effective nitrogen fixers. Commercial inoculant strains may evolve quickly via kairos evolution in the soil (Farooq and Vessey, 2009). Over time, genetic exchange may dilute the beneficial capacities of introduced strains (Bottomley et al., 1994; López-García et al., 2002) or introduced strains may disappear completely without repeated inoculation (Obaton et al., 2002), though it has also been reported that strains are highly adaptable to new environments (Alves et al., 2003; Andrade et al., 2002). There are even reports from Brazil of re-isolated rhizobia that had become more competitive in their new environment while they maintained their nitrogen fixation (Alves et al., 2003). Knowledge of the local soil community is therefore indispensable to assess the potential benefits of an inoculation strategy (Catroux et al., 2001; McInnes et al., 2004; Obaton et al., 2002).

6.3. Bradyrhizobium *diversity*

In the absence of local soybean nodulating bradyrhizobia, e.g. when newly introducing the crop in a particular region, inoculation can be very effective (Botha et al., 2004). However, a resident population of nodulating bradyrhizobia may significantly reduce the chances of a successful competition by inoculant strains (Jansen van Rensburg and Strijdom, 1985; Okogun and Saninga, 2003), with local conditions such as soil composition, pH, temperature and land use also influencing the outcome (Botha et al., 2004; Obaton et al., 2002; Rahmani et al., 2009; Sadowsky, 2000).

Studies characterizing the local rhizobial flora have resulted in the presence in national as well as specialist culture collections of many rhizobial strains that are of local and regional importance, but that are often not identified to species level. These strains represent a major biodiversity resource and are very diverse. However, identifying them has long been a serious challenge because of the slow growth of bradyrhizobia and their poor performance in many standardized biochemical tests. As a consequence, the classification of bradyrhizobia is less well developed than that of most other rhizobia; however, molecular methods are now leading to more comprehensive characterizations (Rivas et al., 2009; Van Berkum and Fuhrmann, 2009). Particularly among soybean symbionts, 17 different serogroups were described by 1965 (Date and Decker, 1965), 12 of them belonging to *B. japonicum* and five to *B. elkanii*. This diversity was confirmed by rRNA internally transcribed spacer (ITS) sequence divergence (Van Berkum and Fuhrmann, 2000) and further analysis with ITS data from *B. liaoningense* revealed a very close relationship of this species with *B.*

japonicum (Willems et al., 2001) with reticulate evolutionary patterns obscuring species boundaries (Van Berkum and Fuhrmann, 2009). Molecular methods now allow a more precise identification and characterization of strains and this will contribute to an improved classification for *Bradyrhizobium* as well as to more successful design of inoculation strategies and quality control of inoculant strains (McInnes et al., 2004; Rickli Binde et al., 2009).

7. Root nodule bacteria for forage legume growth promotion

7.1. Importance of forage legumes

Cow milk and cattle meat rank the first and third, respectively, of food and agricultural commodities in the world (Table 2). Forage legumes are essential for an efficient animal-based agriculture worldwide. They have been the foundation for dairy and meat production for centuries as rich sources of protein, fibre, and energy (Graham and Vance, 2003). Besides providing high quality feed for livestock, they are a key component for sustainability of crop-pasture rotations. Legumes offer the potential to enhance the productivity as well as the sustainability of mixed, inter-cropped and rotational cropping systems (Hardarson and Atkins, 2003). Their value lies greatly in their ability to fix nitrogen (N_2) in symbiosis with soil bacteria of the genera *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Azorhizobium* and *Allorhizobium* (Table1) (Graham, 2008; Graham and Vance, 2003; Gualtieri and Bisseling, 2000; Vance, 1998).

Table 2

Biological N_2 fixation is second only to photosynthesis as the most important biochemical process on earth. It can provide substantial amounts of N_2 to plants and soil, which reduces the need for industrial fertilizers (Carlsson and Huss-Danell, 2003). Use of legumes in pastures and for soil improvement dates back to the Romans, with Varro (37 BC; cited by Fred et al., 1932) noting "Legumes should be planted in light soils, not so much for their own crops as for the good they do to subsequent crops" (Grahamand Vance, 2003).

Perennial forage legumes are usually more effective and derive higher percentages of their N_2 from the atmosphere than most grain legume species (Hardarson and Atlins, 2003). When grown in mixtures with grasses, these species take a large fraction of their N_2 from the atmosphere, with average field measurements over 80% (Carlsson and Huss-Danell, 2003). Reported rates of N_2 fixation in above-ground plant tissues are in the range of up to 373 kg N ha^{-1} $year^{-1}$ in red clover (*Trifolium pratense* L.), 545 kg N ha^{-1} $year^{-1}$ in white clover (*T. repens* L.), 350 kg N ha^{-1} $year^{-1}$ in alfalfa (*Medicago sativa* L.) and 138 kg N ha^{-1} $year^{-1}$ in birdsfoot trefoil (*Lotus corniculatus* L.) (Carlsson and Huss-Danell, 2003; Gregerson et al., 1999: Vance et al., 1988).

In Uruguay, improved pastures integrated by forage legumes, are the primary base of agriculture, dairy and livestock production (Rebuffo et al., 2006). Perennial cultivated pastures cover an area of 1.400.000 has, while natural grasslands over sown with legumes occupy approximately 650.000 ha (DIEA/MGAP, 2009). Total area sown to forage legumes accomplishes over 2 million has, which represents 15% of agricultural land. Dairy farms occupy an area of 750.000 ha, 50% of which is sown with legume-based pastures in mixture with grasses (mainly alfalfa, birdsfoot trefoil and clovers) (DIEA/MGAP, 2009; Rebuffo et al., 2006). Extensive cattle production is sustained on natural grasslands improved with over sown exotic legumes (mainly *Lotus* spp., with *L. subbiflorus* representing 87.6%) DIEA/MGAP, 2002; Rebuffo et al., 2006).

The most remarkable feature in the utilization of forage legumes in Uruguay has been the impact on the effective management of N in the environment through N supply to natural and agro-ecosystems. Their use has largely reduced N fertilization requirements while improving farmer profitability. Consequently, forage legume–rhizobia symbiosis does have a significant effect on Uruguayan economy (Rebuffo et al., 2006). One ton of urea - the most utilized N fertilizer - costs approximately \$420 and supplies 460 kg N. With average estimates of N_2 fixation in forage legumes of about 230 kg N ha^{-1} $year^{-1}$, \$210 are saved per hectare (Montáñez et al. 2003). Thus, considering 2 million hectares sown with perennial legumes, Uruguay accomplishes savings in the order of 420 million dollars per year through reducing imports of N fertilizer (a figure close to 50 % of total annual meat exports).

7.2. Rhizobia as inoculants for forage legumes: the Uruguayan system

The perennial strategy of most temperate forage legumes like alfalfa (*Medicago sativa* L.), trefoils (*Lotus* spp.) and clovers (*Trifolium* spp.), relies on the success of stand establishment and early development of healthy root systems to achieve high dry matter yields and optimal productivity. Microbial-based strategies that improve forage legume productivity, optimize N_2 fixation, conserve soil N and augment the pool of soil N for the benefit of rotational non-leguminous crop, have been exploited worldwide through rhizobial inoculant technology (Brockwell and Bottomley, 1995; Carlsson and Huss-Danell, 2003; Catroux et al., 2001).

Although commercial rhizobial inoculant production and use is worldwide extended, Catroux et al. (2001) concluded that their quality remains poor despite the available technologies. These authors stated that legal requirements and controls can improve the quality of inoculants and thus their efficacy. As observed in countries with standards and government control, the trend is to increase the quality using sterile carriers or liquid inoculants in order to avoid contaminants and to keep high numbers of rhizobia in the packages for at least 1 year storage. Uruguay, together with Brazil, Canada and France, has been recognized as one of the countries with regulatory authorities responsible for the quality control services, supported by appropriate legislation (Brockwell and Bottomley, 1995; Lupawayi et al., 2000).

The Uruguayan system for biological N_2 fixation technology has been recently described by Montáñez et al. (2003). Created in 1960, the key for its success has been the implementation of a national government-supported strategy, based on a strong functional relationship among public research, private industry and farmers. Regulatory authorities (Ministry of Livestock, Agriculture & Fisheries) are supported by appropriate legislation on inoculant registration, quality control and use. Main activities of the official Laboratory of Soil Microbiology and Inoculant Control consist on: (i) characterization and selection of rhizobial strains, (ii) preservation of the culture collection as the source of high quality germplasm for research, (iii) strain supply to the industry and (iv) quality surveillance of commercial inoculants. High quality standards are achieved using sterile peat carrier as well as liquid formulations, with numbers of viable rhizobia in the packages mandatory high (2 x 10^9 rhizobia /g peat), as pointed by Lupwayi et al. (2000). Today, four local manufacturers share the inner market with four imported brands, while Uruguayan high-quality rhizobial inoculants are exported to other South American countries. As a result of research and extension policies, farmers have adopted the inoculation technology to a 100% extent.

As mentioned before, Uruguay bases its improvement of forage supply on the temperate legumes alfalfa, clovers and trefoils. The main rhizobial partner of alfalfa is the fast-growing species *Sinorhizobium* (*Ensifer*) *meliloti* (Vance et al., 1988); the fast-growing species *Rhizobium leguminosarum* biovar *trifolii* nodulates *Trifolium* spp. (Gualtieri and Bisseling, 2000), while the moderately fast-growing *Mesorhizobium loti* and slow growing *Bradyrhizobium* sp. nodulate *Lotus* species (Díaz et al., 2005; Gregerson et al., 1999). Selected strains with superior symbiotic capacities need to be isolated and developed as inoculants (Hardarson and Atkins, 2003; Lupawyi et al., 2000). Periodical assessment of commercial strains under field conditions is essential (Hardarson and Atkins, 2003) and the need to monitor culture variability to maintain the quality of legume inoculants has been also emphasized (Bloem et al., 2002). In Uruguay, *S. meliloti* strain U45 (isolated from alfalfa, Uruguay) was formerly used for alfalfa commercial inoculant. However, variant cultures of this strain exhibited a range of N_2-fixing effectiveness and competitiveness when inoculated onto two alfalfa cultivars (Bloem et al., 2002). Therefore, it has been currently substituted by U143 (MCH3, isolated from alfalfa, Uruguay). *M. loti* strain U226 is used for *Lotus corniculatus*, while strain U531 (NC3, isolated from *L. subbiflorus*, Uruguay) is used for *L. subbiflorus*.

7.3. Diversity of indigenous rhizobia nodulating alfalfa and Lotus *spp.*

The need for rhizobial strains with enhanced N_2 fixation and tolerance to edaphic soil constraints (i.e. pH soil) has been repeatedly emphasized (Catroux et al., 2001 ; Graham and Vance, 2003; Langer et al., 2008). Several authors studied the occurrence, diversity and symbiotic properties of alfalfa-nodulating strains isolated from acid soils of Uruguay and Argentina (Castro-Sowinski et al., 2002a; Del Papa et al., 1999; Segundo et al., 1999). Mid-acid tolerant strains (MAT) able to grow at pH 5.5 but not at pH 5.0 and acid-tolerant (AT) strains able to grow at pH 5.0 were characterized. Ten percent of the indigenous *S. meliloti* population in Uruguayan soils was tolerant to acidic conditions and

PCR analysis of the strains suggested that considerable diversity is present. Symbiotic analysis of the strains confirmed that they have the potential to improve the growth of alfalfa in acidic soils (Castro-Sowinski et al., 2002a) and may be considered for inoculant production (Segundo et al., 1999). Mid-acid tolerant strains have been also characterized for laccase activity and melanin production. Interestingly, plant growth-promoting effect in rice by a laccase-producing *S. meliloti* strain when co-inoculated with *Azospirillum brasilense* was observed [Castro-Sowinski et al., 2002b).

Symbiotic effectiveness and ecological characterization of indigenous rhizobia nodulating *Lotus* spp. has been extensively studied in Uruguay. Immunological, biochemical and genetic properties were described for a large collection of strains (Baraibar et al., 1999; Díaz et al., 1995; Irisarri et al., 1996; Monza et al., 1992; Monza et al., 1997; Monza et al., 2006). Based on colony type and growth rates, isolates from nodules of *Lotus* spp. were separated into two groups corresponding to slow- and fast-growing strains, the first group nodulating *L. subbiflorus* (Irisarri et al., 1996) and the second *L. corniculatus* (Monza et al., 1992; Monza et al., 1997), respectively. Partial 16S rDNA gene sequencing revealed that fast-growing strains could be identified as *Mesorhizobium loti* species and the slow-growers as *Bradyrhizobium* sp. (Monza et al., 2006).

Although specificity is not yet completely defined in the genus *Lotus*, local studies have demonstrated its occurrence within indigenous population, with different level of efficiency Díaz et al., 2005). Irisarri et al. (1996) found that all of the isolates effectively nodulating *L. subbiflorus*, were unable to form effective nodules on *L. corniculatus*. Similarly, Baraibar et al. (1999) and Monza et al. (1992) found that all of the isolates effectively nodulating *L. corniculatus* induced small and ineffective nodules in *L. subbiflorus*. Inability of the inoculant strains to successfully compete with established rhizobia populations in soil has been frequently reported and agronomical implications need to be considered (Díaz et al., 2005). Results reported by Baraibar et al. (1999) also proved that 83% of the indigenous rhizobia nodulating *Lotus* spp. were acid tolerant in culture medium (pH 4.5) and supported the importance of selecting among them the most efficient and resistant strains to be included in the inoculants. As an example, the overwhelming increase in the area of natural grasslands over sown with *L. subbiflorus*, especially adapted to acid soils, has been largely sustained in the selection of the indigenous strain U531 (NC3) for commercial inoculant.

7.4. Final remarks

Research and extension policies valorising rhizobial germplasm study and preservation, farmers training for proper inoculant use and legal enforcement of commercial inoculant quality have proved a successful approach to promote the use of forage legumes while enhancing biological N_2 fixation at a national scale. Interdisciplinary and inter-institutional approaches should be strengthened on a global scale to recognize the ecology of forage legume microbes as a key tool for developing sustainable agricultural systems.

8. Perspectives

The rhizobium-legume symbiosis is important in many fields, not only in agriculture for sustainable production, but also in basic biology as a model for symbiosis, evolution and differentiation. With the global interest in biodiversity rhizobia have also become both friends and foes of taxonomists, and a debate about the naming of species is going on since a few years (Lindström and Young, 2009). The diverging interests (choice of diverse model/type strains) of taxonomists, molecular biologists and agronomists has hampered research into inoculant development, since molecular biologists study certain model strains and species, taxonomist describe new species using other type and representative strains whereas many good inoculant strains are poorly described from molecular and taxonomic points of view. Intellectual property rights will in the future protect also rhizobial biodiversity resources from unfair economical exploitation, but the development might as well lead to increased protectionism which will further hamper free distribution and movement of strains for scientific and agronomic purposes.

However, the examples presented in this article show that much effort is put into increasing the use of BNF and thus sustainability in agriculture. It is our hope that the information about the well-functioning Uruguayan system in combination with the extensive local knowledge in Africa, and the knowledge of current taxonomy as well as filling existing gaps in the knowledge of population genetics, rhizobial genomes and Chronos and Kairos evolution, could help bring the diverse fields of rhizobium research together and prompt further development of the rhizobial commons.

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FIGURE LEGENDS

Fig. 1. Signal exchange in rhizobium-plant symbiosis. Flavonoids produced by the host plant induce rhizobial nod genes. This leads to production of Nod factors. The insert shows an infection thread passing the root cortex toward a cluster of dividing cells that will become a root promordium. (Reprinted from Schultze and Kondorosi 1998, with permission).

Fig.2. Evolution in bacteria: Chronos evolution is the evolution of the bacterial core genome and mediates speciation. Kairos evolution is rapid evolution of the accessory genome and mediates ecological niche adaptation, for example symbiotic adaptation. The filled circles represent bacteria and the small symbols transfer of genetic elements between bacteria.

TABLES

Table 1. Soybean cultivation: area cultivated and quantities produced in 2007. Source: FAOSTAT database accessed at 24 August 2009 (http://faostat.fao.org/site/567/default.aspx#ancor)

	Area (k Ha)	Production (k Tonnes)
World	90.000	220.533
Africa	1.210	1.254
Europe	1.893	2.630
Asia	19.479	27.183
North America	27.131	75.556
South America	40.393	113.747
Oceania	13	32

Table 2. Dairy and meat production: quantities produced in 2007, ranked by value among the 20 most important food and agricultural commodities (value rank number in brackets). Source: FAOSTAT database accessed at 30 September 2009 (http://faostat.fao.org/site/339/default.aspx)

	Cow milk (Tonnes)	Cattle Meat (Tonnes)
World	566850186 (1)	55491236 (3)
Africa	26578917 (4)	4879269 (1)
Europe	208615099 (1)	10721290 (3)
Asia	144423727 (5)	11957639 (10)
North America	92335414 (2)	12422756 (1)
South America	54413949 (4)	12431029 (1)
Oceania	25131511 (1)	680764 (4)
Uruguay	1576000 (2)	572748 (1)

FIGURES

Figure 1.

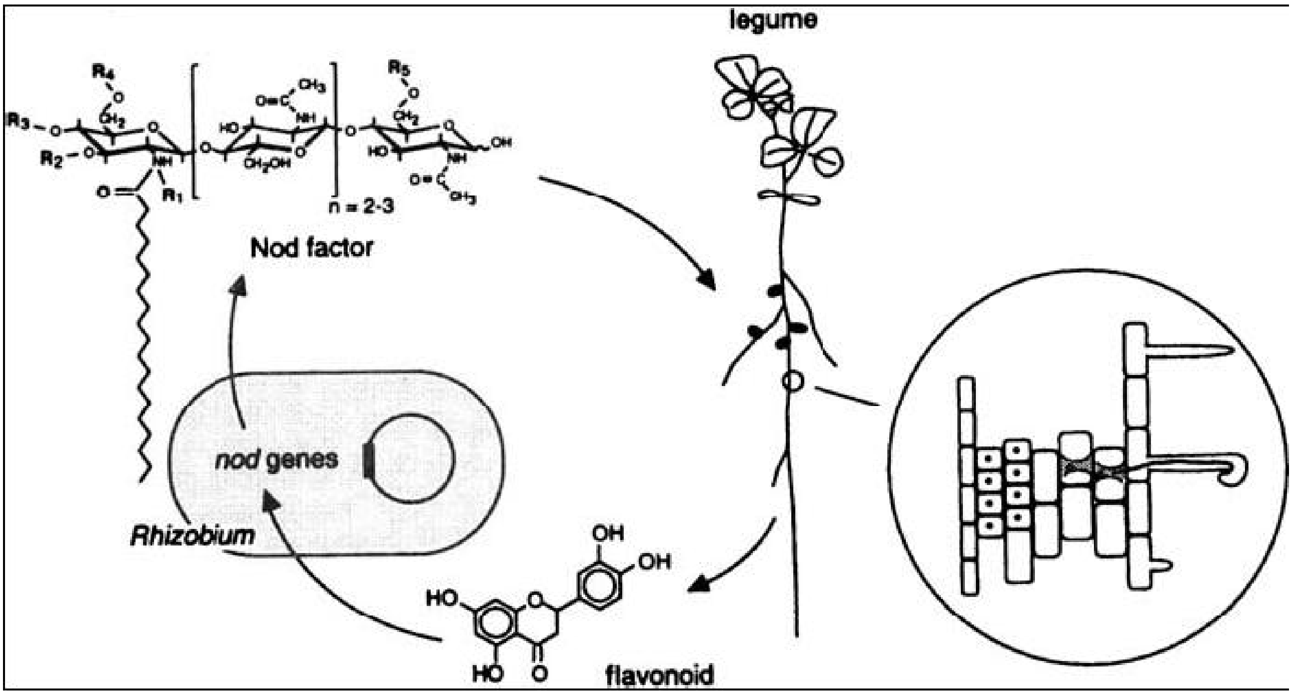

Fig. 1. Signal exchange in rhizobium-plant symbiosis. Flavonoids produced by the host plant induce rhizobial nod genes. This leads to production of Nod factors. The insert shows an infection thread passing the root cortex toward a cluster of dividing cells that will become a root primordium. (Reprinted from Schultze and Kondorosi 1998, with permission).

Supplementary Table 1: Species of rhizobia and the plants they nodulate

Name[1]	**Host plants nodulated**	**References**
Alphaproteobacteria		
Azorhizobium caulinodans	*Sesbania rostrata*	Dreyfus et al., 1988
Azorhizobium doebereinerae	*Sesbania virgata*	Moreira et al., 2006
Bradyrhizobium betae	Isolated from deformed roots of *Beta vulgaris*; unable to reproduce root deformations on *Beta vulgaris*; unable to nodulate *Glycine max* and *Pachyrrhizus ahipa*	Rivas et al., 2004
Bradyrhizobium canariense bv. *genistearum*	*Adenocarpus* spp., *Chamaecytisus proliferus, Spartocytis supranubius, Lupinus* spp., *Teline* spp., *Ornithopus* spp.	Vinuesa et al., 2005
Bradyrhizobium elkanii	*Glycine* spp., *Macroptilium atropurpureum, Vigna* spp.	Kuykendall, 2005
Bradyrhizobium iriomotense	Isolated from nodules of *Entada koshunensis*. Nodulates *Macroptilium atropurpureum*	Islam et al., 2010
Bradyrhizobium japonicum bv. *glycinearum*	*Glycine* spp., *Macroptilium atropurpureum*	Jordan, 1982; Vinuesa et al., 2005
Bradyrhizobium japonicum bv. *genistearum*	*Adenocarpus* spp., *Chamaecytisus proliferus, Spartocytis supranubius, Lupinus* spp., *Teline* spp., *Ornithopus* spp.	Vinuesa et al., 2005
Bradyrhizobium japonicum group I	*Crotalaria pallida, Trifolium repens, Trifolium fragiferum*	Liu et al., 2007
Bradyrhizobium japonicum group Ia	*Crotalaria pallida, Mimosa pudica, Trifolium fragiferum, Trifolium repens*	Liu et al., 2007
Bradyrhizobium jicamae	*Pachyrhizus erosus, Lespedeza* spp.	Ramirez-Bahena et al., 2009
Bradyrhizobium liaoningense	*Glycine soja, Glycine max*	Xu et al., 1995
Bradyrhizobium pachyrhizi	*Pachyrhizus erosus*	Ramirez-Bahena et al., 2009
Bradyrhizobium yuanmingense	*Lespedeza cuneata, Vigna unguiculata, Glycyrrhiza uralensis, Phaseolus lunatus, Vigna mungo, Vigna radiata, Vigna unguiculata*	Appunu et al., 2009; Ormeño-Orrillo et al., 2006; Yao et al., 2002
Devosia neptuniae	*Neptunia natans*	Rivas et al., 2003
Devosia yakushimensis	Isolated from nodules of *Pueraria laobata*; no nodulation reported	Bautista et al., 2010
Ensifer adhaerens (genomovar A)	*Sesbania grandiflora, Medicago sativa, Leucaena leucocephala, Pithecellobium dulce, Lotus arabicus*	Merabet et al., 2010; Willems et al., 2003

Name[1]	Host plants nodulated	References
Ensifer arboris	*Acacia senegal, Prosopis chilensis*	Nick et al., 1999
Ensifer fredii	*Glycine max, Glycine soja, Vigna unguiculata, Cajanus cajan*	Chen et al., 1988
Ensifer garamanticus	*Agyrolobium uniflorum, Medicago sativa*	Merabet et al., 2010
Ensifer kostiense	*Acacia senegal, Prosopis chilensis*	Nick et al., 1999
Ensifer kummerowiae	*Kummerowia stipulacea*	Wei et al., 2002
Ensifer medicae	*Medicago trunculata, Medicago* spp.	Rome et al., 1996
Ensifer meliloti	*Medicago* spp., *Melilotus* spp., *Trigonella*	Dangeard 1926; Rome et al., 1996; Yan et al., 2000
"Ensifer mexicanus"	*Acacia angustissima, Acacia cochliacantha, Leucena leucocephala, Phaseolus vulgaris*	Lloret et al., 2007
Ensifer morelense	*Leucaena leucocephala*	Wang et al., 2002
Ensifer numidicus	*Agyrolobium uniflorum, Lotus creticus*	Merabet et al., 2010
Ensifer saheli	*Sesbania* spp.	de Lajudie et al., 1994
Ensifer terangae	*Sesbania* spp., *Acacia* spp.	de Lajudie et al., 1994
Ensifer terangae bv. sesbaniae	*Sesbania* spp.	Lortet et al., 1996
Ensifer terangae bv.acaciae	*Acacia* spp.	Lortet et al., 1996
Ensifer xinjiangense	*Glycine max*	Chen et al., 1988
Mesorhizobium albiziae	*Albizia kalkora, Albizia julibrissin, Glycine max, Leucaena leucocephala, Phaseolus vulgaris*	Wang et al., 2007
Mesorhizobium alhagi	*Alhagi sparsifolia, Sophora alopecuroides, Glycyrrhiza inflata, Medicago sativa, Indigofera endecaphylla, Vicia cracca, Sophora flavescens*	Chen et al., 2010
Mesorhizobium amorphae	*Amorpha fruticosa, Cicer arietinum*	Rivas et al., 2007; Wang et al., 1999b
Mesorhizobium australicum	*Biserrula pelecinus, Astragalus membranaceus, Macroptilium atropurpureum*	Nandasena et al., 2009
Mesorhizobium camelthorni	*Alhagi sparsifolia, Sophora alopecuroides, Glycyrrhiza inflate, Medicago sativa*	Chen et al., in press.
Mesorhizobium caraganae	*Caragana microphylla, Caragana intermedia*	Guan et al., 2008
Mesorhizobium chacoense	*Prosopis alba*	Velázquez et al., 2001

Name[1]	**Host plants nodulated**	**References**
Mesorhizobium ciceri	*Cicer arietinum*	Nour et al., 1994
Mesorhizobium gobiense	*Astragalus filicaulis, Lotus frondosus, Lotus tenuis, Oxytrophis glabra*	Han et al., 2008a
Mesorhizobium huakuii	*Astragalus sinicus, Robinia pseudoacacia*	Chen et al. 1991; Ulrich and Zaspel, 2000
Mesorhizobium loti	*Lotus* spp., *Anthyllis vulneraria, Lupinus densiflous, Robinia pseudoacacia*	Jarvis et al., 1982, Ulrich and Zaspel, 2000
Mesorhizobium mediterraneum	*Cicer arietinum*	Nour et al., 1995
Mesorhizobium metallidurans	*Anthyllis vulneraria*	Vidal et al., 2009
Mesorhizobium opportunistum	*Biserrula pelecinus, Astragalus adsurgens, Astragalus membranaceus, Lotus peregrinus, Macroptilium atropurpureum*	Nandasena et al., 2009
Mesorhizobium plurifarium	*Acacia* spp., *Leucena* spp., *Prosopis juliflora, Chamaecrista ensiformis*	de Lajudie et al., 1998b
Mesorhizobium robiniae	*Robinia pseudoacacia*	Zhou et al., in press.
Mesorhizobium septentrionale	*Astragalus adsurgens, Phaseolus vulgaris, Glycine max, Leucaena leucocephala, Macroptilium atropurpureum, Lotus corniculatus, Robinia pseudoacacia*	Gao et al., 2004; Han et al., 2008a
Mesorhizobium shangrilense	*Caragana* spp., *Glycyrrhiza uralensis, Astragalus adsurgens, Vigna unguiculata, Vigna radiata, Phaseolus vulgaris*	Lu et al., 2009b
Mesorhizobium tarimense	*Lotus frondosus, Lotus tenuis*	Han et al., 2008a
Mesorhizobium temperatum	*Astragalus adsurgens, Phaseolus vulgaris, Vigna unguiculata, Glycine max, Leucaena leucocephala, Medicago sativa, Lotus corniculatus*	Gao et al., 2004
Mesorhizobium thiogangeticum	Isolated from the rhizosphere of *Clitoria ternatea* although it did not nodulate this host	Ghosh and Roy, 2006
Mesorhizobium tianshanense	*Glycyrrhiza, Sophora, Caragana, Halimodendron, Swainsonia, Glycine, Cicer arietinum*	Chen et al., 1995; Rivas et al., 2007
Methylobacterium nodulans	*Crotalaria* spp.	Jourand et al., 2004
Ochrobactrum cytisi	*Cytisus scoparius*	Zurdo-Pineiro et al., 2007

Name[1]	**Host plants nodulated**	**References**
Ochrobactrum lupini	*Lupinus albus, Lupinus honoratus*	Trujillo et al., 2005
Phyllobacterium trifolii	*Trifolium pratense, Trifolium repens, Lupinus albus*	Valverde et al., 2005
Phyllobacterium ifriqiyense	Isolated from root nodules of *Lathyrus numidicus* and *Astragalus algerianus*; no nodulation data	Mantelin et al., 2006
Phyllobacterium leguminum	Isolated from root nodules of *Astragalus algerianus, Agyrolobium uniflorum*; no nodulation data	Mantelin et al., 2006
Rhizobium alamii	*Medicago ruthenica* (most strains isolated from *Arabidopsis* rhizosphere)	Berghe et al., 2009
Rhizobium alkalisoli	*Caragana intermedia, Caragana microphylla, Phaseolus vulgaris, Vigna radiata*	Lu et al., 2009a
Rhizobium cellulosilyticum	Isolated from poplar saw dust. One strain forms ineffective nodules on *Medicago sativa*	Garcia-Fraile et al., 2007
Rhizobium daejeonense	Isolated from a cyanide treatment bioreactor; nodulates *Medicago sativa*	Quan et al., 2005
R. etli bv. mimosae	*Phaseolus vulgaris, Mimosa affinis, Leucaena leucocephala*	Wang et al., 1999a
R. etli bv. phaseoli	*Phaseolus vulgaris, Phaseolus coccineus*	Segovia et al., 1993; Souza et al., 1994
Rhizobium fabae	*Vicia faba*	Tian et al., 2008
Rhizobium galegae	*Galega officinalis, Galega orientalis*	Lindström, 1989
R. galegae bv. officinalis	*Galega officinalis*	Radeva et al., 2001
R. galegae bv. orientalis	*Galega orientalis*	Radeva et al., 2001
R. gallicum bv. gallicum	*Phaseolus vulgaris, Phaseolus coccineus, Macroptilium atropurpureum, Onobrychis viciifolia, Leucaena leucocephala*	Amarger et al., 1997
R. gallicum bv. phaseoli	*Phaseolus vulgaris, Macroptilium atropurpureum*	Amarger et al., 1997
R. giardinii bv. giardinii	*Phaseolus vulgaris, Macroptilium atropurpureum, Leucaena leucocephala*	Amarger et al., 1997
R. giardinii bv. phaseoli	*Phaseolus vulgaris, Macroptilium atropurpureum*	Amarger et al., 1997
R. giardinii	*Desmanthus illinoensis, Desmanthus leptolobus, Dalea purpurea, Psoralea esculenta, Prosopis juliflora*	Beyhaut et al., 2006

Name[1]	**Host plants nodulated**	**References**
Rhizobium hainanense	*Desmodium* spp., *Centrosema pubescens, Stylosanthes guyanansis, Tephrosia candida, Acacia sinuate, Zornia diphylla, Macroptilium lathyroides*	Chen et al., 1997
Rhizobium huautlense	*Sesbania herbacea, Leucaena leucocephala*	Wang et al., 1998
Rhizobium indigoferae	*Indigofera spp.*	Wei et al., 2002
Rhizobium (*Agrobacterium*) *larrymoorei*	Isolated from aerial tumours of *Ficus benjamina*. No nodulation data	Bouzar and Jones, 2001
Rhizobium leguminosarum	*Pisum* spp., *Lathyrus* spp., *Vicia* spp., *Lens* spp., *Phaseolus* spp., *Trifolium* spp., *Robinia pseudoacacia, Carmichaelia australis, Clianthus puniceus, Sophora chathamica*	Frank 1889; Ramirez-Bahena et al., 2008; Ulrich and Zaspel, 2000; Weir et al., 2004;
Rhizobium loessense	*Astragalus* spp.	Wei et al., 2003
Rhizobium lusitanum	*Phaseolus vulgaris, Macroptilium atropurpureum, Leucaena leucocephala*	Valverde et al., 2006
Rhizobium mesosinicum	*Albizia julibrissin, Kummerowia stipulacea, Kummerowia striata, Dalbergia* spp.	Lin et al., 2009
Rhizobium miluonense	*Lespedeza* spp.	Gu et al., 2008
Rhizobium mongolense[2]	*Medicago ruthenica, Phaseolus vulgaris*	van Berkum et al., 1998
Rhizobium multihospitium	*Lotus* spp., *Alhagi toum, Astragalus* spp., *Halimodendron halodendron, Oxytropis* spp., *Robinia pseudoacacia, Sophora pecuriodes, Caragana jubata, Lathyrus odoratus, Vicia hirsuta*	Han et al., 2008b
Rhizobium oryzae	Isolated from surface-sterilized roots of *Oryza alta*. Strains nodulate *Phaseolus vulgaris* and *Glycine max*	Peng et al., 2008
Rhizobium phaseoli	*Phaseolus vulgaris*, *Trifolium repens*	Ramirez-Bahena et al., 2008
Rhizobium pisi	*Pisum sativum*, *Trifolium repens, Phaseolus vulgaris*	Ramirez-Bahena et al., 2008
Rhizobium (*Agrobacterium*) *radiobacter*	Pathogenic strains on various plants as well as non-pathogenic strains. No nodulation reported[3]	Young et al., 2005
Rhizobium (*Agrobacterium*) *rhizogenes*	Pathogenic strains on various plants as well as non-pathogenic strains. No nodulation reported[3]	Young et al., 2005

Name[1]	**Host plants nodulated**	**References**
Rhizobium (*Agrobacterium*) *rubi*	Pathogenic on *Rubus* spp. No nodulation data	Young et al. 2005
Rhizobium selenitireducens	no data	Hunter et al., 2007
Rhizobium soli	Isolated from soil, no plant hosts reported	Yoon et al., in press.
Rhizobium sullae	*Hedysarum coronarium*	Squartini et al., 2002
Rhizobium tibeticum	*Trigonella archiducis-nicolai, Medicago lupulina, Medicago sativa, Melilotus officinalis, Phaseolus vulgaris, Trigonella foenum-graecum*	Hou et al., 2009
Rhizobium tropici	*Phaseolus vulgaris*, *Leucaena* spp., *Robinia pseudoacacia, Gliricidia sepium*	Acosta-Durán et al., 2002; Martínez-Romero et al., 1991; Ulrich and Zaspel, 2000
Rhizobium tubonense	Isolated from *Oxytropis glabra* but unable to renodulate this host. Effective nodules on *Vigna unguiculata* and *Medicago sativa*	Zhang et al., in press.
Rhizobium (*Allorhizobium*) *undicola*	*Neptunia natans*	de Lajudie et al., 1998a
Rhizobium vignae	*Astragalus dahuricus, A. oxyglottis, Vigna radiata, Desmodium microphyllum*	Ren et al., in press.
Rhizobium (*Agrobacterium*) *vitis*	Non-pathogenic or pathogenic on *Vitis* spp. or other dicotyledonous plants. No nodulation data	Young et al., 2005
Rhizobium yanglingense[2]	*Gueldenstaedtia multiflora, Coronilla varia, Amphicarpaea trisperma*	Tan et al., 2001
Shinella kummerowiae	Isolated from the root nodules of *Kummerowia stipulacea*, but unable to renodulate this host	Lin et al., 2008
"Sinorhizobium abri"[4]	*Abrus precatorius*	Ogasawara et al., 2003
Sinorhizobium americanum[4]	*Acacia* spp.	Toledo et al., 2003
"Sinorhizobium indiaense"[4]	*Sesbania rostrata*	Ogasawara et al., 2003
Betaproteobacteria		
Burkholderia caribensis	*Mimosa pudica, Mimosa diplotricha*	Chen et al., 2003; Vandamme et al., 2002
Burkholderia dolosa	One strain isolated from *Alysicarpus glumaceus*	Vandamme et al., 2002; Vermis et al., 2004
Burkholderia mimosarum	*Mimosa pigra, Mimosa scabrella*	Chen et al., 2006
Burkholderia nodosa	*Mimosa bimucronata, Mimosa scabrella*	Chen et al., 2007
Burkholderia phymatum	*Machaerium lunatum, Mimosa invisa*	Elliott et al., 2007b; Vandamme et al., 2002
Burkholderia sabiae	*Mimosa caesalpiniifolia*	Chen et al., 2008

Name[1]	**Host plants nodulated**	**References**
Burkholderia tuberum	*Aspalathus carnosa*, *Cyclopia* spp., *Macroptilium atropurpureus*	Elliott et al., 2007a; Vandamme et al., 2002
Cupriavidus taiwanensis	*Mimosa pudica, Mimosa diplotricha, Mimosa pigra*	Chen et al., 2001; Chen et al., 2003; Chen et al., 2005
Herbaspirillum lusitanum	*Phaseolus vulgaris*	Valverde et al., 2003

[1] Names in quatation marks have been published outside Int. J. Syst. Evol. Microbiol. and have not yet been validated.

[2] Silva et al. (2005) proposed that *R. mongolense* and *R. yanglingense* be considered as a biovar (bv. orientale) of *R. gallicum.*

[3] Strains formerly classified as agrobacteria are sometimes present in nodules, but have not been shown to induce nodulation on their own unless a symbiotic plasmid is present (Velazquez et al., 2005; Wang et al., 2006).

[4] This species belongs to the *Ensifer* phylogenetic cluster but was described before the transfer of *Sinorhizobium* to *Ensifer* (Young et al., 2003).

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