Critical Reviews in Plant Sciences, 21(4):323–378 (2002)

Advances in *Rhizobium* Research

A. Sessitsch,[1] J.G. Howieson,[2] X. Perret,[3] H. Antoun,[4] and E. Martínez-Romero[5]*

[1]ARC Seibersdorf research GmbH, Division of Environmental and Life Sciences, A-2444 Seibersdorf, Austria; [2]Centre for *Rhizobium* Studies, Murdoch University, Murdoch 6150, Western Australia; [3]Laboratoire de Biologie Moléculaire des Plantes Supérieures, Université de Genève, 1292 Chambésy, Geneva, Switzerland; [4]Recherche en Sciences de la Vie et de la Santé, Université Laval, Quebec, Canada; [5]Centro de Investigacion Sobre Fijacion de Nitrogeno, UNAM, Cuernavaca, Morelos, Mexico

Referee: Prof. Dr. Dietrich Werner, FG Zellbiologie und Angewandte Botanik, Fachbereich Biologie, Philipps-Universität Marburg, Karl-von-Frisch-Strasse, D-35032 Marburg, Germany

* Author for correspondence. (Tel: +43 50550 3509; fax: +43 50550 3444; e-mail: angela.sessitsch@arcs.ac.at)

ABSTRACT: Rhizobia are well known for their capacity to establish a symbiosis with legumes. They inhabit root nodules, where they reduce atmospheric nitrogen and make it available to the plant. Biological nitrogen fixation is an important component of sustainable agriculture, and rhizobial inoculants have been applied frequently as biofertilizers. In this review we present recently developed technologies and strategies for selecting quality inoculant strains by taking into consideration the complex interaction between the edaphic environment with the genotypes of both the legume and its microsymbiont. Enhanced competitive ability in an inoculant strain is a key requirement for successful colonization of plant roots, nodule formation, and subsequent N_2-fixation. We discuss several avenues for the management and manipulation of rhizobial competition as well as genes that influence competition in the rhizosphere. The use of molecular techniques has greatly contributed to our knowledge of nodule-bacterial diversity and phylogeny. Approaches to the study of rhizobial diversity as well as mechanisms for the evolutionary diversification of nodulating bacteria are presented. *Rhizobium* genomes ranging from 5.5 to 9 Mb have been sequenced recently and deposited in public databases. A comparison of sequence data has led to a better understanding of genes involved in the symbiotic process as well as possible mechanisms responsible for horizontal transfer of genetic elements and symbiosis genes among rhizobia. Furthermore, rhizobia are frequent rhizosphere colonizers of a wide range of plants and may also inhabit nonleguminous plants endophytically. In these rhizospheric and endophytic habitats they may exhibit several plant growth-promoting effects, such as hormone production, phosphate solubilization, and the suppression of pathogens.

KEY WORDS: biological nitrogen fixation, rhizobial competition, diversity, genome structure, plant growth promotion.

I. INTRODUCTION

Rhizobia encompass a range of bacterial genera, including *Rhizobium*, *Bradyrhizobium, Sinorhizobium, Mesorhizobium, Allorhizobium,* and *Azorhizobium*, which are able to establish a symbiosis with leguminous plants. They elicit the formation of specialized organs, called nodules, on roots or stems of their hosts, in which they reduce atmospheric nitrogen and make it available to the plant. Symbiotic nitrogen fixation is an important source of nitrogen, and the various legume crops and pasture species often fix as much as 200 to 300 kg nitrogen per hectare (Peoples et al., 1995). Globally, symbiotic nitrogen fixation has been estimated to amount to at least 70 million metric tons of nitrogen per year (Brockwell et al., 1995). In 1999, world consumption of fertilizer nitrogen was 88 million tons and apart from the consumption of nonrenewable energy sources, environmental pollution from fertilizer nitrogen escaping the root zone is high because in many cases nitrogen fertilizers are not used efficiently by crops (Peoples et al., 1994). Therefore,

0735-2689/02/$.50

biological nitrogen fixation is an important and integral component of sustainable agricultural systems. Furthermore, biological nitrogen fixation from legumes offers more flexible management than fertilizer nitrogen because the pool of organic nitrogen becomes slowly available to nonlegume species (Peoples et al., 1995). Concomitant with N_2-fixation, the use of legumes in rotations offers control of crop diseases and pests (Robson, 1990; Graham and Vance, 2000).

Inoculation of legumes with rhizobial strains selected for high N_2-fixing capacity can improve nitrogen fixation in agriculture, particularly when local rhizobial strains are absent from soils or ineffective. However, newly introduced strains often fail to compete with well-adapted indigenous populations. Substantial efforts have been undertaken to improve nodulation by trying to understand the factors that affect the interactions between rhizobia and soil fauna, between macro- and microsymbionts, as well as between these components and the edaphic environment. Strategies to facilitate decision making regarding the choice of legume and — if required — the choice of an appropriate inoculant strain of rhizobia are addressed in Section II of this review.

Our understanding of the vast diversity of nodulating bacteria has increased extraordinarily due to the application of molecular markers in bacterial taxonomy. The search for novel rhizobia has not only expanded our knowledge on the evolution of nodule bacteria, but also provided very valuable information on strains with varying nodulating and N_2 fixation capabilities as well as different physiological properties. These aspects are discussed in section IV. Furthermore, considerable knowledge has been obtained on the diversity and beneficial effects of rhizobia living in association with nonleguminous plants. The plant growth-promoting effects of free-living rhizosphere rhizobia as well as of endophytic strains are reviewed in Section VI.

The interaction between legume and rhizobia has been studied intensively at a molecular level. This interaction starts with a signal exchange between both partners. Plant roots secrete specific flavonoids that interact with the bacterial NodD protein, resulting in the activation of rhizobial *nod* genes and synthesis of Nod factors. Perception and transduction of these key signal molecules for nodule organogenesis has been reviewed recently (Broughton et al., 2000; Miklashevichs et al., 2001). Genes involved in the nodulation process or in determining competitive ability have also been identified recently and are addressed in this review (see Section III), as are several attempts to improve nitrogen fixation by genetic engineering. Furthermore, the sequence information of whole rhizobial genomes (Kaneko et al., 2000; Galibert et al., 2001) or symbiotic islands (Freiberg et al., 1997; Göttfert et al., 2001) has become available, providing a more complete picture of rhizobial genomes, which is discussed in Section V.

The objective of this review is to synthesize fundamental results from very early research reports with modern outputs. We hope the outcome of our efforts is to stimulate research at many levels, from the field to the laboratory, in this vital process of symbiotic N_2-fixation.

II. ADVANCES IN TECHNOLOGIES AND STRATEGIES FOR SELECTING INOCULANT QUALITY RHIZOBIAL STRAINS

In this section, we review advances in technologies and strategies for selecting inoculant quality strains of root-nodule bacteria for the purpose of maximizing legume derived N_2-fixation in agriculture. This objective has been a pursuit of scientists for over 100 years, but it is especially relevant in today's world because biological N_2-fixation is considered an essential element of agricultural sustainability. The Bellagio conference on N_2-fixation (Kennedy and Cocking, 1997) acknowledged that with the decline in the price of manufactured fertilizer in the 1990s, biological N_2-fixation with legumes and rhizobia, was most likely to remain in extensive, rather than intensive, agricultural systems. However, Graham and Vance (2000) warned that a world decline in agricultural dependence on biological nitrogen fixation was incompatible with the need to increase world protein production from a notably deteriorating area of global arable land. The urgency for advancement in the quality and utilization of

rhizobial inoculants (and legumes) in both intensive and extensive forms of world agriculture was promulgated with some force and clarity. The potential economic benefits of improving global N_2-fixation in agriculture by 10% have been estimated at close to US$ 1 billion annually (Herridge and Rose, 2000).

Relative to the almost weekly advances made in our understanding of the genetics of the root nodule bacteria, which are covered in later sections of this review, advances made in rhizobial ecology appear pedestrian. This is not an indictment of the outputs from rhizobial ecologists, but more an affirmation of the complexities of dealing with the multiple interactions of the edaphic environment (E) with the genotypes of both the legume (G_l) and its microsymbiont (G_r).

There are a number of routes to improved N_2-fixation, including plant breeding and selection, legume adoption, and correct inoculant usage. However, preeminent rhizobial ecologists have suggested that 90% of all rhizobial inoculants applied are of no practical benefit to the productivity of legumes (Brockwell and Bottomley, 1995). While this analysis probably underestimates the 'insurance factor' in second crop inoculation, our challenge in achieving an increase in global N_2-fixation may well commence with a dissection of the rationale for this latter claim.

Brockwell and Bottomley (1995) considered a combination of flawed inoculant technology, substandard inoculant strains and poor decision making in the use of inoculants as the key factors contributing to the inefficient global usage of rhizobial inoculants. Current inoculant technologies have been reviewed recently by Lupwayi et al. (2000) and Date (2001). Therefore, in this section of the review we concentrate on decision making prior to an assessment of inoculation requirement, then prior to the commencement of a rhizobial selection program, following which we review methodologies appropriate for the selection of inoculant quality rhizobia. In so doing, we hope to develop a rationale that, if followed, will improve global N_2-fixation and overcome the constraints to effective inoculant usage identified by Brockwell and Bottomley (1995).

A. Decision Making Relative to the Adoption of Legumes and in Assessment of the Requirement to Inoculate

The integration of legumes into farming systems is acknowledged to have wide-ranging benefits, the most important being N_2-fixation and the control of cereal crop diseases and pests (Robson, 1990; Graham and Vance, 2000). Eight other ancillary benefits to legume cultivation have been listed by Howieson et al. (2000a). Although legumes have been used since antiquity in human diets on all continents (Saxena, 1988), and they represent a significant component of many of the unimproved grasslands of the world (Snaydon 1987; Cocks and Bennett, 1999), legumes only represent a minor proportion of the plants in most managed agricultural enterprises. Given their obvious value, why should this be the situation?

In the developing world, productivity in the less managed and extensive semiarable regions may be underpinned by a legume base that is in ecological equilibrium with grasses and herbs (Snaydon, 1987). However, bio-economic analysis applied to moderate-sized farms in the developed world indicates that legumes are only adopted where they are transparently profitable (Pannell and Bathgate, 1991). Furthermore, and most relevant to this review, the on-farm decision to adopt legumes may be impacted by the necessity to inoculate with root-nodule bacteria specific to that legume. For example, a fixed cost of inoculant materials of between $5 and $10 per hectare combined with the opportunity cost of labor required in the inoculation operation is sufficient to alter the decision-making process against the adoption of legumes in low-input agricultural enterprises (Howieson, 1995). Similarly, in small landholdings in the developing world, legume adoption is clearly enhanced if inoculation is not required (Mpepereki et al., 2000). With this as background, it can be seen that the decision to embark on a research program to develop legumes and their inoculants for farming systems requires substantial premeditation. It is futile to begin if the chances of legume adoption (or inoculation) are low because of economic or other constraints after their use.

B. Separating the Decision Making into Components

A series of flow diagrams illustrating the decision-making processes required to increase N_2-fixation in agriculture is shown below. Figure 1 provides an overview of these routes for decision making. The first step in the process is to analyze whether legume usage is required in the particular farming system of focus and whether a suitable legume is commercially available (Figure 1 and Table 1—component A). The second step is to assess whether legume inoculation is required, whether it can be implemented or whether it can be avoided. This may necessitate a simple field or pot experiment being conducted at a representative site (Figure 2—component B), and if a positive response to inoculation is detected, then a strategy for increasing N_2-fixation must be developed (Figure 3—component C). The techniques for this activity as illustrated are covered in component D. These strategies must be holistically reconciled with the capacity to deliver appropriate inoculant technology to the farming system and its prospects for adoption.

C. Developing and Introducing Legumes to Farming Systems — Is it Profitable? (Table 1—Component A)

Cereals, oilseeds, grasses, and herbs produce higher protein grains and higher yields when grown after, or in conjunction with, legumes (Rowland et al., 1988; Armstrong et al., 1997; Dakora and Keya, 1997). The transfer of symbiotic N through soils to nonlegumes can readily be quantified with modern isotopic techniques (Unkovich and Pate, 2000; van Kessel and Hartley, 2000). Because of this, in many farming systems legumes are not grown as continuous crops or pastures, but are rotated with a non-leguminous crop such as cere-

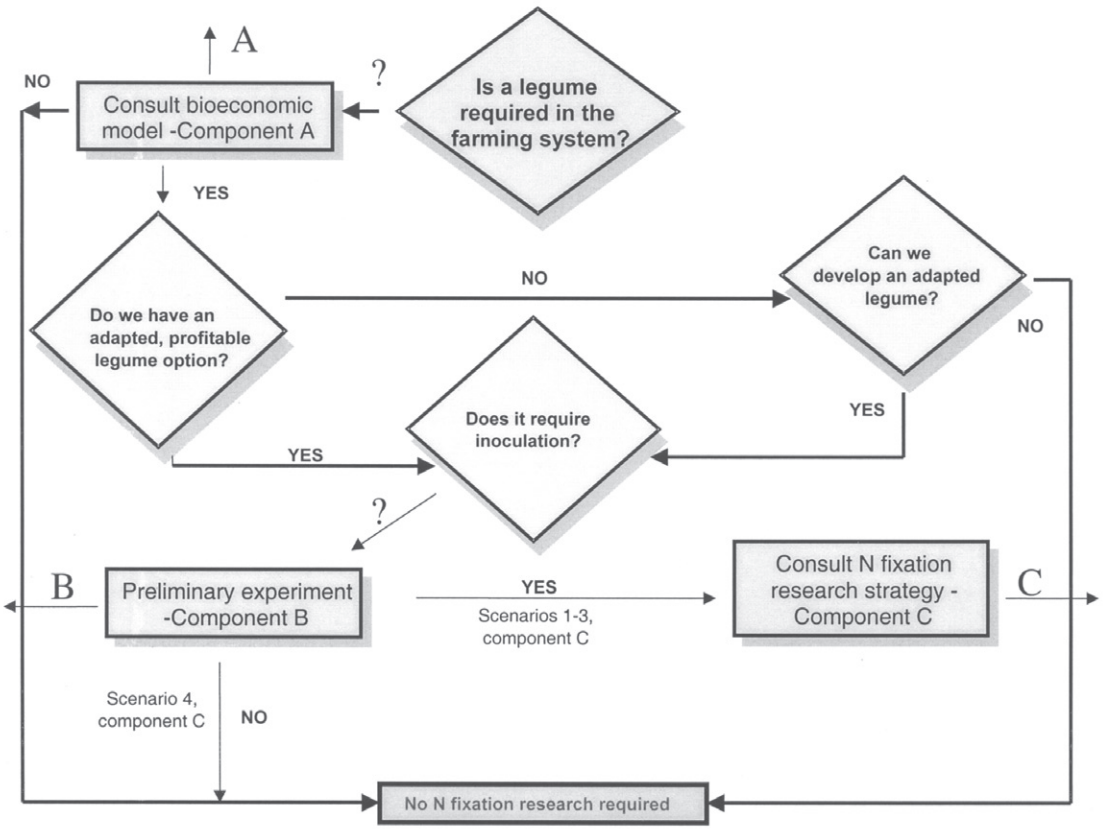

FIGURE 1. A flow chart illustrating the range of decisions required to be considered prior to initiating a legume or rhizobial selection program.

TABLE 1
Component A. The Most Profitable Land Use Options for a Hypothetical Farm in a Low Input Dryland Mediterranean Environment. The Negative Values Represent the $/ha That This Option Is Less Profitable Than the Optimal Land Use, the Number of ha of Which Is Represented in Shaded Type

	soil 1	soil 2	soil 3	soil 4	soil 5	soil 6	soil 7
	500	500	250	250	375	500	125
PPPP	500,00	-10,08	250,00	-16,39	-12,86	500,00	-46,93
PPPW	-11,79	-12,78	-4,85	-12,40	-21,76	-16,95	-51,29
PPPO	-3,69						
PPW	-16,03	-16,62	-12,22	-17,31	-22,76	-19,49	-52,69
PPO	-2,78						
PWPW	-17,24	-32,90	-25,28	-23,80	-30,11	-34,31	-57,80
POPO	-15,92						
PPWW	-21,68	-17,27	-17,10	-17,15	-18,70	-25,48	
PPCWW		-10,21	0,00	-7,68	-5,00	-23,63	-27,46
PWW	-33,55	-30,99	-35,17	-33,31	-38,74	-26,90	-46,92
PWB		-40,21	-39,97	-27,15	-37,11	-38,12	-44,74
PWWW	-42,36	-39,24	-36,44	-26,92	-38,14	-49,93	-50,38
PWWB		-40,21	-37,80	-24,22	-36,92	-44,57	-42,93
PPPT	-22,29						
WWWW	-56,59	-57,31	-48,73	-34,09	-34,78	-45,21	-63,12
WWWB		-64,41	-56,51	-38,12	-38,46	-62,91	-44,89
CWBLD		-9,49	-8,74	-10,75	-23,99		
CWBL		-8,64	-8,37	-10,91	-24,55		
CWWLD		-0,85	-2,87	-6,26	-22,04		
CWWL		500,00	-2,51	-6,43	-23,95		
WCBLD		-11,30	-11,81	-11,26	-27,90		
WCBL		-10,45	-11,56	-11,42	-28,46		
WCWLD		-6,19	-8,50	-12,96	-31,46		
WCWL		-5,34	-8,26	-13,12	-33,37		
CWPWLD		-9,37	-10,63	-20,51	-40,79		
CWPWL		-8,69	-10,44	-20,00	-40,61		
WF				-31,19	-22,19	-52,70	-28,29
WWF				-9,56	-4,13	-36,08	-12,18
WBF				-15,88	-9,64	-34,37	-11,13
WWWF				-11,83	-7,93	-38,98	-16,57
WWBF				-10,48	-6,22	-33,20	-8,71
WCBF				-4,07	-2,11	-35,59	-5,28
CWBF				-3,86	-1,96	-30,62	0,00
WK				-28,07	-22,76	-49,02	-32,74
WWK				-8,42	-5,51	-34,90	-16,25
WBK				-18,90	-15,23	-37,29	-19,30
WWWK				-11,33	-9,37	-38,69	-20,10
WWBK				-10,44	-8,16	-32,66	-12,66
WCBK				-0,21	-0,14	-31,87	-5,28
CWBK				250,00	375,00	-26,23	125,00
WV				-24,34	-24,48	-45,20	-34,14
WWV				-7,60	-8,32	-34,01	-18,84
WBV				-13,91	-13,87	-32,25	-17,74
WWWV				-1,41	-2,17	-30,70	-12,74
WWBV				-10,44	-10,89	-33,29	-15,22
WCBV				-0,21	-2,82	-31,15	-7,79
CWBV				0,00	-2,68	-26,19	-2,52

Note: P, pasture; W, wheat; B, barley; L, lupin; LD, lupin sown dry; C, canola; K, chickpea; F, field pea; V, vetch; O, oats.

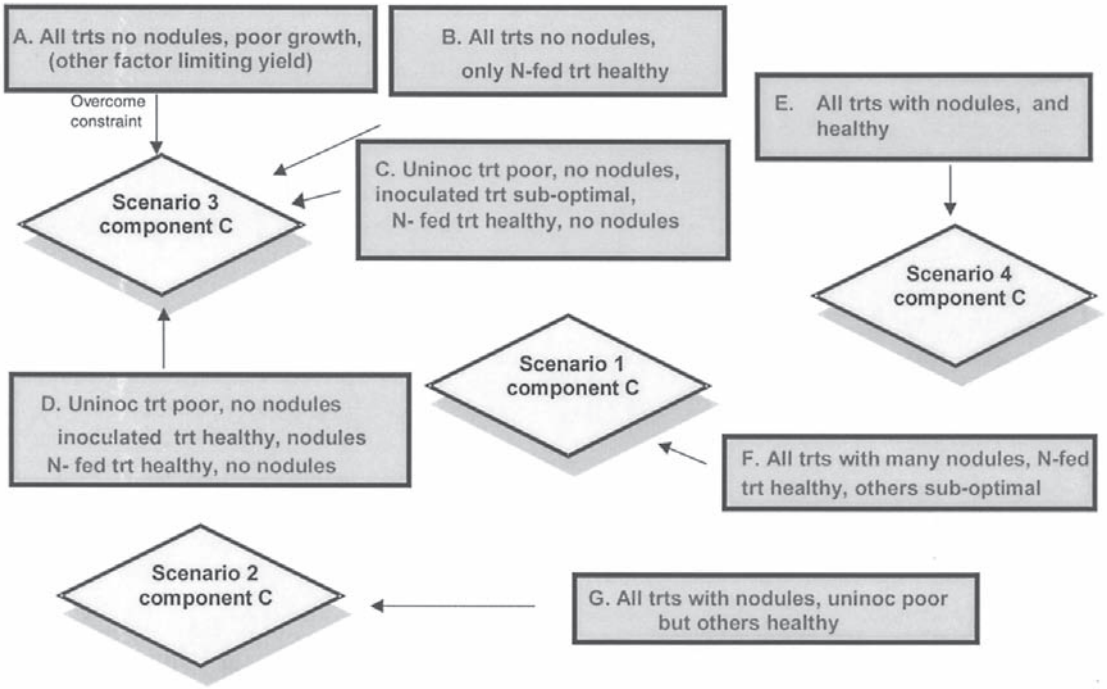

FIGURE 2. Component B — the possible outcomes of a preliminary inoculation experiment to determine if a legume requires inoculation in a particular soil. The experiment has three legume treatments-uninoculated, inoculated with a "best bet" strain, and N-fertilized (after Date, 1977). The ensuing research requirements are represented in component C, Figure 3.

als or oilseeds. However, different soil types are more or less conducive to legume production, and therefore yields may vary, with concomitant impact on the following crop production and profitability. Because of their complexity, it is convenient to model these quantifiable biotic and abiotic interactions to aid in answering the question "can a legume be profitable if introduced to the farming system?"

Table 1 (component A) represents a set of options for utilizing legumes in a hypothetical dryland farm in a Mediterranean environment (average growing season rainfall 229 mm) as predicted by the MIDAS bio-economic model version 4 (Pannell and Bathgate, 1991).

The model farm has seven soil types increasing in clay content from less than 10% (soil 1) to more than 50% (soil 7). The numbers in shaded type in Table 1 represent the hectares selected by the model for the most profitable rotational option on that soil type. On five of the seven major soil types represented in this model, cereal–legume rotations are the most profitable land use; however, several alternative options are only marginally less profitable (as indicated by the shadow costs that show the lower profitability for this option in $/ha). For example, on soil 4 the model selects 250 ha of a cereal-canola-chickpea rotation (CWBK) as being the most profitable. However, cropping wheat with a vetch crop every fourth year (WWWV) is only $1.41/ha less profitable in every year. Thus, a significant increase in the cost of the chickpea component would see WWWV become the most profitable option. In this scenario, if second crop inoculation in the chickpea phase was removed (at a saving of approximately $10 per ha.) the selected option would become even more financially attractive, particularly if productivity was unaffected.

On the poorest acid sandy soils (1 and 3) permanent pasture is identified as the most profitable option because the soils are too infertile for reliable grain production. On soil 3, the introduction of a canola-cereal-lupin rotation (CWWL) would be competitive with permanent pasture if increased profitability of less than $3/ha. was achieved. The selection of an effective and persistent inoculant strain of lupin rhizobia would be an

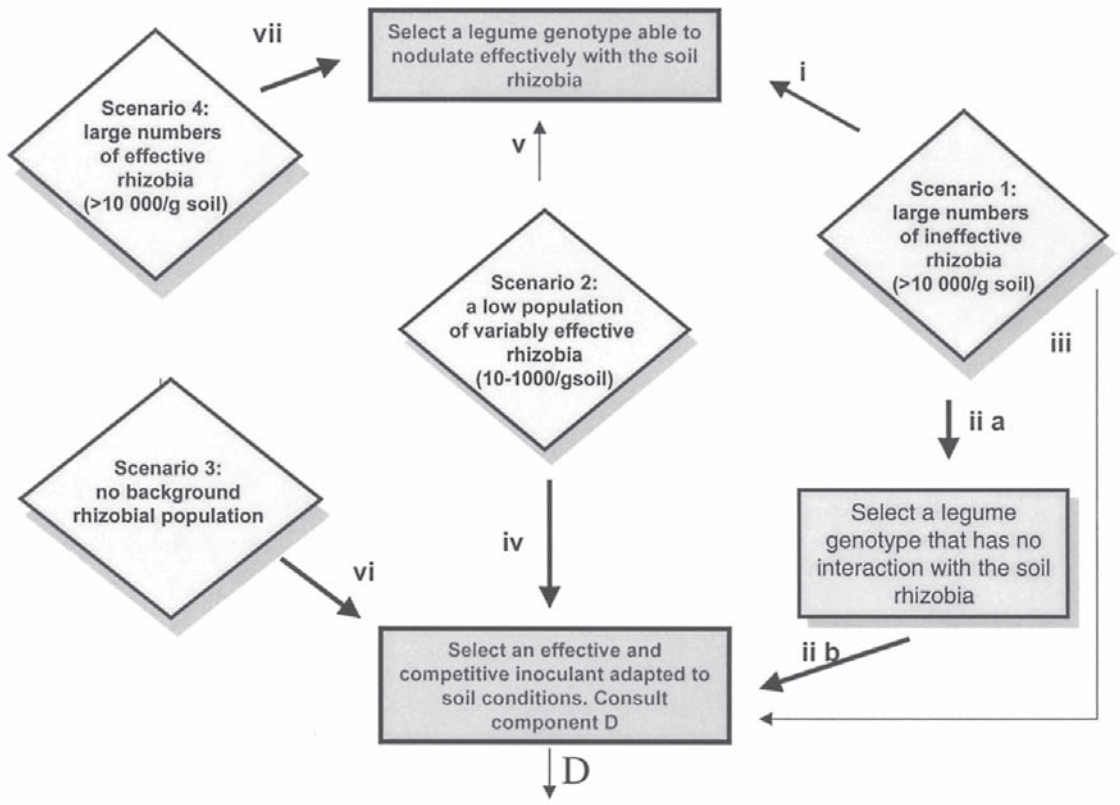

FIGURE 3. Component C — research strategies for increasing N_2-fixation (after Howieson and McInnes, 2001). Examples of successful outcomes from research strategies I-VII are shown in Table 2.

imperative if this scenario were to be economically competitive. Repeat inoculation would not be economically pragmatic.

Thus, for both of these examples, the selection of an inoculant strain that was sufficiently well adapted to the edaphic environment to obviate second crop inoculation would markedly increase the profitability of legume usage. The cost of inoculation could be effectively amortized over several successive legume phases.

In putting these model predictions into a research context, if a legume is to be introduced to the farm, or remain part of the most profitable farming system on these soil types, the cost of rhizobial inoculant must be minimized. To achieve this, the legume must nodulate effectively, in the long term, with the soil population of root-nodule bacteria that (ideally) will have developed following an original inoculation with an elite strain. This, then, would be a sensible research objective directed at overcoming some of the constraints to effective inoculant usage, such as poor decision making in the use of inoculants, as identified by Brockwell and Bottomley (1995).

However, effective and abundant second crop nodulation can never be assumed. Evaluating this aspect of legume science has been a fundamental preoccupation of rhizobial ecologists for many years. To investigate whether a legume can indeed nodulate and achieve an optimal symbiosis with soil-borne rhizobia, the focus of the decision making process shifts to Figure 2—component B.

D. Assessment of Inoculation Requirement (Components B and C)

The principles for investigating legume response to inoculation have not varied substantially since Hellriegel (1886) and Hellriegel and Wilfarth (1888) demonstrated the unequivocal role of bacteria in nodule formation. Inoculation of legumes with a thin coating of soil, taken from a field where the crop had previous been grown

successfully, rapidly became a standard practice (Fred et al., 1932). Inoculation was often not pursued when a legume was to be grown in the same field for a second time. One hundred years later, Thies et al. (1991) proposed a predictive model for inoculation response based on the N status of the soil combined with the size of the rhizobial population in the soil. The response to inoculation appeared to decline as the number of compatible rhizobia in the soil rose above 10 cells/g — the cell number in soil that represents the lower limit for detection in the most probable number (MPN) technique for enumerating rhizobia (Brockwell, 1971). As with most biological models, the limitation to the wider application of the Thies et al. (1991) model is in the amount of work required to provide data for its substantiation. The enormous variation inherent in the edaphic component in many agricultural settings (E — see later discussion) and its interaction with rhizobial populations (G_r —see later discussion) has limited the adoption of this model, yet its principles remain valid. These principles and those enunciated by the previous workers (Brockwell, 1971; Bell and Nutman, 1971) are represented in a simplified flow chart (Figure 2 — component B) to aid decision making with respect to the requirement for inoculation. The simple experiment described in the chart is a necessary precursor to legume and inoculant development, if that indeed was the outcome of consultation with component A. The very same experiment, principles, and interpretations apply when investigating the persistence of rhizobia previously introduced to the soil as a legume inoculant. The experiment has three treatments:

- the uninoculated legume
- the legume inoculated with a "best guess" inoculant strain
- the uninoculated legume supplied with fertilizer N

This experiment can be conducted at a field site representative of the target environment for legume introduction, a site where previous inoculant strains have been introduced, or it can be conducted in a controlled environment with the legume grown in pots containing soil from the target environment. In the latter, several soils can be collected and the treatments factorialized with soil type to gain a broader understanding of the likely legume reaction. A further modification might be to grow the legume in pots containing an inert substrate inoculated with a small portion of soil from target sites (Brockwell et al., 1988). This modification requires greater axenic control of the growing environment to avoid rhizobial contamination.

While the experiment described is relatively simple, interpretation of its outcomes is not a menial task. There are seven potential outcomes from a successfully conducted experiment (component B), and four of these led to a specific research direction (scenario 3 of component C in Figure 3). Thus, where there is no background population of rhizobia capable of effective nodulation (A and B), or where the "best guess" strain is only moderately effective (C) there exists a requirement to select an effective, inoculant strain of rhizobia adapted to the target edaphic environment. Even where the "best guess" strain has proven effective (D), there may still be a requirement to assess its adaptation to the soil. The scenario where the soil is devoid of a backround rhizobial population capable of interacting with the legume of choice is commonly encountered where legumes are introduced to regions outside their center of evolution (Howieson et al., 2000a).

A further outcome, where all treatments have healthy nodules because of the presence of an effective background rhizobial population (E), directs us to a legume selection program (scenario 4) that requires minimal microbiological input. This is a common outcome with pulse legumes grown in tropical Asia (Singleton et al., 1992) and was the philosophical foundation for the successful soybean selection program in southern Africa (Mpepereki et al., 2000).

The last two outcomes of the experiment, where variably effective background rhizobial strains are present in the soil in varying number (F and G), lead us to future research scenarios 1 or 2 in Figure 3 (component C). These represent perhaps the most common (albeit intractable) situations in world agriculture. The presence of competitive but variably effective populations of rhizobia in the soil frequently compromises N_2-fixation,

because resident soil rhizobia are often successful in nodule formation (Thies et al., 1991). There are dual research strategies to pursue when faced with these difficult situations, requiring the initiation of parallel rhizobial and legume selection programs. Thus, overall, six of the seven potential outcomes from the preliminary experiment in component B can be interpreted through component C in a manner that suggests the development of a selection program for root-nodule bacteria. Methodologies and strategies for this are discussed in component D. Table 2 lists examples, from

TABLE 2
Recent Examples Where Applying the Research Pathways i-vii in Figure 3 Have Been Successful in Developing Improved Symbiotic N_2-Fixation in Agriculture

Pathway	Legume	Reference	Comments
i	*Medicago littoralis*	Ballard and Charman, 2000.	cv. Pildappa well adapted to the medic rhizobial population present in Australian soils.
	Glycine max (promiscuous)	Mpepereki et al., 2000.	Soyabean well adapted to indigenous rhizobia in African soils.
iia, iib	*Biserrula pelecinus*	Howieson et al., 1995.	A new legume which avoids interaction with a poorly effective rhizobial population in Australian soils pH 5-9.
	Glycine max (specific)	Mpepereki et al., 2000.	Soyabean that restricts entry of indigenous rhizobia in African soils.
iii	*Arachis hypogaea*	H.V.A. Bushby and R.A. Date (personal communication)	Competitive peanut inoculants developed for northern Australia.
iv	*Trifolium* spp.	Watkin et al.,. 1999, Howieson et al.,2000b	A broad host range, acid tolerant strain WSM409 for aerial seeding trifoliums and sub-clover in Australia.
	Glycine max	Hungria and Vargas, 2000.	Acid tolerant soyabean inoculant for Brazilian Cerrados.
	Medicago polymorpha	Howieson and Ewing, 1986.	An acid tolerant medic inoculant selected to colonise acid soils in Australia.
	Phaseolus vulgaris	Vargas and Graham, 1988.	*R. tropici* selected for acidic soils in S. America.
v	*Medicago murex*	Howieson and Ewing, 1989.	A symbiotically competent legume, *M. murex* was developed in parallel with an acid tolerant inoculant strain in Australia.
vi	*Cicer, Hedysarum* spp. *Scorpiurus, Lotononis bainesii*	Brockwell and Gault, 1972. Diatloff 1977.	The current scenario with many legumes introduced outside their centre of origin.
vii	*Vigna spp.*	Singleton et al., 1992.	The scenario with many pulse legumes in Asia.
	Ornithopus, Lupinus	A. McInnes (personal communication)	The scenario with serradella and lupins in S.W. Australia.

both temperate and tropical agriculture, where the research steps i-vii in component C have resulted in the development of improved nitrogen fixation in legumes.

E. Component D — Selecting and Improving Root-Nodule Bacteria to Maximize N_2-Fixation

There are two somewhat opposed schools of thought. Some authors consider that a legume, when faced with competition for nodulation by variably numerous and effective rhizobial strains in the soil (scenarios 1 and 2, component C), can actively select for an effective microsymbiont (Robinson, 1969; Renwick and Gareth-Jones, 1986). Alternatively, it has been proposed that rhizobial numbers are the primary, if not absolute, determinant of the number of nodules formed (Thies et al., 1991; Evans et al., 1996). We propose that rhizobial genotypes are differentially adapted to soil conditions, and it is this adaptation (rather than the relationship with the legume) that primarily determines the outcome of competition for nodule occupancy (Sprent, 1994; Howieson et al., 2000b). There is experimental evidence to support all these views; however, the research strategy pursued to select appropriate inoculant-quality rhizobia can vary substantially according to one's preference.

Before discussing this further, it is appropriate to review some terminology and concepts. Consider the host genotype to be represented as G_l and the rhizobial strain genotype as G_r. In an otherwise nonlimiting environment, we can consider N_2-fixation to be a product of the two genotypes, viz G^2. However, the environment (E) is rarely nonlimiting, thus net N_2-fixation can be represented as $G^2 \times E$. E, which has both biotic and abiotic components, can impact on either G_r or G_l. G^2 can be assessed in the absence of significant interaction with E in a controlled environment glasshouse or growth room. Plants may be grown in an inert substrate and adequately supplied with all nutrients except N. If one supports the concept that legumes have the capacity to select an effective microsymbiont from a pool of strains, then perhaps maximizing G^2 represents the end point of research. The selected genotypes can be introduced to the soil. If, however, one supports the view that $G_r \times E$ more strongly impacts N fixation, then rhizobial strain selection research inevitably must graduate from the controlled environment to the field environment. In so doing, the release of substandard inoculant strains that are poorly adapted to their target environment, a constraint to increased N_2-fixation identified by Brockwell and Bottomley (1995), might be minimized.

Breeding for enhanced N_2-fixation in legumes ($G_l \times E$) with a focus on legume physiology and agronomy rather than interaction with rhizobial genotype is outside the scope of this review. The reader is directed to recent reviews by Hungria and Vargas (2000), Herridge et al. (2001), and Herridge (2002).

Several attempts have been undertaken to improve the N_2-fixation efficiency of rhizobial strains by genetic engineering. However, the release of genetically improved strains is often restricted by national regulation and potential ecological effects due to the release of transgenic organisms are frequently discussed by the public. Nevertheless, some recombinant rhizobial strains have been commercialized, such as *S. meliloti* strain RMBPC-2, which has been approved by the US Environmental Protection Agency in 1997. This genetically engineered bacterium contains additional copies of *nifA* and *dctABD* to increase nitrogen fixation and thus yield of alfalfa (Bosworth et al., 1994). The improvement was based on the fact that *nifA* plays a positive regulatory role in the expression of nitrogen fixation genes and that dicarboxylic acids from the plant are required as a carbon and energy source. The recombinant strain was tested at four field sites in Wisconsin, where yield was significantly increased by 12% compared with the biomass obtained with the wild-type strain at a site where soil nitrogen and organic matter content were low. However, the recombinant strain did not affect yield at sites with either high indigenous competitors or high nitrogen levels (Bosworth et al., 1994).

Ramírez et al. (1999) introduced the *Vitreoscilla* sp. hemoglobin gene (*vhb*) into *R. etli* strain CE3 to improve its symbiotic performance on common bean. Hemoglobin is an oxygen-binding protein, and there-

fore the recombinant bacterium showed an increase in respiratory activity, chemical energy content, and expression of the nitrogen fixation gene *nifH*. Plants inoculated with the engineered strain showed significantly enhanced nitrogenase activity and total nitrogen content compared with plants inoculated with the wild-type strain (Ramírez et al., 1999). The authors concluded that the hemoglobin synthesis stimulated the respiratory efficiency of free-living rhizobia as well as of symbiotic bacteroids leading to higher levels of nitrogen fixation. Recently, a Tn*5* mutant of *R. tropici* was obtained that also showed enhanced respiration as well as significantly improved symbiotic performance (Marroquí et al., 2001). This mutant had increased levels of the cytochromes c_1, CycM, and aa_3, and the mutated gene was shown to be a glycogen synthase (*glgA* gene). Results suggested that *glgA* mutants may be used to enhance symbiotic nitrogen fixation in the field (Marroquí et al., 2001).

Overexpression of the *B. japonicum* terminal oxidase cbb_3 in a *R. etli ntrC* mutant led to an increase in nitrogen fixation (Soberón et al., 1999). As symbiotic nitrogen fixation is an energy-consuming process that takes place under microaerophilic conditions, a cbb_3 terminal oxidase, efficiently coupled to ATP production, is produced during symbiosis. However, due to the presence of oxygen, the cbb_3 gene is not expressed in the free-living state. In addition to O_2, NtrC — a transcriptional activator protein that modulates gene expression in response to nitrogen — represses the free-living cell production of the cbb_3 terminal oxidase (Soberón et al., 1999). Therefore, overexpression of cbb_3 in a *ntrC* mutant of *R. etli* led to a significantly improved symbiotic performance, as determined by nitrogenase activities (Soberón et al., 1999).

Specific DNA amplification was applied by Castillo et al. (1999) to construct *S. meliloti* strains that contain different copy numbers of a symbiotic gene region covering a regulatory gene (*nodD1*), the common nodulation genes (*nodABC*), and a gene essential for nitrogen fixation (*nifN*). Average copy numbers ranged from 2 to 7. Strains with a moderate increase in copy number of the symbiotic gene region showed significantly improved nodulation, nitrogenase activity, plant N content, and plant growth (Castillo et al., 1999).

Some rhizobia, mainly strains belonging to the genus *Bradyrhizobium*, possess a hydrogen uptake (Hup) system that is able to recycle the hydrogen evolved by nitrogenase, resulting in a more efficient use of energy (for a review see Ruiz-Argüeso et al., 1999). In order to improve nitrogen fixation in Hup^- strains, Báscones et al. (2000) introduced the Hup gene cluster into various *Rhizobium* strains. This was done by using Tn*5*-derived minitransposons that allow stable integration into the chromosome. With some strains high levels of hydrogenase activity were achieved, whereas others showed only poor expression of the Hup system. In order to generate strains suitable for field release, a procedure was developed for eliminating the antibiotic resistance gene as well as for the characterization of the minitransposon insertion site (Báscones et al., 2000).

Approaches to genetically improve the competitive ability of rhizobial strains are discussed in Section III.

F. Component D — Methodologies to Optimize G^2 Using Natural Strain Biodiversity

The application of molecular intervention to improving G^2 certainly has merit for those symbioses where optimizing N_2-fixation has been intractable. The most problematic symbioses in this regard appear to be *Phaseolus, Vigna,* and *Glycine* (Herridge, 2002). However, for many symbioses of commercial relevance there is an almost unlimited supply of rhizobial strains that, when inoculated onto their host legume, produce N_2-fixation in the range 20 to 105% of an appropriate N-supplied control plant (Howieson et al. 2000 a,b). The challenge for these symbioses is to align the genotype of the host with the optimal genotype of the microsymbiont.

The nitrogen fixation process in legumes requires the coordinated expression of a set of genes that become active in both the legume and prokaryotic micro-symbiont postinfection (Kaminski et al., 1998). The manifestation of this coordinated gene activity can be routinely evaluated under controlled conditions, where all the ele-

ments essential for plant growth, nodulation, and N_2-fixation (temperature, CO_2 concentration, light, pH, root aeration, and mineral nutrients except combined N) may be optimized. Under these circumstances, plant growth becomes, in isolation, a function of the specific set of genes available for N_2-fixation. Conditions appropriate for nonlimiting nodule function and culture of the legume, covering many substrates, have been described previously: aerated agar (Barrett-Lennard and Dracup 1988), hydroponics (Mytton et al., 1984), vermiculite (Vincent, 1970), sand (Howieson et al., 2000b), and semienclosed systems (Gibson, 1963). All methodologies have their limitations or advantages and the choice of method depends on the legume as well as the environment available for working. However, the same set of principles applies to all methods: conditions for plant growth, nodulation, and subsequent nodule function must be nonlimiting and the appropriate control of contamination by unwanted root-nodule bacteria must be implemented. Under these circumstances, host-strain interactions in the capacity for N_2-fixation, G^2, can be examined and elite combinations discovered.

A particular advantage with sand culture systems is that more than one legume species can be assessed against a specific rhizobial strain contained in a single pot. This approach has been useful in the investigation of the interactions between annual and perennial *Trifolium* spp. when exposed to single rhizobial isolates (Yates et al., 2002). With subtle modifications, standardized glasshouse techniques can also be adapted for focussed investigation of some components of $G^2 \times E$. Cheng et al. (2002) report an adaptation of the techniques of Howieson et al. (1995), where in a split-plot factorial design an acid sand substrate was combined with rates of rhizobial inoculation, rhizobial strains, and two species of *Medicago* to investigate differential symbiotic tolerance to acidity in the nodulation phase.

G. Component D — Methodologies to Optimize $G_r \times E$

The principal abiotic stresses that impact $G_r \times E$ may be generalized because any conditions outside the routine edaphic ranges of pH, moisture content, salinity, temperature, clay content, organic matter content, and mineral nutrition that the microsymbiont may normally be exposed to in its saprophytic state. The challenge in screening strains for adaptation in the field is in defining these 'routine' conditions and in predicting where variance to these conditions may impact G_r x E.

Some of these abiotic stresses and their impacts on root nodule bacteria have been well defined (Sadowsky and Graham, 1998). For example, the mineral nutritional constraints on root nodule bacteria and symbiotic N_2-fixation are well understood *in vitro* and reflect a combination of the essential needs of either the free-living root nodule bacteria, the growth of the legume, or the N_2-fixation process itself (Robson, 1978; O'Hara, 2001). We know cobalt is an essential nutrient required by root nodule bacteria, boron is essential for legumes (but not root nodule bacteria), and molybdenum is essential for nitrogen fixation because of its specific role in nitrogenase. However, because mineral deficiencies in root nodule bacteria can affect a multitude of physiological functions (nutrient uptake, growth regulation, gene function, genetic exchange, survival, and the viable nonculturable state), the impacts of nutrient deficiencies on root nodule bacteria in their free-living state are probably underestimated (O'Hara, 2001).

Root-nodule bacteria throughout their diverse centers of origin have probably evolved to tolerate prevailing conditions that nevertheless might be considered stressful to root nodule bacteria in other environments. $G_r \times E$ stress in this sense is probably a manifestation of man's endeavours to exploit legumes and root nodule bacteria in regions outside their natural origins. For example, in the tropics root-nodule bacteria such as the species *R. tropici* (Graham et al., 1994) appear comfortable in the pH range 4.0 to 5.0 in soils whose ancient geology and prolonged weathering have rendered them low in clay content (<10%), low in exchangeable divalent cations (<10 meq / 100 g), high in Al saturation, and low in P content (<2 ppm; Uehara, 1978). These very same conditions are considered inhospitable to root-nodule bacteria found in the fine-textured soils of the Mediterranean basin, with the exception of the

lupin-nodulating *Bradyrhizobium* sp. *(Lupinus).* Conversely, while many rhizobial species of the Mediterranean basin in the genera *Rhizobium, Mesorhizobium,* and *Sinorhizobium* persist and nodulate their hosts optimally in fine-textured, calcareous soils of pH 8.0, these same conditions are highly prejudicial to nodulation of lupins (Tang et al., 1992; 1993). In a similar sense, while *Bradyrhizobium* spp. (*Lupinus*) may survive long periods of exposure to high temperature in a desiccated, presumably anhydrobiotic state, on the unprotected surface of seeds of their host *Ornithopus* (Bowman et al., 1995), these same conditions are rapidly lethal to many temperate (Bushby, 1982) as well as tropical (Hungria and Vargas, 2000; Date, 2001) rhizobial species. Hence, in optimizing $G_r \times E$ for its abiotic parameters, we must be aware that different rhizobial species and genera have probably evolved intrinsic adaptive mechanisms that suit them to their prevailing conditions.

Understanding and defining with precision the abiotic conditions that impact $G_r \times E$ offers some opportunity for selecting genotypes of root nodule bacteria that might tolerate them. Perhaps the best understood abiotic stress on root nodule bacteria is acidity. There are several clear examples in the literature of successful selection programs for acid tolerance in root nodule bacteria that have produced outcomes adopted by commerce. The discovery of acid-tolerant strains of *R. phaseoli* by Graham et al. (1982) gave rise to *Phaseolus* inoculants successful on acid soils (Vargas and Graham, 1988). These acid-tolerant strains have been widely utilized in South America (Sadowsky and Graham, 1998) and have been classified subsequently as belonging to the novel species *R. tropici* (Graham et al., 1994). The selection of acid-tolerant strains of *S. meliloti* from naturally occurring pockets of acid soils in the Mediterranean basin (Howieson and Ewing, 1986) combined with species of *Medicago* more capable of nodulation under acid stress (Howieson and Ewing, 1989) allowed the cultivation of *Medicago* spp. on one million hectares of acid soils in southern Australia, leading to productivity increases of up to 51% (Howieson et al., 1991). Finally, scientists at EMBRAPA Brazil selected strains of soybean bradyrhizobia well adapted to the acid and Al-rich soils of the Brazilian Cerrados, a region of some 207 million hectares in total (Hungria and Vargas, 2000). In these examples, the selection of elite strains was accomplished *in situ* following some rudimentary prescreening in the laboratory. Can our present knowledge of the physiological and genetic basis of acid tolerance in bacteria expedite future selection for acid tolerant inoculants?

Acid stress (low pH) on root nodule bacteria is a relatively simple condition to reproduce in the modern laboratory, and many attempts have been made to do so since Fred and Davenport (1918). However, despite our capacity to maintain a stable pH with appropriate buffers in the face of metabolic repudiation, the physiological and genetic complexity of acid tolerance in rhizobia makes it extremely difficult for the character to be genetically manipulated, or even fully simulated, in the laboratory.

Dilworth et al. (2001) listed a variety of challenges that acid-sensitive bacteria face when exposed to acidity. Some bacterial responses to acid-stress include maintenance of intracellular pH (pH_I) in order to protect internal cell components, modification or abandonment of external structures exposed to acidity (such as periplasmic proteins or flagella) and resisting stresses (such as heavy metals) whose interaction with pH may be more lethal than pH per se. An alternative, but probably concomitant, strategy to resist acid pH may be the accumulation of compatible solutes (Aarons and Graham, 1991; Graham et al., 1994). Insertional mutagenesis to create either acid-sensitive mutants or mutants with pH-dependent reporter expression has identified three genes intrinsically involved in pH tolerance (*actP, exoH, actA*), a sensor-regulator gene pair (*actS-actR*) that is required for acid tolerance and approximately 12 genes that show pH-dependent expression (e.g., *lpiA, phrR, fixNO, kdpB;* Reeve et al., 1997; 1998; 1999; Tiwari et al., 1993; 1996a,b). Using a proteomics approach, the same group has identified 52 rhizobial proteins whose concentration changes after exposure to acidity. Many of these proteins have been identified recently from the genome database of Rm1021 (Reeve et al., 2002). Recently, differential pH-induced proteins

have been identified in *R. tropici* and *R. etli* (Peick et al., 1999). In that study, proteins were identified that had high similarity to enzymes involved in the synthesis of exopolysaccharides.

The net effect of acid tolerance genes acting simultaneously in root nodule bacteria may be the ability to generate an 'acid tolerance response' (ATR; Foster and Hall, 1990). An ATR has been described in root nodule bacteria when cells grown under mildly acid conditions exhibit much greater resistance to severe acidity than corresponding cells pregrown at neutral pH (O'Hara and Glenn, 1994). Not all strains possess an ATR, and there is some indication that acid soil-tolerant strains of *R. leguminosarum* bv. *trifolii* possess an ATR, whereas sensitive strains do not (Watkin et al., 2000).

Because of the low level of understanding of the concomitant effects of biotic and abiotic stresses on rhizobia living in acid soils, we do not believe we can select acid soil-tolerant rhizobia in the laboratory alone. However, the combined body of information on the effects of pH on rhizobia may provide a number of 'hoops' through which strains must be able to jump if they are to succeed in acid soils. First, it seems logical to source putatively acid-tolerant strains from where they occur naturally in acid soils. Second, a strain must presumably be able to grow at low pH in the presence of heavy metals (perhaps Cu and Zn) and at low Ca concentration (200 to 500 μ*M*) if it is to survive in acid soils. It also seems probable that strains with the capacity to develop an ATR are more likely to succeed in acid soils than those that do not.

The same approach that has uncovered this wealth of knowledge regarding the effects of acidity on root-nodule bacteria can probably be applied to understanding the physiological and genetic response to many of the other important abiotic stresses (desiccation, salinity) that root nodule bacteria might face in a hostile soil environment. However, if we overlay the biotic stresses of predation, antibiosis, organic nutrition, and competition on these abiotic parameters, is it likely that inoculant strains with optimal $G_r \times E$ will ever be selected from within the laboratory? We think not, in the short term, and hence adequate field techniques for screening the adaptation of inoculant-quality rhizobia in the soil must be developed.

H. Component D — Field Screening for $G_r \times E$

While our understanding of stress tolerance increases with intensive laboratory studies, such as those described above, field methodology for selecting strains adapted to the edaphic environment of the host legume have also been refined. For many symbioses, the greatest challenge is to develop a consistent nodulation pattern for the legume in the agricultural environment. Therefore, it is important that the selection process is focussed on identifying a pool of effective rhizobia for the target legume (i.e., high G^2) and then differentiates between them on the basis of $G_r \times E$. An approach such as this might overcome one of the key constraints to improved inoculant usage identified by Brockwell and Bottomley (1995), and that is of the use of suboptimal inoculant strains.

One resilient methodology for testing survival and colonization by rhizobia in target soils is the "cross-row" technique, as described by Howieson and Ewing (1986), which is a modification of that developed by Chatel et al. (1968). Briefly, strains are introduced to the soil as inocula at a site of appropriate chemical and physical characteristics and generally free of the rhizobial species of interest. The pH of the site should be in the range targeted for the host-legume, particularly if this is likely to be a constraint on rhizobial survival. Soils with a sandy texture (5 to 20% clay) expedite recovery of roots for examination of the nodules and also place increased selection pressure on inoculant survival through periods of temperature stress and desiccation (Bushby and Marshall, 1977). However, if legumes are targeted for clay soils (e.g., the black cracking basaltic clays of South America, Europe, and eastern Australia), then it may be necessary to take cores over root systems and recover roots and nodules through a process of soaking and sieving (Date, 1982). Clay soils may also contain high levels of soil N that mask inoculation responses. In this case strain selection is best

performed on soils where N levels have been depleted by nonlegume crop or pasture species.

In the "cross-row" bio-assay the plots are sown as 2-m lines of inoculated legume seed, separated by 1-m buffers and fertilized with all necessary macro- and micro-nutrients except N. Plants are allowed to grow through a full season during which top dry weight and N_2-fixation can be assessed. If the target soils are low in available N, the biomass production of the tops is an excellent indicator of symbiotic performance. This can provide valuable information given that preselection of the elite combinations of G^2 in component D was based on N fixation under optimal conditions. If the soil contains appreciable N, then the N^{15} natural abundance method (Unkovich and Pate, 2000) can reliably indicate strain symbiotic performance. It is likely that strains differ in their relative abilities to survive after inoculation on the seed surface or in the legume rhizosphere in difficult soils. Hence, data on *in situ* performance gathered in the year of establishment of the experiment can provide valuable additional information in the search for elite strains.

Following a season in the soil in the cross-row experiment, the individual strains are traced for their survival and movement away from the line of introduction to the soil using a nodulation bio-assay. In this assay, uninoculated but surface-sterilized seeds are sown across the original line at two or three points within the original 2-m length. Individual plants are excavated 10 to 12 weeks after sowing and the nodulation pattern recorded. Strains that achieve nodulation consistently at distances up to 20 cm away from their original placement are considered to have colonized the soil and thus tolerated the existent edaphic conditions. Strains that are not recovered are considered to have failed, and thus strains are differentiated on the basis of $G_r \times E$.

Experimental design can be as randomized blocks, or adjusted to take advantage of spatial analysis techniques (Cullis and Gleeson, 1991). If the experiment is sown into a soil containing background rhizobia capable of nodulating the bio-assay host, then nodule occupancy can be determined using molecular techniques (Thies et al., 2001). The implications for N_2-fixation of competition for nodulation by background strains are reviewed in the following section.

I. Conclusions

To realize the enormous potential for increased global N_2-fixation from legumes requires the selection and development (or construction) of elite inoculant strains of root-nodule bacteria that suit both their legume host and the target edaphic environment. There are numerous research pathways to achieving this, requiring appropiate decision making and understanding of the socio-economic constraints to both legume adoption and inoculation. This section has emphasized some aspects of the research methodologies and bio-economic components relevant to the development of elite inoculant strains of root-nodule bacteria. If followed, these strategies should overcome two of the constraints to optimizing global inoculant usage in agriculture identified by Brockwell and Bottomley (1995) — poor decision making and substandard inoculant strains.

III. THE *RHIZOBIUM* COMPETITION PROBLEM

Competition for nodule occupancy occurs whenever two or more rhizobial strains have the opportunity for infection of a susceptible legume plant. Nodulation by unwanted strains has been an intractable issue to rhizobiologists for many years (Ireland and Vincent, 1968; Brockwell et al., 1982) and continues to be so (Denton et al., 2000; Brockwell, 2001). Competition is most severe in the case of pasture legumes growing in long-term pasture phases, or in self-regenerating rotations with cereal crops (Reeve and Ewing, 1993), where the regenerating legume must nodulate from within the soil population of rhizobia after the year of establishment. Competition is less severe in annually sown crop legumes or forages where the seed may be inoculated prior to sowing, an action that gives the inoculant strain a positional, if not numerical, advantage.

Numerous rhizobial strains have been identified that show high nitrogen-fixing ability with their target host legume (e.g., Parker and Oakley, 1963; Howieson et al., 2000a). Nevertheless, attempts to increase legume yields in agricultural fields by inoculation with superior strains have

often failed (Brockwell and Bottomley, 1995). This may be due to inappropriate inoculation technology (Date, 2000; Lupwayi et al., 2000), but it is often the result of the inability of inoculant strains to compete with indigenous rhizobia for nodule formation on the plant host. Considerable efforts have been undertaken to understand rhizobial competition and various factors contributing to inoculation success have been reviewed previously by Dowling and Broughton (1986), Bottomley (1992), Streeter (1994), and most recently Sadowsky and Graham (1998). In this section the management and manipulation of rhizobial competition will be addressed, as well as genetic factors known to be involved in competition.

A. Managing Rhizobial Competition

One of the greatest difficulties in managing rhizobial competition for nodulation is that we do not actually have a comprehensive understanding of which phases in the nodulation process are exposed to competition, or indeed which of these phases are the most sensitive to external influences (Phillips et al., 1996). If we are to manage rhizobial competition effectively, a starting point is to identify which step in the nodulation process is most impacted by competition. This may, of course, vary for different symbioses.

Root colonization by a particular strain (perhaps the final outcome of competition) probably involves several phases of a rhizobial life cycle. If achieving numerical superiority is pivotal to success in a competitive environment (Thies et al., 1991; Carter et al., 1995), then rhizobial multiplication and survival in the bulk soil (saprophytic competence, Chatel et al., 1968), tolerance to antibiotic agents (Triplett, 1990), chemotaxis (Bauer and Caetano-Anolles, 1990), motility (Wadisirisuk et al., 1989), the efficiency of attachment to roots and growth rate (Smit et al., 1992; Hartwig et al., 1991), as well as multiplication from the nodule environment after root senescence (Murphy et al., 1995), may all be considered components of competition. However, which of these components is the most susceptible to external influence? In the following part of this section we examine several opportunities for managing competition by influencing some of these phases. In general, the approaches can be thought of as falling into two broad categories — those that directly enhance the numerical superiority of a given rhizobial genotype in a given phase, or those that provide an exclusive and advantageous benefit to a particular host legume-rhizobial combination.

B. Manipulating Competition through Conventional Approaches

Changing the soil chemical environment by addition of phosphate and lime altered nodule occupancy of clover by indigenous rhizobial strains (Renwick and Gareth-Jones, 1986; Almendras and Bottomley, 1987). Although the mechanisms for this were not immediately clear, it has been shown since that changing external pH, calcium, and phosphate concentrations affected relative strain success in the attachment of *S. meliloti* to roots of *Medicago* spp. (Howieson et al., 1993). These same chemical factors were also shown to interact differentially on strains in their effects on growth rate (Howieson et al., 1992; Reeve et al., 1993). Hence, in the research of Almendras and Bottomley (1987) the altered chemical environment may have impacted several phases of the rhizobial life cycle that we believe are important to competition, including strain multiplication and attachment to legume roots.

It has been shown that the location of an inoculant strain together with a carbon source, such as glycerol in microgranules, conferred a competitive advantage to the inoculant bacterium (Duquenne et al., 1999). Additionally, soil textural and structural properties affected rhizobial competition (Moawad and Bohlool, 1984; Palanipappan et al., 1997). Strains have been shown to differ in their efficiency of nodulation according to soil temperature (Roughley, 1970; Montañez et al., 1995) and the choice of inoculation method (Danso and Bowen, 1989; Hardarson et al., 1989). Conventional agricultural practices such as liming and fertilization, as well as tillage, green manuring, and removal of stubbles (which all impact soil temperature and structure) therefore may be manipulated to affect rhizobial com-

petition for nodulation. In the foreseeable future these practices may be managed to take advantage of particular desirable strains.

Other conventional approaches have been applied successfully to overcome competition by ineffective indigenous rhizobia. By selecting the genotype of the host legume to nodulate preferentially with effective background rhizobial strains, Mpepereki (2000) greatly improved N_2-fixation in *Glycine* in Africa. In an alternative approach to increase legume production in soils of southern Australia where variably effective naturalized rhizobia were competitive for nodulation with *Trifolium,* Howieson et al. (1995) introduced a previously unexploited pasture legume genus *Biserrula,* together with its specific root-nodule bacteria (Nandasena et al., 2001). Abril et al. (1997) demonstrated that inoculation of chickpea with native and well-adapted rhizobia resulted in a better response than inoculation with exotic, nonadapted strains. The potential for exploiting genetic diversity to improve agricultural production from legumes is discussed more fully in Section IV of this review.

C. Manipulating Competition Using Less Conventional Approaches

Factors that 'catalyze' rhizobial growth are more likely to affect competition than factors that provide broad-spectrum energy sources (Phillips et al., 1996). This 'genotype-specific activity' is the basis of the rhizopine concept of Murphy and Saint (1992), of the selective aromatic catabolism proposed by Rynne et al. (1994), and of the biotin synthesis/uptake concept of Phillips et al. (1995), all of which seek to enhance strain competitiveness. The latter concept has been used to promote competitive root colonization of *S. meliloti*, and as the growth of *S. meliloti* is frequently limited by the availability of biotin (Streit et al., 1996), recombinant strains with extra biotin synthesis capability were developed (Streit and Phillips, 1996). Transconjugant strains achieved a higher cell density on a defined medium, but in the rhizosphere the biotin-producing strains showed delayed growth and competed poorly against the parental strain (Streit and Phillips, 1996). Possibly the additional metabolic load due to biotin synthesis inhibited other cell functions important for competition in the rhizosphere. However, the concepts mentioned above offer the opportunity to manipulate competition, either in the rhizosphere or the bulk soil, by delivering numerical superiority to rhizobial genotypes previously selected or engineered for catabolism of a specific substrate. We should also include under this heading the numerous proposals for utilizing different isoflavone fractions from legume seed and root exudates as precursors for the synthesis of legume genotype-specific rhizobial metabolites (Loderio et al., 2000). While metabolism of these exudates can result in increased rhizobial growth (Hartwig et al., 1991), this approach may not provide significantly superior numbers of the desirable strain. It may instead offer the desirable strain a key to the door for legume infection. Various *Rhizobium* genes involved in nodulation such as the common *nodABC* and the regulatory *nodD* genes are induced by flavonoid signals from the plant host. The protein products of *nod* genes synthesize Nod factors, which are signal molecules that define the host range of the microbial symbiont (Freiberg et al., 1997). Nod factors also regulate nodule initiation and morphogenesis (Denarié et al., 1996). Recently, it has been shown by Lamrabet et al. (1999) that mutants of *S. fredii* producing altered Nod factors exhibit significantly decreased competitiveness to nodulate soybeans.

Historically, it has been logistically difficult to undertake screening procedures for competitiveness across a large number of strains. Amarger (1981) reported a novel means to select competitive rhizobia based on the final plant dry weight when a range of effective strains were mixed individually with an ineffective strain of known competitiveness. A greater plant weight indicated increased competitive ability. A modification of this experimental technique that would allow its completion prior to the manifestation of differential N_2-fixation might be to identify successful competitors based on a PCR analysis of nodule occupants (Wilson, 1995; Tas et al., 1996; Malek et al., 1998). To avoid repetitive isolations, another alternative might be to label the rhizobial strain of known competitive ability with a marker gene such as *lacZ* (Krishnan and

Pueppke, 1992; Kamboj et al., 1996), *gusA,* or *celB* (Wilson et al., 1994; Wilson et al., 1995; Streit et al., 1995; Sessitsch et al., 1996; Sessitsch et al., 1998). The activity of the gene is sufficient to color nodules when exposed to an appropriate substrate and the proportion of colored nodules indicates the outcome of the competition for nodulation (Wilson, 1995; Sessitsch et al., 1997c; Vásquez-Arroyo et al., 1998). Furthermore, the availability of differently marked strains allows simultaneous detection of several strains on a single plant as well as the detection of multiple nodule infections (Sessitsch et al., 1996; de Oliveira et al., 1998). To assist this approach, Reeve et al. (1999) identified insertion sites in the *S. meliloti* chromosome that are only transcribed when the bacteria are in the nodule environment. Sites such as these might be valuable for rhizobial competition studies with marker genes as described above because the gene insertion has less potential for affecting that substantial part of the rhizobial life cycle completed external to the nodule. Similarly, rhizobia that carry *gusA* or *celB* constructs with *nifH* gene promoters express the marker gene only under microaerophilic conditions such as within nodules (Wilson et al., 1995; Sessitsch et al., 1996).

In an acid environment, strain competitiveness might be enhanced by the insertion or upregulation of genes involved in acid tolerance. There has emerged a substantial body of knowledge of genes conferring acid tolerance in *S. meliloti* over the last decade using an insertional mutagenesis approach (Tiwari et al., 1993, 1996a, b; Reeve et al., 1998, 1999), although Dilworth et al. (2001) speculated that there is still much to learn before this knowledge can be applied pragmatically. This latter attitude is perhaps vindicated when we apply a proteomic approach to understanding acid tolerance and learn that 50 or more proteins might be involved in bacterial acid tolerance (Reeve et al., 2002). Nonetheless, manipulating acid tolerance in rhizobia selected for an acid environment represents one of the few opportunities for managing rhizobial competition by influencing the saprophytic, or free living, stage of the rhizobial life cycle.

D. Genetic Determinants of Rhizobial Competition in the Rhizosphere

Rhizobia generally achieve their maximum population density in, or around, the rhizosphere of legumes (Rovira, 1961). When rhizobia achieve high numbers in the rhizosphere, their metabolic byproducts or antibacterial secretions may be in sufficent concentration to influence competition for nodulation. One of the few characterized factors contributing to the competitive ability of rhizobial strains in the rhizosphere are rhizopines, which are *myo*-inositol derivatives. Approximately 10% of the strains of *R. leguminosarum* bv. *viciae* and *S. meliloti* appear to produce and catabolize the rhizopine L-3-*O*-methyl-*scyllo*-inosamine (Wexler et al., 1995). Bacteroids of these strains synthesize rhizopines, which are then exclusively catabolized by free-living cells of the same strain in infection threads and rhizospheres (Murphy et al., 1995; Wexler et al., 1995). Rhizopine strains enjoy a competitive advantage in nodulation, perhaps because of the availability of this exclusive substrate, and they have been shown to outcompete mutants containing a Tn*5* transposon insertion in the rhizopine gene region (Gordon et al., 1996). Heinrich et al. (1999) reported that a rhizopine strain of *S. meliloti* remained at a competitive nodulation advantage even after a period of 4 years in the soil. The genes involved in rhizopine synthesis have been termed *moc* and *mos* genes, respectively, and are regulated by *nifA*/*ntrA* regulatory genes, which are maximally expressed in bacteroids (Murphy et al., 1995). However, low levels of rhizopines are also synthesized at the early stages of the symbiotic interaction, probably resulting from microaerobic induction in free-living bacteria (Heinrich et al., 2001). These recent observations may explain the rhizopine effects on competition. It had hitherto been conceptually difficult to ascribe a competitive advantage to rhizopine production and catabolism in rhizospheres or decayed nodule tissue, both of which are unlikely to be substrate limited environments.

The catabolism of rhizopines is dependent upon a functional *myo*-inositol catabolic pathway (Bahar et al., 1998; Galbraith et al., 1998), and recently it has been demonstrated that the capability of rhizobial strains to degrade *myo*-inositol

is essential for efficient nodulation (Fry et al., 2001; Jiang et al., 2001). Furthermore, *myo*-inositol is abundant in soils and rhizospheres, and the ability to metabolize inositol confers a competitive advantage to bacteria colonizing soils and plant roots (Wood and Stanway, 2001). A functional *myo*-inositol dehydrogenase gene (*idhA*) is required for efficient nitrogen fixation and competitiveness of *S. fredii* (Jiang et al., 2001). The competitive ability of *idhA* mutants proved to be severely affected, and they were outcompeted by the parent strain, even when the mutant was applied at a 10-fold numerical advantage (Jiang et al., 2001). In *R. leguminosarum* bv. viciae the expression of *iolA* and *iolD* is required for growth on *myo*-inositol, and mutations in these genes resulted in a substantially decreased nodulation competitiveness (Fry et al., 2001). Thus, our understanding of the rhizopine concept and associated catabolism of *myo*-inositol, like the development of marker genes and the knowledge of acid tolerance in rhizobia, has made remarkable progress in the last half-decade. These advances in basic science may yet provide an opportunity for genetic intervention in rhizobial competition.

A second well-characterized determinant of nodulation competitiveness is the production of trifolitoxin (TFX), which is a potent antirhizobial peptide that is produced by some strains of *R. leguminosarum* (Bosworth et al., 1993; Breil et al., 1993). TFX inhibits members of a specific clade of the α-Proteobacteria that includes legume micro-symbionts as well as plant and animal pathogens (Triplett et al., 1994). Although TFX is rapidly broken down in soil (Robleto et al., 1997), it has been shown that TFX production significantly enhances strain competitiveness (Triplett et al., 1987; Triplett, 1988; Triplett, 1990; Robleto et al., 1997). Furthermore, genes responsible for TFX production have been transferred successfully to nonproducing strains to increase competitive ability (Robleto et al., 1997). A recombinant, TFX-producing *R. etli* strain has been shown to significantly increase nodule occupancy values in non sterile growth chamber experiments (Robleto et al., 1997) as well as under agricultural conditions. A field experiment was carried out in which the transgenic strain was tested over 2 years for nodule occupancy and over 3 years for assessment of the effect of TFX production on grain yield. The recombinant strain exhibited at least 20% greater nodule occupancy than the wild-type strain in both years, and no effect on grain yield was observed (Robleto et al., 1998).

Similarly, *R. leguminosarum* strains have been shown to produce bacteriocins — antibiotics that are active against closely related strains or species. *Rhizobium* bacteriocins have been characterized as small, medium, and large, based on their size and diffusion characteristics (Schwinghamer and Brockwell, 1978; Hirsch, 1979). Only a few strains produce medium bacteriocins, and symbiotic plasmids have been shown to carry determinants for their synthesis (Hirsch et al., 1980). A recent report revealed that medium bacteriocins are related to RTX proteins, which include hemolysin and leukotoxin, and contribute to nodulation competitiveness (Oresnik et al., 1999).

There are further avenues for manipulating competition by selective substrate utilization in the rhizosphere. Roots of alfalfa, the host plant of *S. meliloti*, exude proline as well as proline-releasing compounds such as betaines and stachydrine (Phillips et al., 1998). The enzyme proline dehydrogenase, encoded by *putA*, catalyzes the oxidation of proline to glutamate and has been found to be essential for the nodulation efficiency and competitiveness of *S. meliloti* (Jiménez-Zurdo et al., 1995; Jiménez-Zurdo et al., 1997). Recently, transgenic strains carrying multicopy plasmids with the *putA* gene in combination with a strong constitutive promoter proved to be more competitive for alfalfa nodulation in the greenhouse as well as under agricultural conditions than their parental strains (van Dillewijn et al., 2001).

The physiology of the bacterial surface itself may also be a factor influencing rhizobial competition. Mutants of *R. etli* with altered colony morphology induced nodules and reduced acetylene, but showed decreased competitive abilities (Araujo et al., 1994). Similarly, mutants of *S. meliloti* with altered lipopolysaccharides (LPS) (Lagares et al., 1992) and of *B. japonicum* with altered exopolysaccharides (EPS) (Parniske et al., 1993) were compromised in their competitive abilities. Whereas most rhizobia carrying mutated *exo* genes (responsible for the biosynthesis of exopolysaccharides) show decreased nodulation

competitiveness, an *exo* mutant of a *S. fredii* strain was more competitive than its parental strain (Krishnan and Pueppke, 1998).

Rhizobium spp. typically carry two to eight stable plasmids that can constitute up to 40% of the rhizobial genome (Honeycutt et al., 1993) and that have been linked to competitive ability. Plasmid-cured strains often fail to compete with wild-type strains (Hynes et al., 1990; Brom et al., 1992; Moènne-Loccoz and Weaver, 1995), and a substantial decrease in competitivity for nodulation was found in multiple plasmid-cured strains when compared with single plasmid-cured strains (Brom et al., 2000). It must be understood, however, that the deletion of such a significant amount of DNA has the capacity to impact a wide range of cell activities that together may be manifested as reduced competitiveness. Furthermore, genetic exchange among rhizobia in soil (Schofield et al., 1987) and the often reported genomic instability of *Rhizobium* (Romero and Palacios, 1997; Romero et al., 1997) may lead to altered competitiveness. Transient increases in nodulation competitiveness were obtained by introducing cryptic plasmids from *R. tropici* into *R. etli* (Martínez-Romero and Rosenblueth, 1990).

Genes required for the utilization of a range of carbon sources are located on plasmids, and it has been found that plasmid-encoded genes involved in the degradation of rhamnose play a role in competition of *R. leguminosarum* bv. *trifolii* (Oresnik et al., 1998). Rhamnose utilization mutants showed impaired competitive abilities. Furthermore, rhamnose catabolic genes were induced by root extracts of the host plant, suggesting that rhamnose catabolism plays an important role in the early interaction between macro- and microsymbiont (Oresnik et al., 1998). Mimosine, a toxin produced by the tree legume *Leuceaena,* provides a competitive advantage to mimosine-degrading rhizobia (Soedarjo and Borthakur, 1998). Furthermore, stomatin-like proteins required for nodulation competitiveness have been identified (You et al., 1998). In addition, nodule formation efficiency genes (*nfe*) located on plasmids have been found in *S. meliloti* (Sanjuan and Olivares, 1991; Soto et al., 1993), whereas *B. japonicum* hosts a *nfe* gene that is located on the chromosome (Chun and Stacey, 1994).

R. leguminosarum strains were constructed for the biological control of *Sitonia* larvae by introducing the *Bacillus thuringiensis* δ-endotoxin gene (*cryIIIA*) (Giddings et al., 2000). Although the recombinant strain did not show the intended trait, it exhibited a significantly enhanced competitive ability for nodule formation when compared with the parental strain. Although it is often assumed that any secondary effects of transformation events will result in a reduction of fitness or competitive ability, this study illustrates this is not always the case. In addition, it has been shown that the competitive ability for nodulation is not necessarily impaired due to the additional metabolic load imposed by expression of the introduced DNA and synthesis of the corresponding enzyme (Sessitsch et al., 1997c).

There are multiple avenues emerging for the manipulation of competition between rhizobia in the soil (summarized in Table 3). These avenues provide the guidelines for continued exploration of rhizobial competition at the ecological, biochemical, and molecular levels. It may not be too long before we are able to eventually influence rhizobial competition for nodulation in agricultural practices to the net benefit of N_2-fixation.

IV. DIVERSITY OF NODULE BACTERIA FROM LEGUMES

Bacteria that form nitrogen-fixing nodules in the roots and stems of legumes have different growth rates, biosynthetic pathways, catabolic capabilities, habitats, and morphologies. Among these bacteria differences are well recognized in fatty acid profiles, sequences of proteins and genes, structures of lipopolysaccharides and exopolysaccharides, protein and enzyme patterns, as well as in the size of the chromosomes and in the genome organization. Nevertheless, nodule-forming bacteria are not as phylogenetically diverse as nitrogen-fixing bacteria (Phillips and Martínez-Romero, 2000), suggesting that nitrogen fixation is an earlier bacterial characteristic than nodulation.

In this section we have tried to avoid overlaps with previous reviews on *Rhizobium* diversity and related topics (Young and Haukka, 1996; van Berkum et al., 1999; Sadowsky and Graham, 1999-

TABLE 3
Approaches to the Manipulation of Rhizobial Competition

Characteristic	Reference
Selection of competent soil saprophytes, strains with enhanced motility, or more efficient attachment properties	Chatel et al., 1968; Wadisirisuk et al., 1989; Smit et al., 1992
Altering the soil chemical or physical environment through management practices to advantage desirable strains	Almendras and Bottomley, 1987; Renwick and Gareth-Jones, 1986; Hungria and Vargas, 2000
Inoculation technology that provides a positional superiority to the desired strain	Duquenne et al., 1999; Danso and Bowen, 1989
Selecting legume hosts capable of expressing a preference for their micro-symbiont	Mpepereki et al., 2000; Robinson, 1969
Selecting strains with genotype specific catabolic properties that confer an advantage to the desired strain under nutrient limitation	Phillips et al., 1996; Heinrich et al., 1999; Rynne et al., 1994
Selection of strains tolerant of particular edaphic stresses such as acidity, high temperature, salinity	Howieson et al., 2000; Vargas and Graham, 1988
Selecting or engineering strains producing antirhizobial peptides or bacteriocins	Triplett et al., 1987; Robleto et al., 1998; Oresnik et al., 1999
Manipulating rhizobia for selective substrate utilization to deliver a nodulation advantage	Van Dillewijn et al., 2001

2001; Terefework et al., 2000; Wang and Martínez-Romero, 2000; Sprent, 2001), but we follow some of the ideas presented earlier in *Rhizobium* phylogenies and bacterial genetic diversity (Martínez-Romero and Caballero-Mellado, 1996).

Much emphasis and interest has been given recently to the molecular diversity of the nodule-bacteria and this has, with some significant exceptions, provided an extension and confirmation of the results of previous phenotypic analyses. However, the molecular tools have contributed to the confidence level for the identification of these bacteria and enlarged the scope of our understanding of nodule-bacteria diversity. The surprising discovery of novel nodulating groups, classified within the β-Proteobacteria, is based on the analysis of the 16S rRNA gene sequences (Moulin et al., 2001). In this case, the phenotypic comparison to their relative *Burkholderia* spp. has not yet been published. It is puzzling how the β-Proteobacteria were mistaken as *Bradyrhizobium* (Boone et al., 1999), which have extremely different growth rates. *Burkholderia* strains are fast growers (much faster than *Rhizobium* strains), while *Bradyrhizobium* strains are slow-growing bacteria. It remains to be established if the nodulation capacity is extended to other β-Proteobacteria that are more commonly known as plant-associated bacteria, human or plant pathogens. It may be that they are a single group among nonnodulating bacteria, as seems to be the case for *Azorhizobium caulinodans* (Dreyfus et al., 1988), which up to now constitutes the single nodulating species among the *Xanthobacter*-like organisms.

Nodulating *Methylobacterium* strains have been reported recently from *Crotalaria* nodules (Sy et al., 2001). These bacteria grow in methanol and have been designated *M. nodulans*, and are probably the same organism previously reported

to nodulate *Lotononis bainesii* (Norris 1958). Seven other *Methylobacterium* species tested by Sy et al. (2001) had neither *nodA* genes nor nodulated *Crotalaria* plants. Similarly, six red-pigmented *Methylobacterium* strains isolated from *Lotononis bainesii* in South Africa and Uruguay were unable to nodulate Australian native *Crotalaria retusa* and *C. cunninghamii*, yet nodulated *C. juncea* ineffectively (Abreu and Howieson, unpublished data). Previously, no nodulating bacteria within the *Methylobacterium* or the β-Proteobacteria (Moulin et al., 2001) had been described. Other novel nodule isolates (de Lajudie et al., 1998a; Tan et al., 2001a) resemble former *Agrobacterium* strains (now reclassified as *Rhizobium*, Young et al., 2001). Thus, it seems worth searching among the vast majority of nonanalyzed legumes for their symbiotic bacteria to reveal additional nodulating bacteria and expand our understanding of the evolution of this phenotype in bacteria.

Beyond the chromosomal diversity there exists symbiotic diversity within single groups or species and significant diversity in the Nod factor structures produced (reviewed in Dénarié et al., 1996; Perret et al., 2000; Spaink et al., 2000) even by bacterial symbionts of a single legume. Up to now the diversity of rhizobia is still largely determined from nodule bacteria, and so the diversity of soil rhizobia has not been described comprehensively. The few studies available reveal a larger diversity of rhizobia strains in the soil (Louvrier et al., 1996 and references therein) and the amplification of *nodD*, the nodulation regulatory gene, directly from soil showed that nodules contain selected genotypes from those existing in soil (Zézé et al., 2001).

The diversity of nodule bacteria seems to be driven by crop domestication (Martínez-Romero and Caballero-Mellado, 1996) and by the history of the land use (Wang et al., 1999a; Palmer and Young, 2000). Diversity may be constrained by some agricultural practices such as chemical fertilization (Caballero-Mellado and Martínez Romero, 1999) or slurry amendments (Labes et al., 1996). However, the biodiversity of nodulating bradyrhizobia in soils that have carried a lupin-cereal rotation for 25 years in southwestern Australia remains very high (McInnes, 2002).

In recent years a few novel genera and several species have been reported. The current status of legume nodule-bacteria taxonomy may be consulted at the following web site: **http://www.honeybee.helsinki.fi/users/lindstro/Rhizobium/index.htm.** When the diversity of nodule bacteria was reconsidered some years ago with the dawning of the molecular era, it was anticipated that many genera and species would be required to accommodate all the diversity. In contrast, it has been considered recently that the overall diversity of tropical rhizobial species may be restricted (Moreira et al., 1998; Parker, 2001) in view of the fact that few bacterial genotypes with low specificity nodulate several tropical legumes (Lafay and Burdon 1998; Parker 2001). In addition, common genera of root-nodule bacteria were recovered from the survey of a large number of tropical legumes (Moreira et al., 1998). The observation of local legumes sharing symbionts has been made not only in cases of symbiosis with *Bradyrhizobium* but also with *Rhizobium* such as those of *R. hainanense* (Chen et al., 1997) and *R. yanglingense* (Tan et al., 2001b), and the advantages of local legumes sharing microsymbionts has been discussed (Parker, 1999). A larger diversity of legume plants is encountered in tropical areas (Sprent 2001), and many tropical plants have also been described as promiscuous (Pueppke and Broughton, 1999). In contrast, many of the Mediterranean legumes are considered to be highly specific in their rhizobial requirements. We see evidence of an increase in the number of rhizobial species being identified with molecular analysis, as more legumes are being examined for their agricultural potential (Nandasena et al., 2001; Kishinevsky et al., 2002). It may be that a greater increase in the taxonomic diversity within the *Mesorhizobium, Sinorhizobium,* and *Rhizobium* will emerge from Mediterranean and temperate regions of the world than from the tropics.

A. Origins of Diversity

The comparison of whole genomes, or significant part of genomes, is now possible for some

strains. A global comparison of the *M. loti* chromosome and the *S. meliloti* genome has been reported (Figure 3 in Galibert et al., 2001). *S. meliloti* and *M. loti* seem to have diverged 400 to 500 million years ago (Morton, 2001). It has been considered that old diverging lineages may account for the extensive diversity in *Rhizobium* (Piñero et al., 1988). Despite the large divergence of *S. meliloti* and *M. loti*, the remnants of an ancestral genome, modified over time by insertions, transpositions, and inversions, may still be recognized (Morton, 2001). Estimating the time of group divergence, although speculative, is a fascinating task. Molecular differences provide hints to the time bacteria shared an ancestor and undertook their diverging pathways. From the sequence of glutamine synthetase, the divergence of the genera *Bradyrhizobium* and *Rhizobium* was estimated at over 500 million years ago (Turner and Young, 2000).

There is greater diversity among bacteria than among eukaryotes (Doolittle, 1999). It has been theorized that bacterial diversity appeared very early in the primitive life on Earth, perhaps tracing back to the last common ancestor (Doolittle, 1999). Other lineages such as those leading to nodule bacteria may trace back to the origin of plants, whereas those like *Salmonella* and *Escherichia* spp. (mammal dependent) seem to have diverged much later. The prevalence of diverging clones with limited elimination by bacterial extinction (Dykhuizen, 1998) contributes to diversity. "Diversity arises and is maintained through interplay between ecological and genetic factors" (Spiers et al., 2000).

In rhizobia, their wide geographical distribution (see later) and the different plant hosts and niches they occupy have also been proposed as causes of their diversification (Martínez-Romero and Caballero-Mellado, 1996). At present, it is recognized that rhizobia are aquatic (Chaintreuil et al., 2000; Wang and Martínez-Romero, 2000), epiphytic (Boivin et al., 1997), and endophytic (see Section VI) in addition to being soil bacteria. They are also encountered in seeds (Pérez-Ramírez et al., 1998). Furthermore, different bacterial genera and species may be obtained from a single plant species. Diverse *Sinorhizobium*, *Mesorhizobium,* and *Rhizobium* spp. have been obtained from *Leucaena leucocephala* (Wang et al., 1999a), *Acacia farnesiana* (de Lajudie et al., 1994, 1998b), and *Astragalus adsurgens* (Gao et al., 2001). It seems that some nodule occupants may be favored by environmental conditions or even the depth of the roots (Dupuy and Dreyfus, 1992; Dupuy et al., 1994). It is remarkable that both *Sinorhizobium* and *Bradyrhizobium* have been reported nodulating native *Acacia* spp. in Africa, Australia, and in the Americas (de Lajudie et al., 1994; Frioni et al., 1998; Marsudi et al., 1999). It is tempting to speculate that diversification has taken place from a common ancestor since their geographical isolation as a consequence of genetic drift and/or adaptation of bacteria to plants. The bacterial genetic adaptation may include recombination with local bacteria. The differences encountered between *R. gallicum* in Europe and in the Americas may be related to their geographical isolation (Sessitsch et al., 1997a).

Human domestication of legumes may have selected particular rhizobial lineages that were suited for their nodulation. Alternatively, there is the observation that the more rare a legume species, the more specific rhizobia it has (Thrall et al., 2000). Interestingly, legumes that are considered more modern are nodulated solely by fast growing rhizobia and not by *Bradyrhizobium,* and this seems to support an earlier proposal that *nod* genes evolved in bacteria more similar to *Bradyrhizobium* then further spread to *Rhizobium* ancestors (Martínez-Romero, 1994; see Diversity Epilogue). The genetic diversity of hosts has been found to relate to the polymorphism of their symbionts (Spoerke et al., 1996; Wilkinson et al., 1996)

Other soil bacteria such as *Pseudomonas* are highly diverse, and the ecological causes of their diversity have been discussed (Spiers et al., 2000) and are coincidental to some of the considerations made for rhizobia.

B. Lateral Transfer as a Diversification Mechanism

Lateral transfer of genetic information seems to contribute to the generation of novel rhizobial

genotypes, and the long evolutionary histories of bacteria would allow ample opportunity for lateral transfer. The most convincing example of transfer of genetic information in the field is that reported by Sullivan et al. (1995), who, 7 years after applying a *Mesorhizobium loti* strain as an inoculant, recovered bacteria other than the inoculant from *Lotus* nodules. The novel symbionts carried the symbiotic genetic information from the inoculant strain yet originated from soil *Mesorhizobium* strains (Sullivan et al., 1996) that acquired the so-called "symbiotic island" from the introduced strain by lateral transfer. Their results also showed the existence of nonsymbiotic *Mesorhizobium* strains similar to those reported previously in *R. etli* (Segovia et al., 1991). In congruence with the documented lateral transfer, it has been observed that *Mesorhizobium loti* strains correspond to different *Mesorhizobium* lineages revealed by their 16S rRNA gene sequences and DNA relatedness, indicating that the symbiotic islands have naturally spread in *Mesorhizobium*. Similar cases of symbiotic lateral transfer are deduced to occur in *Mesorhizobium* having other specificities, such as in those nodulating *Astragalus sinicus* (Zhang et al., 2000), *Amorpha fructicosa* (Wang et al., 1999c), and *Cicer arietinum* (Nour et al., 1995). In all these cases, a diversity of *Mesorhizobium* groups have been recognized from nodules of each plant species, and in some cases the identity (or near-identity) of *nod* gene sequences has been demonstrated in these dissimilar bacteria (see *Mesorhizobium* diversity). In *S. meliloti* chromosomal regions exhibiting a lower GC content may have been acquired from lateral gene transfer, and there is a suggestion that *vapAD* genes might be relics of the ancient integration of plasmids into the chromosome (Capela et al., 2001).

In *Rhizobium* species the genes for symbiosis are plasmid borne, while they are located in the chromosome of *Bradyrhizobium* and *Mesorhizobium* species. The exceptions in *Mesorhizobium* are *M. amorphae* (Wang et al., 1999c) and *M. huakuii* (Guo et al., 1999), which have symbiotic plasmids. The mobile "symbiotic island" of *M. loti* integrates into the chromosome of the recipient strain (Sullivan and Ronson, 1998). Transfer of plasmids among species and genera of nodule bacteria has been obtained repeatedly in the laboratory (Martínez et al., 1987 and references therein; Rogel et al., 2001), and from the comparative analysis of chromosomal and plasmid markers it has been suggested that plasmid transfer also occurs in stems (Laguerre et al., 1993; Perret and Broughton, 1998).

Among *Rhizobium* and *Sinorhizobium* strains nodulating *Phaseolus vulgaris* there are indications that genetic transfer of symbiotic information has occurred because *nodA* gene sequences are quite similar among these strains (Laguerre et al., 2001). This may also explain the *nifH* and *nod* gene tree topology obtained from different *Sinorhizobium* strains. Large differences were observed in these genes in the American isolates compared with the African isolates (Haukka et al., 1998). The American *Sinorhizobium* isolates seemed to cluster with the American *Rhizobium* isolates by analyzing *nod* and *nif* gene sequences. Because *nod* and *nif* genes are plasmid borne, it is possible that lateral transfer of plasmids has occurred among these genera. Also common insertion elements were found in *S. meliloti* and former *Agrobacterium* strains (Deng et al., 1995).

From the genome sequencing projects (Freiberg et al., 1997; Kaneko et al., 2000), it has been observed that similar plasmid genes exist in different genera and species, suggesting that plasmids have transferred among strains. Interestingly, in some strains the genetic information located on plasmids is not confined to plasmids in other strains (Haugland and Verma, 1981; Galibert et al., 2001). Examples of the spontaneous integration of plasmids and megaplasmids into chromosomes have been reported (Mavingui et al., 2002), revealing the plasticity in genome organization of nodule bacteria.

Plasmid transfer between bacteria has been recognized as a mechanism potentiating their rapid adaptation and colonization of different niches (Reanney, 1976; Mazodier and Davies, 1991; Souza and Eguiarte, 1997). In *R. etli*, the colonization of bean nodules or of maize as an endophyte seems to correlate with the plasmid content of the bacteria and not with chromosomal characteristics (Gutiérrez-Zamora and Martínez Romero, 2001).

It has been shown on several occasions that bacteria incapable of forming nodules are close relatives to symbiotic ones (Martínez-Romero

et al., 2000; Gándara et al., 2001). This is also the case with the novel *Methylobacterium* and β-Proteobacteria described previously. An ancient lateral transfer of nodulation genes is the most viable explanation for the presence of *nod* genes in the β-Proteobacteria (Moulin et al., 2001) and in *Azorhizobium*. It seems that the earlier proposal to describe nodule-bacteria phylogenies or relationships as nets (Martínez-Romero and Caballero-Mellado, 1996) instead of trees to account for lateral transfer is pertinent.

C. Approaches to Diversity Studies

Despite the controversy surrounding the definition of bacterial relatedness and phylogenies on the basis of 16S rRNA gene sequences (Young and Haukka, 1996; Martínez-Romero et al., 2000), 16S rRNA gene sequences constitute the largest database for the comparison of new isolates, and in the majority of cases the results derived from such analyses provide a very good indication of the affiliation of the isolate that is usually confirmed by independent data using alternative methods. It is worth mentioning that there can be heterogeneity of the 16S rRNA gene copies within a single species, for example, in *S. saheli* with two different sequences more different than those among *S. saheli* and *S. terangae* and also in a *Sinorhizobium* isolate from *Acacia senegal* (Young and Haukka, 1996). A PCR-RFLP analysis of 23S rRNA genes was not coherent with the 16S rRNA-derived trees (Terefework et al., 1998), and partial sequences of 23S rRNA genes also show some discrepancy to 16S rRNA gene sequences (van Berkum et al., 1999) but complete 23S rRNA gene sequences of some strains confirmed in general the 16S rRNA gene sequence-based relationships (Pulawska et al., 2000). The 5' end of the 23 S rRNA gene contains an intervening sequence that is cleaved during rRNA processing and has also been used to classify rhizobia (Selenska-Pobell et al., 1997).

Because ribosomal RNA gene sequences are too conserved to reveal differences among closely related bacteria, intergenic sequences of ribosomal genes (ITS) have been analyzed (Sessitsch et al., 1997b; Vinuesa et al., 1998; Tan et al., 2001a). In addition, the banding patterns of some tRNAs and of 5S rRNA are proving useful to recognize rhizobia groups and species (Velázquez et al., 1998, 2001a and b).

To better describe rhizobial diversity and propose robust bacterial phylogenies, genes other than the ribosomal RNA genes have been analyzed, such as glutamine synthetase (GSI, Turner and Young, 2000, and GSII, Wernegreen and Riley, 1999; Turner and Young, 2000), *recA* and *atpD* (Gaunt et al., 2001). Interestingly, the analyses derived from the groupings were in agreement with those proposed by the 16S rRNA gene sequence data analysis, namely, *Sinorhizobium* was distinguished as independent from *Rhizobium*, *Mesorhizobium,* and *Bradyrhizobium* (Wernegreen and Riley, 1999; Turner and Young, 2000; Gaunt et al., 2001). *Azorhizobium* was clearly separated as well. The picture that emerged generated confidence in the 16S rRNA gene sequence based trees but left a small margin of doubt for the existence of lateral transfer of genetic information among genera to explain the anomalous placing of the GSII sequences of *B. japonicum* and of *M. loti* and *M. haukuii*. With this analysis the *M. loti* and *M. huakuii* did not cluster with the other *Mesorhizobium* strains (Turner and Young, 2000). Two copies of GSII gene sequences have been found in *S. meliloti*, one in the chromosome and the other in megaplasmid B (Capela et al., 2001). The number of copies of GSII in other bacteria is unknown, and it remains to be established if possible recombination events among the gene copies may affect their phylogenetic information.

Undoubtedly the proportion of the *Rhizobium* genome sampled is still very small. How many more genes need to be sequenced to better define rhizobia phylogenies? Do key genes exist that may reflect the evolution of these bacteria? It is clear that new approaches are required to depict more realistic evolutionary trends of these bacteria. Global approaches such as microarray analysis and proteomics would be of value to address nodule-bacteria diversity, and these will be used frequently in the future. The determination of (not necessarily 100% complete) genomic sequences from several rhizobia is feasible and will be relevant for diversity studies.

D. *Mesorhizobium* Diversity

Mesorhizobium species have been encountered in Africa, Asia, Australia, Europe, South and North America, and even in the Arctic (Poinsot et al., 2001). They nodulate various legumes in the Mimosoideae and Papilionoideae subfamilies of the Leguminosae. The genus was described as being intermediate between *Rhizobium* and *Bradyrhizobium* (Jarvis et al., 1997). There is a range of growth rates within *Mesorhizobium* strains, but in general they grow slower than *Rhizobium* and faster than *Bradyrhizobium*.

The DNA-DNA relatedness among all the described *Mesorhizobium* species is 2 to 35%, and protein pattern correlation coefficients of 75 encompass all the *Mesorhizobium* species. The Asian species *M. huakuii* and *M. tianshanense* are not more closely related to each other than to the other *Mesorhizobium* species by 16S rRNA gene sequence or by patterns of metabolic enzymes (Wang et al., 1999c). The size of the chromosome of *M. loti* MAFF 303099 is 7.03 Mb and this strain carries two additional plasmids that do not harbor the *nod-nif* genes, which are located on the chromosome (Kaneko et al., 2000). It remains to be established if the other mesorhizobial species or *M. loti* strains have similar chromosome sizes.

In the genus *Mesorhizobium* there seems to exist symbiotypes moving or flowing over different genotypes based on the following data: (1) seven Chinese *Mesorhizobium* strains from *Astragalus sinicus* representing different 16S and 23S ribosomal DNA genotypes (one of them corresponding to *M. huakuii*) had identical *nodA* sequences (Zhang et al., 2000), (2) besides *M. mediterraneum* and *M. ciceri* (Nour et al., 1994) there are other mesorhizobial genomic groups nodulating *Cicer arietinum* (Nour et al., 1995). The *nodA* gene sequences of *M. mediterraneum* and *M. ciceri* are almost identical (Zhang et al., 2000), suggesting the existence of gene transfer between these two species, (3) in addition to *M. amorphae*, two additional *Mesorhizobium* genotypes were found to nodulate *Amorpha fruticosa,* and they all had a symbiotic plasmid of identical size with common *nifH* gene hybridization patterns, (4) *M. plurifarium* encompasses a diverse but continuous group of bacteria that nodulate *Acacia* spp., *Prosopis* and *Leucaena* (de Lajudie et al., 1998b), and (5) *M. loti* symbiotic islands may be transferred and contained within different *Mesorhizobium* genotypes (Sullivan et al., 1996). Other data derived from the analyses of *nod* genes and GSII sequences (Wernegreen and Riley, 1999) also support the absence of genetic barriers for lateral transfer within *Mesorhizobium* lineages. There might have been an ancient exchange of genes between a *Rhizobium* species and *M. haukuii* (Turner and Young, 2000), and a *Mesorhizobium* isolate from *Astragalus adsurgens* that exhibits a mosaic sequence of 16S rRNA genes that carry characteristic signatures of *Rhizobium* strains (Gao et al., 2001).

The symbiotic islands of two *M. loti* strains (Sullivan and Ronson, 1998; Kaneko et al., 2000) are different in size and have around half of the nucleotide sequences in common. Conserved sequences were interrupted by insertions and deletions (Ronson et al., 2001). The symbiotic islands of *M. loti* have been classified as belonging to the family of conjugative transposing elements called CONSTINs (Hochhut and Waldor, 1999). Furthermore, genomic islands harboring other genes seem to exist in *Mesorhizobium* (Ronson et al., 2001), which could also be involved in moving large pieces of DNA among *Mesorhizobium* strains. The linear comparison of the *M. loti* whole genome with *S. meliloti* reveals some regions (around 6) present in *M. loti* but not in *S. meliloti*. These could have been acquired in *M. loti*. If there are several genomic islands that confer ecological adaptation and these were to move frequently between *Mesorhizobium*, then these bacteria could be highly chimeric (with no species boundaries) resulting from a mixture of genomes. Clearly, this requires further research.

E. *Bradyrhizobium* Diversity

Bradyrhizobium species are encountered in Africa, Asia, North and South America, Europe, and Australia. They nodulate a wide diversity of legumes in the Mimosoideae, Caesalpinoideae, and in the Papilionoideae. Many native tropical legumes from the Amazons, Africa, and Central America (Doignon-Bourcier et al., 1999; Parker and Lunk,

2000; Parker 2001), but also legumes in Mediterranean and temperate areas (Lange 1961; Barrera et al., 1997; Parker, 1999) are nodulated by *Bradyrhizobium*. Photosynthetic *Bradyrhizobium* strains have been isolated from stem and root nodules of *Aeschynomene* (So et al., 1994; Molouba et al., 1999). More recently, *Bradyrhizobium* strains have been found inside rice plants in Asia (Engelhard et al., 2000) and Africa (Chaintreuil et al., 2000). A characteristic of the genus is its slow growth and alkali production in some media (Graham and Parker, 1964). *B. liaoningense* was described as a particularly slow grower (Xu et al., 1995), a characteristic shared with some isolates of *Acacia* (Barnet and Catt, 1991) and *Phaseolus lunatus* (Ormeño et al., unpublished) or a number of Caesalpinoidea isolates from Brazil (Moreira, 2000). In *Bradyrhizobium* there are strains tolerant to high temperatures, desiccation, acidity (reviewed in Graham, 1992), and strains that fix nitrogen as free-living bacteria under low levels of oxygen (Keister, 1975). The size of the genome of *B. japonicum* is 8.7 Mb, whereas the size of other species is unknown.

In Africa, native *Bradyrhizobium* strains were recruited to nodulate adapted soybean cultivars, eliminating the need for inoculation (Abaidoo et al., 2000). Soybean symbionts used as inoculants in Brazil were identified as *B. elkanii* (Rumjanek et al., 1993) or *B. japonicum* and *B. elkanii* (Boddey and Hungria, 1997). Both *B. japonicum* and *B. elkanii* have been recovered from soybean nodules in the USA (Keyser et al., 1984), in Paraguay (Chen et al., 2000), and in Japan (Minamisawa et al., 1992). *B. japonicum* strains with different interstrain nodulation competitive abilities that belong to different serogroups were recognized (Moawad et al., 1984; van Berkum et al., 1993).

Few species have been described in *Bradyrhizobium,* and three of them correspond to soybean symbionts despite the fact that *Bradyrhizobium* strains are symbionts of a wide range of legumes. These symbionts were ascribed to "cowpea" bradyrhizobia in the past (Jordan, 1984). Recently, novel bradyrhizobia groups have been reported. Legumes from the Canary Islands in Spain are nodulated by *Bradyrhizobium* spp. (Vinuesa et al., 1998). A number of *Bradyrhizobium* strains from peanut (van Rossum et al., 1995; Zhang et al., 1997), from *Lupinus* spp. (Barrera et al., 1997), and from *Phaseolus lunatus* (Ormeño et al., unpublished) were found to be related to *B. japonicum*. The *Lupinus* isolates from the cold uplands in Mexico and the *P. lunatus* isolates from their site of origin in Peru had low DNA relatedness (around 35%) to *B. japonicum* despite a high identity of their 16S rRNA genes. As well, the commercial *Lupinus* isolate in Australia, WU425, is widely divergent from *B. japonicum* and *B. elkanii* by fatty acid methyl ester analysis (FAME) (Graham et al., 1995). The *Bradyrhizobium* from peanuts in China resemble *B. japonicum* by 16S rRNA gene sequence but not by FAME (Chen et al., personal communication). Some isolates from tropical legumes in Panama were related to *B. japonicum* USDA 110 or to bradyrhizobia in the Phillipines (Parker and Lunk, 2000). North American isolates from *Amphicarpae* (Phaseoleae) and from *Apios* and *Desmodium* resembled *B. elkanii* by 16S rRNA gene sequences (Parker, 1999) but differed from it by isozyme alleles, *nod* genes sequences, and symbiotic behavior (Marr et al., 1997; Sterner and Parker, 1999). It has been discussed previously that the high conservation of 16S rRNA genes in (Barrera et al., 1997) hampers species distinction, and this may be related to the fact that there is only one ribosomal operon in *japonicum* (Kündig et al., 1995; Göttfert et al., 2001) compared with three copies in *Rhizobium* and *Sinorhizobium* (Capela et al., 1999) and two in *Mesorhizobium* species (Kaneko et al., 2000).

A number of alternative approaches that have been useful for other bacteria have been explored to describe *Bradyrhizobium* diversity. These approaches include AFLP analysis (Willems et al., 2000; Chen et al., unpublished results), pyrolisis mass spectrometry (Barrera et al., 1997), low-molecular-weight RNA profiles (Velázquez et al., 1998), FAME (Graham et al., 1995; Zhang et al., 1997), REP-PCR fingerprints (Chen et al., 2000), ribosomal intergenic analysis (Vinuesa et al., 1998; van Berkum and Fuhrmann, 2000; Doignon-Bourcier et al., 2000; Tan et al., 2001a), and others (Minamisawa et al., 1992; Ladha and So, 1994). All these approaches reveal more diversity

than that shown by ribosomal RNA gene sequence data.

By DNA-DNA hybridization a complex and seemingly continuous array of *Bradyrhizobium* strains from diverse legumes was revealed (Willems et al., 2001a, and b), and AFLP analysis showed 34 distinct groups, although phenotypic differences could not be attributed to these groups (Willems et al., 2000).

There is a notorious lack of correlation between phenotypic and genetic analyses in bradyrhizobia (So et al., 1994; van Rossum et al., 1995), and this has led to questioning of the usefulness of the polyphasic approach in this group (So et al., 1994). The sequence of the symbiotic region (chromosomal) in *B. japonicum* has been reported (Göttfert et al., 2001), and there was evidence of horizontal transfer of symbiotic genes in *Bradyrhizobium* (Isawa et al., 1997). Recombination among a *B. japonicum*-like strain and *B. elkanii* has been postulated to occur in nature to explain the mosaic observed in the 5' fragment of the 23S rRNA gene sequence in a *B. elkanii*-related strain isolated in Panama (Parker, 2001). The similarity of the *B. elkanii* 16S rRNA gene signature sequence to *Mesorhizobium* has been interpreted as evidence of recombination (Lafay and Burdon, 1998). The possibility that "*Bradyrhizobium* strains may be affecting each other's evolution through exchange of various... genes" has been discussed (Parker, 2001).

Are there genomic islands or conjugative transposons (in analogy to those in *Mesorhizobium*) in *Bradyrhizobium* species? The existence of genomic islands in strains of the genus *Bradyrhizobium* could fit with their large chromosome size, the continuity of genotypes, the finding that their plant-host specificity is scattered among genotypes and with the lack of congruence of phenotypic and genetic data. This deserves future research.

F. *Rhizobium* and (Former) *Agrobacterium* Diversity

Rhizobium species are found worldwide; they interact with a large diversity of plants even outside the Leguminosae and have been found as endophytes (see section VI). For a long time *Rhizobium* and *Agrobacterium* have been recognized as being closely related genera (Graham, 1964). Several proposals for the inclusion of *Agrobacterium* in *Rhizobium* have emerged (Graham, 1964; Sawada, 1993; Pulawska et al., 2000 and references in Gaunt et al., 2001). The close relatedness of both genera has been confirmed with different molecular approaches and with novel isolates, and a formal proposal for the amalgamation of both genera has been published (Young et al., 2001).

The similarity of *Agrobacterium* and *Rhizobium* species has been revealed using a range of analyses. For example, *R. tropici* and *Agrobacterium* bv. 2 strains resemble one another in morphology, growth rate, tolerance to acid pH, DNA-DNA hybridization (Martínez-Romero, 1994) and in the sequences of 16S (Willems and Collins, 1993) and 23S rRNA genes (Pulawska et al., 2000). We also found sequences homologous to *R. tropici teu* genes (Rosenblueth et al., 1998) in *Agrobacterium* plasmids (*A. rhizogenes* Ri plasmid NC 002575, GenBank). *R.* (former *Allorhizobium*) *undicola* are nodule isolates from *Neptunia natans* that are closely related to the former *A. tumefaciens* (de Lajudie et al., 1998a). *R. galegae* and *R. huautlense* are sometimes placed in the neighborhood of *Agrobacterium* (Wang and Martínez-Romero, 2000), but their phylogenetic position is perhaps uncertain (Wang et al., 1998). Other nodule bacteria closely related to *R. radiobacter* (formerly *Agrobacterium*) have been described by 23S rRNA-based PCR-RFLP analysis (Terefework et al., 1998) and by 16S rRNA gene sequences (Tan et al., 2001a).

Differences in plasmid content may explain to a large extent the different behavior of symbionts and pathogens. In *Agrobacterium* that form tumors in *Chrysanthemus*, plasmids in addition to the tumorogenesis (Ti) plasmid have effects on virulence (Ogawa and Mii, 2001). In *Rhizobium* species, plasmids other than the *nod-nif* plasmid have roles in symbiosis (reviewed in Toro, 1996; García de los Santos et al., 1996), and in species of the genus *Rhizobium* (including former *Agrobacterium*) there exist cured derivatives that lose symbiotic or pathogenic characteristics (Segovia et al., 1991).

Plasmids in *Rhizobium* species may carry genes of catabolic pathways (García de los Santos et al., 1996; Oresnik et al., 1998), and the distri-

bution of the *repABC* origins of replication in *Rhizobium, Sinorhizobium,* and *Mesorhizobium* species symbiotic and tumorogenesis plasmids suggest their common evolutionary origin (Turner et al., 1996; Rigottier-Gois et al., 1998). The existence of plasmid replicons related to the symbiotic plasmids in bacteria such as *Paracoccus* (Bartosik et al., 1997) and *Rhodobacter* species indicate perhaps that *nod-nif* plasmids could be encountered in these bacteria (Gaunt et al., 2001).

In *Rhizobium* and *Sinorhizobium* strains there may exist diverse symbiotic specificities within a single species in relation to the different symbiotic plasmids carried by the bacteria. The epithet biovars are used to refer to this symbiotic diversity within a species and they have been described for *R. leguminosarum* (bvs. *viciae*, *trifolii* and *phaseoli*, Jordan, 1984), *R. etli* (bvs. phaseoli and *mimosae*, Wang et al., 1999b), *S. terangae* and *S. saheli* (bvs. acaciae and sesbaniae, Lortet et al., 1996), *R. galegae* (bvs. *orientalis* and *officinalis*, Radeva et al., 2001), and *R. gallicum* and *R. giardinii* (bvs. *phaseoli* and *gallicum*, Amarger et al., 1997). The existence of different symbiotic plasmids in a common genetic background may be explained by plasmid exchange occurring in nature.

The genus *Rhizobium* encompasses bacteria with a large biological diversity that may be explained by being an older lineage or by having more efficient mechanisms of genetic exchange or simply by being polyphyletic.

G. *Sinorhizobium* Diversity

Sinorhizobium species have been encountered in Asia, Africa, Europe, and North and South America. There is *S. meliloti* in Australia, which may have co-evolved with the indigenous *Trigonella* or may have been introduced, as is the case in China for *Medicago* isolates that exhibit a limited genetic diversity (Yan et al., 2000).

Sinorhizobium strains from *Medicago sativa*, *M. falcata,* and *M. truncatula* were recognized as two diverging but related groups (Eardly et al., 1990; Rome et al., 1996a) and later divided into two different species (Rome et al., 1996b). There are indications that there is no genetic recombination or plasmid transfer between these two closely related species (Rome et al., 1996a). Other *Medicago* isolates from *M. ruthenica* have an affiliation to *Rhizobium* strains (van Berkum et al., 1998) but also to *Sinorhizobium* strains by other criteria (van Berkum et al., 1999).

A number of related lineages have been encountered nodulating *Acacia* and *Sesbania* in Africa (de Lajudie et al., 1994; Haukka et al., 1998; Nick et al., 1999). It is possible that these species represent the spreading and divergence from some common ancestor.

In Asia *S. fredii* strains nodulate soybean; a strain seemingly related to it, NGR234 (isolated from New Guinea), has the largest host range ever reported, including several species in the Caesalpinioideae subfamily (Pueppke and Broughton, 1999). Interestingly, *S. fredii*-like bacteria have been recovered from *Phaseolus vulgaris* in nodules in Spain (Herrera-Cervera et al., 1999), and as nodule isolates of *Acacia* native species from a reserve area in Mexico (Toledo et al., unpublished results). The Spanish and Mexican *S. fredii* isolates do not nodulate soybean, and we suggest they have been geographically isolated from their Asian relatives for million of years.

Recently, *Ensifer adhaerens* was recognized as being related to the genus *Sinorhizobium* based on its 16S rRNA gene sequence (Balkwill, 2001). It has DNA-DNA homology in the range of other *Sinorhizobium* species (Rogel et al., 2001) and is most closely related to novel *Sinorhizobium* groups identified recently (Wang et al., 2002). *E. adhaerens* strains are predatory soil bacteria (Casida, 1982; Germida and Casida, 1983). They did not form nodules on the hosts tested or do they have *nod* genes (Rogel et al., 2001), but they may become nodulating bacteria when acquiring the symbiotic plasmids from *R. tropici* (Rogel et al., 2001). *E. adhaerens* may be transferred to *Sinorhizobium* if more evidence becomes available.

In *Sinorhizobium* species, the *nod* and *nif* genes are located on plasmids of around 500 kb, with the exception of *S. meliloti* Rm 1021, which has two megaplasmids of 1354 kb and 1683 kb (Galibert et al., 2001) that may be the product of a co-integration event. The size of the chromo-

some of *S. meliloti* 1021 is 3.7 MB (Capela et al., 1999) and that of NGR234 is nearly 3.5 MB (Viprey et al., 2000).

H. *Azorhizobium* Diversity

Azorhizobium strains were isolated in Africa (Dreyfus et al., 1988) and also in Asia from rice (Engelhard et al., 2000). The remarkable characteristic of *Azorhizobium* strains is that they can form photosynthetic stem-nodules and fix nitrogen as free-living bacteria. Only one species is recognized, and it is closely related to *Aquabacter* and to *Xanthobacter* species (Rainey and Wiegel, 1996). All of them have been assigned to a new family (Kuykendall et al., 2002).

Azorhizobium is the genus with the fewest different isolates of nodulating bacteria. The search for other stem nodulating bacteria has rendered bacteria belonging to *Bradyrhizobium* (So et al., 1994).

In *A. caulinodans*, *nod* genes are located on the chromosome, but they have a different GC content and are bordered by insertion elements. These findings led to the proposal that nodulation genes were acquired by lateral transfer from other nodule bacteria. Interestingly, the Nod factors produced by another *Sesbania* symbiont, *S. terangae* bv. sesbaniae, are similar to those produced by *A. caulinodans*.

I. Diversity Epilogue

The more we have sampled the diversity of a nodule-bacteria group, the more difficult it is to define species for such clusters because more contiguous and continuous lineages or branches are then revealed. This is the case with the groups closely related to *R. etli*, with the intermediate bacteria seemingly recombinants between *R. tropici* A and B (Martínez-Romero, 1996), and is becoming the case in the genera *Bradyrhizobium*, *Mesorhizobium,* and even *Sinorhizobium.* Sometimes species delimitations may be artifactual in these bacteria.

V. SIZE, STRUCTURE, AND FEATURES OF RHIZOBIAL GENOMES

More than 3 years after the publication of the sequence of pNGR234*a*, the symbiotic plasmid of *Rhizobium* sp. NGR234 (Freiberg et al., 1997), we now have available the DNA sequences of a 410-kb symbiotic-gene-rich region of *B. japonicum* 110 (Göttfert et al., 2001), the 7.6 and 6.7 Mb genomes of *M. loti* strain MAFF303099 and *S. meliloti* strain 1021, respectively (Kaneko et al., 2000; Galibert et al., 2001) and that of the closely related plant pathogen *Agrobacterium tumefaciens* strain C58 (unpublished; see Table 4). This overwhelming flow of sequence data is probably not over, because the symbiotic plasmid of *R. etli* sequenced more than a year ago will eventually be deposited in databases, and the genome of *B. japonicum* will be completed in Japan in the near future. With sizes ranging from 5.5 to almost 9 Mb, genomes of rhizobia and related bacteria are large when compared with those of other microorganisms and often encode more proteins (Table 4) than the 6300 predicted on the 15 chromosomes (≅12 Mb) of *Saccharomyces cerevisiae*. Compared with the rest of the genome, genes directly involved in symbioses with legumes (nodulation and nitrogen fixation loci) or in the formation of crown gall by *Agrobacterium* represent only a small fraction of all encoded peptides.

The structure of these genomes varies considerably: from the single 8.7-Mb chromosome of *B. japonicum* (Kündig et al., 1993) to strains containing circular and linear chromosomes as well as plasmids (e.g., *A. tumefaciens* C58) (Allardet-Servent et al., 1993), or those having megaplasmids larger than many bacterial genomes and encoding essential functions as in *S. meliloti* 1021 (Finan et al., 2001). Accordingly, the number of replicons ranges from one (*B. japonicum* strain 110) to at least seven as in *R. etli* CFN42 (Quinto et al., 1982), and symbiotic genes can be found either on chromosomes and/or on plasmids. This diversity in size and structure of genomes correlates well with the diversity found in the taxonomy of rhizobia. It also indicates that the position of symbiotic loci on one or the other of the cell's replicons has no or little effect on their phenotypic expression (Mavingui et al., 2002).

TABLE 4
Structure, Size, and Major Features of Genomes of Members of the Rhizobiaceae

Strain	Replicon	Size (bp)	Predicted Proteins
***Agrobacterium tumefaciens* strain C58**			
	circular chromosome	2,841,581	2,721
	linear chromosome	2,074,782	1,833
	plasmid AT	542,869	547
	plasmid Ti	214,331	198
Total	4	5,673,563	5,299
***Mesorhizobium loti* strain MAFF303099**			
	circular chromosome	7,036,074	6,746
	plasmid pMla	351,911	320
	plasmid pMlb	208,315	209
Total	3	7,596,300	7,275
***Sinorhizobium meliloti* strain 1021**			
	circular chromosome	3,654,135	3,341
	megaplasmid pSymb	1,683,333	1,570
	plasmid pSymA	1,354,226	1,294
Total	3	6,691,694	6,205

Source: NCBI genome database
(http://www.ncbi.nlm.nih.gov:80/PMGifs/Genomes/micr.html)

A. RIMES, Mosaic Elements and Other DNA Repeats

Rhizobial genomes often contain families of reiterated sequences. The probing of restricted genomic DNA of two *Rhizobium etli* strains (*R. phaseoli* CFN42 and CFN285), *S. meliloti* 2011, and *A. tumefaciens* C58 with clones of random libraries showed the presence of up to 200 families of DNA repeats per genome (Flores et al., 1987). Repeated sequences range in size from 50 bp (Perret et al., 2001) to several kilobases (Perret et al., 1987) and represent duplications of uncoding regions (mosaic elements) (Perret et al., 2002) or of genes involved in house-keeping functions (e.g., operons coding for ribosomal RNAs), symbioses, and N_2-fixation with legumes (*nodD* and *nifHDK* loci) (Quinto et al., 1982) and transposition (genes of insertion sequences — IS). Duplicated regions can be identical or highly conserved, such as many of the IS elements found in NGR234 (Freiberg et al., 1997; Perret et al., 1997), or they can show a varying degree of sequence similarity like the hundreds of RIMEs (*Rhizobium* Intergenic Mosaic Elements) found in *S. meliloti* strain 1021 (Galibert et al., 2001, Østeras et al., 1995) and NGR234 (Perret et al., 2001). Interestingly, the distribution of these repeated elements is not regular. For example, RIME1 and RIME2 sequences are found preferentially on the chromosomes of *S. meliloti* and NGR234 (Galibert et al., 2001; Perret et al., 2001). Conversely, many IS elements accumulate on plasmids rather than on chromosomes (Perret et al., 1997; Galibert et al., 2001). Although duplication/amplification of symbiotic genes may improve the symbiotic competence of rhizobial strains (Mavingui et al., 1997), the effects of an

increased number of IS and RIMEs on the phenotypes of modified rhizobia is not clear. The presence of many ancient duplications in *S. meliloti* strain 1021 (42% of all genes belong to paralogous families) (Galibert et al., 2001) suggests that genomes of rhizobia may sustain large numbers of repeated sequences without the noticeable effects on bacterial growth and survival. The occurrence of dispersed and large DNA repeats has direct consequences on the stability of the genome architecture, however. Recent analyses using NGR234 as a model system have confirmed that highly conserved and repeated sequences such as those found on pNGR234*a* become preferential sites for homologous recombination at elevated frequencies (Flores et al., 2000). Although this phenomenon can be used by researchers to generate strains with specific symbiotic properties (Flores et al., 2000), the effect of genome rearrangements on the phenotypes of rhizobia occurring in soil is not well understood. Nevertheless, major genome rearrangements such as the integration of both, the c.a. 2.2-Mb megaplasmid pNGR234*b* and the 536-kb symbiotic plasmid pNGR234*a*, into the chromosome of NGR234 have no obvious effect on the growth and symbiotic properties of the modified strains (Mavingui et al., 2002). This suggests that despite obvious problems linked to replication and modifications in the copy number of many genes carried by these replicons, rhizobial genomes are flexible and probably more unstable than previously thought.

B. Symbiosis Islands

Despite the number of bacterial genes required for the establishment of functional symbioses with legume hosts, and the need to preserve their coordinated and timely expression, recent data have confirmed that symbiotic loci can be transferred to nonsymbiotic soil bacteria by means other than mobilization of symbiotic plasmids. Isolation of microsymbionts from nodules of *Lotus corniculatus* grown for 7 years in fields originally devoid of nodulating *Mesorhizobium loti* resulted in the identification of various strains, all of them distinct from the original inoculant strain ICMP3153 (Sullivan et al., 1995). Interestingly, genetic analyses showed the nodulation and nitrogen fixation genes acquired by the local and nonsymbiotic rhizobia were laterally transferred from the inoculant strain via a 500-kb "symbiotic island" (SI) that integrated into the *phe*-tRNA gene of the recipient bacteria (Sullivan and Ronson, 1998). Like other elements of the family of "genomic islands", SI do not carry origins of replication and must integrate into a replicon (in general the chromosome) in order to persist within the host genome. Like other elements of the flexible gene pool, SI share characteristics of mobile genetic elements but carry larger numbers of genes than transposons and phages (Hacker and Carniel, 2001). As genomic islands often have guanine plus cytosine (G+C) contents and codon usage distinct from that of the host genome, bioinformatic analyses of complete genome sequences can lead to their identification.

Using such a procedure, a large symbiosis island was identified in the genome of the Japanese microsymbiont *M. loti* MAFF303099 (Kaneko et al., 2000). With a G+C content 4% lower than the rest of the genome (Table 4), the island of MAFF303099 is 100 kb larger than that of *M. loti* R7a and carries additional genes such as those coding for a complete type III secretion system. Although the DNA sequence of the *M. loti* R7a island has not been fully released, a comparison of the few sections accessible in GenBank with the complete sequence of MAFF303099 showed that both islands are quite different. Except for IntS, a P4-like integrase that possibly mediates integration of *M. loti* SI, the rest of the regions bordering the element in R7a (GenBank accession number AF049242 and AF0249243) are completely different from those in the MAFF303099 chromosome. Interestingly, *in silico* probing of MAFF303099 genome with a 13.2-kb DNA sequence of *M. loti* R7a (GenBank accession number AF311738) that carries a functional *nifA* as well as genes required for the biosynthesis of biotin and nicotinate (Sullivan et al., 2001) confirmed the presence of two highly homologous loci in MAFF303099. As expected, the most conserved region is located on the symbiosis island (genes mll5837 to mll5826), whereas the second locus that does not contain a *nifA* copy (genes mll9106 to mll9093) is carried by pMla, the largest of the two plasmids found in

MAFF303099. In addition, *bioA*, *bioB*, *bioF*, and *bioD* (involved in the biosynthesis of biotin) on the one hand, and *nifA* on the other hand are also duplicated elsewhere in the symbiosis island, suggesting that mutation of one copy of *nifA* or *bioZ* in MAFF303099 would not be sufficient to obtain the respective symbiotic and auxotrophic phenotypes observed with the Tn*5* mutants of R7a (Sullivan et al., 2001).

Even in the absence of a complete DNA sequence of R7a in the database, several conclusions can be drawn from the limited information available. First, like symbiotic plasmids of various rhizobial strains the structure and genetic organization of symbiosis islands seem to vary considerably. Duplications of loci, such as the key symbiotic regulator *nifA* and the biotin and nicotinamide biosynthetic genes, may account for some of the 100-kb difference in size between the islands of *M. loti* R7a and MAFF303099. Furthermore, the absence from the genome of MAFF303099 of *orfC* and *orfD* adjacent to *intS* of R7a indicate that symbiosis islands carry additional genes to those essential or directly related to nodulation and nitrogen fixation. Similarly, many genes encoded by the MAFF303099 island have no ortholog in *S. meliloti* (Galibert et al., 2001), suggesting that these loci may play accessory roles and increase the fitness of recipient strains in other ecological niches than the rhizosphere of legume hosts. If this is true, SI should perhaps be regarded as more general "fitness islands" rather than elements devoted exclusively to symbioses with legumes (Hacker and Carniel, 2001). Secondly, symbiotic genes carried by a genomic island may have distinct origins, as suggested when comparing the homologies obtained for NifA1 (mll5837) and NifA2 (mll5857) of *M. loti* MAFF303099: the mll5837 encoded product is very similar to NifA of *M. loti* R7a and *B. japonicum*, whereas NifA2 closest homologues are proteins of *R. etli*, *R. leguminosarum,* and *Rhizobium* sp. NGR234. Finally, at least in the case of *M. loti* MAFF303099, a large fraction (19.6%) of the genes and gene fragments encoded by the 611-kb island are related to functions of transposable elements (Kaneko et al., 2000). This elevated density of IS-like sequences that resembles the situation in pNGR234*a* (Freiberg et al., 1997) is not found in pMla, pMlb, and the rest of MAFF303099 chromosome. Thus, it is tempting to speculate that symbiotic plasmids/islands shuttle IS sequences across different hosts and promote propagation of these transposable elements (Perret et al., 1997). As many IS that often duplicated elsewhere in the genome of NGR234 are targets for site-specific recombination leading to alternate genome structures within a population of otherwise "clonal" cells (Flores et al., 2000; Mavingui et al., 2002), symbiosis islands such as that of MAFF303099 may also be prone to important genomic rearrangements. This can lead to the acquisition by the island of additional genetic functions (e.g., the gain of a second copy of *nifA*), or alternatively to the transfer of loci from the laterally acquired island and their integration elsewhere in the host genome.

C. Lateral Transfer of Genetic Information and Acquisition of Symbiotic Functions

A recurrent feature in rhizobia is the presence of genetic elements (plasmids, genomic islands, etc.) with G+C contents significantly different from the rest of the genome. Reported for megaplasmid pSymA of *S. meliloti* 1021 (60.4 mol% vs. 62.7 mol% for the chromosome) (Galibert et al., 2001), this is also true for the symbiotic plasmid of NGR234, plasmids pMla, pMlb, as well as the symbiosis island of *M. loti* MAFF303099 (Table 5). It is generally accepted that distinct codon usage and significant differences in G+C content when compared with the rest of the genome are features of regions that have an evolutionary history that differs from the vertically inherited genes (Ochmann et al., 2000). Once introduced, laterally transferred DNA sequences begin a process of amelioration through mutation, becoming gradually similar in G+C content with the rest of the host genome (Martin, 1999). Altogether these data suggest that large DNA segments were horizontally transferred to many rhizobial genomes, and that transfers occurred relatively recently (as for *M. loti* R7a) because the acquired sequences could be still distinguished from the genome of the new host.

In *S. meliloti* strain 1021 it has been postulated that nodulation genes have two distinct ori-

TABLE 5
Characteristics of Large and Fully Sequenced Symbiotic Regions

Strain	Region /Replicon	Size (bp)	Predicted Proteins	% G+C of Replicon	% G+C of Genome
***Bradyrhizobium japonicum* 110**	chromosomal region	410'573	388	58	61-65
***Mesorhizobium loti* MAFF303099**	symbiosis island	610'975	580	59.3	63.7
	pMla	351'911	320	59.3	63.7
	pMlb	208'315	209	59.9	63.7
***Rhizobium* sp. strain NGR234**	symbiotic plasmid	536'165	416	58.5	62

gins: horizontal gene transfer for most loci encoded by pSymA, and resident gene duplications for *nodM* and *nodPQ* (Galibert et al., 2001). In NGR234, most nodulation genes involved in the synthesis of Nod factors have G+C contents 7 to 17 mol% lower than chromosomal loci, also suggesting that symbiotic functions were acquired via the lateral transfer of pNGR234*a* (Freiberg et al., 1997). At c.a. 59 mol%, the nitrogen fixation genes (*nif* and *fix*) also encoded by pNGR234*a* have G+C contents that resemble more those of chromosomal loci, suggesting a distinct evolutionary history from nodulation genes (Dobert et al., 1994). Another striking feature is the mosaic structure of pNGR234*a*. In contrast to *nif* and *fix* homologues clustered between nucleotides 434,000 to 489,000, loci involved in nodulation are dispersed in at least six regions. Furthermore, the strong similarities with *A. tumefaciens* genes required for replication and conjugal transfer of pNGR234*a* and the presence of a cytochrome P450 gene cluster from *B. japonicum* (orfs y4kS to y4lD) highlights the diverse origins of the genetic elements that compose the symbiotic plasmid of NGR234. However, the fragmented evolution of rhizobial replicons is not limited to NGR234. As mentioned previously, similar features have been inferred from genomic sequence data in *S. meliloti* and *M. loti*. For instance, the presence of a 16-kb gene cluster being 80% identical at the nucleotide level to pNGR234*a* in plasmid pMla of strain MAFF303099 is most intriguing (Figure 4). Preceded by conserved NifA - σ^{54}-like sequences, these loci are expressed inside nodules formed by NGR234 on the roots of *Vigna unguiculata* (Perret et al., 1999). Although it is not clear whether these genes contribute directly to nitrogen fixation in *M. loti*, their location on pMla clearly shows that putative symbiotic loci are not necessarily restricted to so-called symbiotic islands/plasmids. The presence of IS-like sequences between the three major operon structures showing the highest degree of conservation (see Figure 4) also indicates an important role for transposable elements in the evolution of this region. Therefore, many forces probably contribute to shape the genomes of microsymbionts. Among these, horizontal transfer of plasmids and genetic islands allow the integration of symbiotic functions into new soil bacteria possibly better adapted to local environmental conditions or specific host-plants. In turn, and mostly through homologous recombination between duplicated sequences (such as conserved IS elements), major genome rearrangements (amplifications, deletions, replicon-fusions) modify the laterally transferred genetic elements allowing the integration (or the loss) of new (or old) symbiotic functions. Both mechanisms contribute to the evolution of symbiotic replicons/islands, a process possibly much more dynamic than was previously thought.

VI. *RHIZOBIUM* AS ENDOPHYTES AND AS PLANT GROWTH-PROMOTING RHIZOBACTERIA OF NON-LEGUMINOUS PLANTS

A. Endophytic Rhizobia

The definition of endophytes and the technical considerations for their isolation and characterization have been reviewed extensively (Reinhold-Hurek and Hurek, 1998a and b;

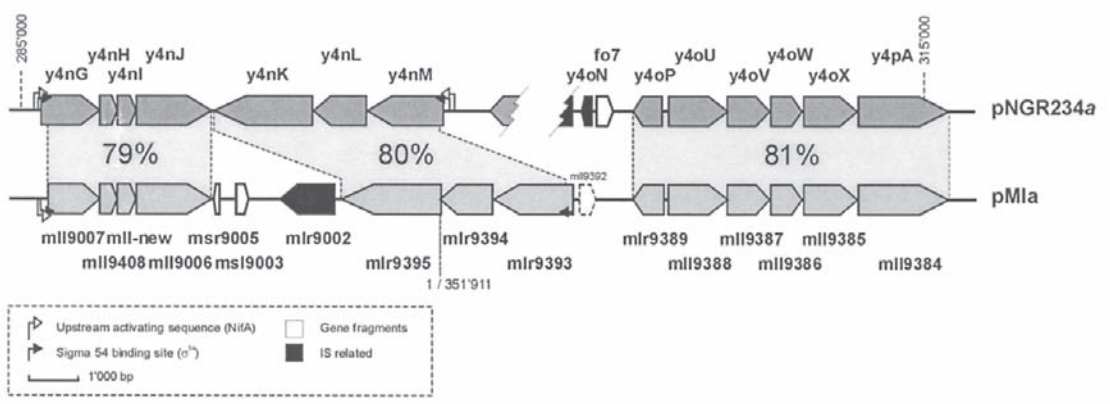

FIGURE 4. Comparison of DNA sequences encoded by pNGR234*a*, the symbiotic plasmid of *Rhizobium* sp. NGR234, and pMla, a 352-kb plasmid of *Mesorhizobium loti* strain MAFF303099. Highly conserved regions are shown with the levels of DNA sequence identity given in %.

Hartmann et al., 2000; James, 2000). In nonnodulating legumes (Sprent, 2001) rhizobia were commonly encountered inside roots without forming nodules (Allen and Allen, 1981). This is perhaps the first report of rhizobia as endophytes. Rhizobia have also been been found as common rhizosphere colonizers of a wide range of plants (Rovira, 1961; Höflich et al., 1995; Chabot et al., 1996) and also as endophytic bacteria not only of legumes (Sturz et al., 1997; O'Callaghan, 1999) but also of nonlegumes; in these cases rhizobia share their rhizospheric and endophytic habitats with many other bacteria (Piceno and Lovell, 2000). Could it be that rhizobia were first rhizospheric, then endophytic, and then nodule-forming bacteria? Rhizobia show effects of plant growth promotion (Höflich et al., 1995; Yanni et al., 2001) that may be due to phosphate solubilization, hormone production, and other traits. *R. undicola* strains (formerly *Allorhizobium*) were isolated as endophytes of banana plants and have been found to be plant growth-promoting rhizobacteria (PGPR) of plantlets derived from tissue culture (Lucía Martínez, personal communication).

There are indications of some degree of adaptation between local plants and endophytic rhizobia (Gutiérrez-Zamora and Martínez-Romero, 2001). Beneficial effects of local endophytes on crop plants have been shown in Brazil, whereas when several Chinese isolates were tested with Californian rice varieties, no increases resulted (Phillips et al., 2000). In that study decreases in plant development were obtained with a *Herbaspirillum* strain (identified on the basis of its 16S rRNA gene sequence). When the same Californian varieties were tested with locally isolated endophytes, plant growth promotion was observed with isolates such as *Pantoea* (Phillips et al., 2000; unpublished results). A large diversity among rice endophytes has been recorded recently (Watanabe et al., 1979; Ueda et al., 1995; Barraquio et al., 1997; Engelhard et al., 2000; James, 2000). In addition, azorhizobia were found in a survey of endophytic bacteria of native races of rice in Nepal (Engelhard et al., 2000). In this case there was no clear association of the rhizobial endophyte with legumes. In Africa, photosynthetic bradyrhizobia were found as natural endophytes of wild rice (Chaintreuil et al., 2000). These photosynthetic bradyrhizobia colonized the root surface and also intercellular spaces but were rarely found intracellularly. Furthermore, azorhizobia (Ladha et al., 1989), former *Agrobacterium*-like and *Bradyrhizobium* strains (Tan et al., 2001a), isolated from *Sesbania aculeata* and *Aeschynomene fluminensis* colonized rice endophytically. Their contribution to rice growth remains to be established.

Continuing the discussion of co-selection and adaptation of plants with bacteria, traditional legume-cereal associations have been studied with the hypothesis that rhizobia enriched in these soils could have served as inocula of nonlegumes for years. In Egypt, a 700-year-old tradition of cultivating rice in rotation with berseem clover provided the rationale for searching for rhizobia as rice endophytes (Yanni et al., 1997). The rhizo-

sphere of field grown rice cultivated in rotation with berseem clover contained about 1.7×10^6 indigenous *R. leguminosarum* bv. *trifolii* per gram of soil. These clover-nodulating rhizobia naturally invaded rice roots and achieved an internal population density of about 1.1×10^6 endophytes per gram of fresh weight of rice (Yanni et al., 1997). In these fields there were records of lower N-fertilizer requirements in the rice-clover rotation than in rice monoculture. Nevertheless, the contribution of N_2-fixation by rhizobia to rice does not seem to be substantial and other plant growth promotion effects have been implicated. A comprehensive review on this multinational project has been published indicating that the rice-clover rotation replaced 25 to 33% of the N-fertilization required in the rice-only fields (Yanni et al., 2001).

R. etli is a natural maize endophyte in traditional fields in Mexico, where there is an enormous richness of *R. etli* strains that nodulate *Phaseolus vulgaris* beans (Piñero et al., 1988 and reviewed in Martínez-Romero, 2002). Bean and maize might also have been co-domesticated as in Peru where bean and maize are grown in intercropping systems with seeds of both species sown together (Pineda et al., 1994). Mexican Indian and Peruvian traditional agriculture is still based on maize-bean associations, and in this way maize serves as a stalk for the climbing bean. This type of agriculture requires a fine developmental compatibility of maize and bean and its advantages have been documented (Souza et al., 1997). Unfortunately, modern agriculture has promoted maize and bean in monocultures in order to facilitate harvest mechanization. There is evidence that the application of N-fertilizers has inadvertedly favored beans with low capacities to fix nitrogen (Martínez-Romero, 2002). It may be that maize cultivars unfavorable for endophytic interactions have also been selected and that the delicate association of bean-*Rhizobium*-maize has been disrupted. Very good responses to inoculation have been obtained with *R. etli* in original maize races as well as in unimproved maize varieties when cultivated in growth chambers and in the greenhouse. The effects may be caused by plant growth-promoting substances such as hormones or lumichrome (Gutiérrez-Zamora and Martínez-Romero, 2001) or by a suppression of pathogens. The role of nitrogen fixation in this association is currently under study.

Maize, like sugarcane, is a C4 plant that may have less photosynthetic constraints to support bacterial nitrogen fixation than C3 plants. The discovery of nitrogen fixation in sugarcane opened a new avenue of research in this area (Ruschel et al., 1975; Boddey et al., 1991; Urquiaga et al., 1992). To attain considerable levels of N_2 fixation in sugar cane, plants require nearly optimal conditions of water, temperature, phosphate, and other nutrients (Boddey et al., 1995). The determinant role of the plant genotype on N_2-fixation has been recognized not only for sugarcane (Boddey et al., 1995; Urquiaga et al., 1989) but also for rice (App et al., 1986; Watanabe et al., 1987; Yoneyama et al., 1997). The capacity of sugarcane to fix nitrogen has been attributed to the fact that low levels of chemical N-fertilization have been used for this crop in Brazil for a long time. There are several reviews on sugarcane endophytes (Boddey et al., 1995; Baldani et al., 2000) and reports on their *in planta* localization (James et al., 1994; James and Olivares, 1998; Fuentes-Ramírez et al., 1999). It seems that bacterial communities are responsible for the nitrogen fixation process (Baldani et al., 2000).

The possible mechanisms of entry of endophytic bacteria into plant tissues have been reviewed (James et al., 1994; Reddy et al., 1997). It is a common finding that plant cells occupied by endophytes are dead (Hurek et al., 1994; Sprent and James, 1995; Reddy et al., 1997), and this constitutes an important contrast to the situation in nodules. Interestingly, saprophytic intracellular rhizobia have been found in old alfalfa nodules (Timmers et al., 2000). Little is known of the rhizobial genes required to penetrate the plant as an endophyte, but it seems that *nod* genes are not required (Gough et al., 1997), and that flavonoids enhance this process (Gough et al., 1997; Webster et al., 1998). The physiology of *Rhizobium* as an endophyte is unknown. Saprophytic *S. meliloti* cells in alfalfa nodules exhibited a limited expression of *nifH* genes but did not fix nitrogen within the limit of detection (Timmers et al., 2000). Expression of *nif* genes was detected on rice roots with *Azoarcus* (Egener et al., 1998) and with *Pseudomonas stutzeri* (Krotzky and Werner, 1987;

Vermeiren et al., 1998). Similarily, expression of the *R. etli nifB* gene on maize roots has been demonstrated (Martínez-Romero, unpublished).

While some genetic and physiological similarities between endophytic and pathogenic bacteria has been suspected, rhizobia as endophytes do not normally induce pathogenic symptoms in plants. The genetic differences distinguishing pathogens from endophytes would be worthy of future analysis. Looking to the future, *Arabidopsis thaliana* seems to be a promising model plant with which to study endophytic interactions (Gough et al., 1997; Triplett, personal communication) because of the availability of existing mutants and the knowledge of its genome. *A. caulinodans* and even *S. meliloti* have been found to colonize *A. thaliana*, however, *S. meliloti* cells were detected only in low numbers (Gough et al., 1996, 1997).

The relative contribution of rhizosphere and endophytic rhizobia to plant growth promotion is unknown. Do endophytes, being sometimes less numerous than their rhizospheric counterparts (Gutiérrez-Zamora and Martínez-Romero, 2001), provide real benefits to the plants? This is a question deserving of priority research in endophytic associations. In this regard, it is worth mentioning that in other systems bacteria-degrading recalcitrant compounds seem to be enriched in endophytic populations but not in the rhizosphere of plants in contaminated sites (Siciliano et al., 2001), and that endophytic bacteria of red clover are considered responsible for the negative allelopathic effects of clover on maize (Sprent and James, 1995; Sturz and Christie, 1996). Endophytic associations are gaining interest in the traditional field of nitrogen fixation research.

B. Rhizobia as Plant Growth-Promoting Rhizobacteria (PGPR)

Plant growth promoting rhizobacteria (PGPR) are a very small portion (2 to 5%) of rhizosphere bacteria that are able to promote plant growth or health when reintroduced in large numbers by inoculation (Antoun and Kloepper, 2001). Early studies on PGPR were performed mainly with fluorescent pseudomonads and root crops such as potato or beet. Plant growth promotion has been observed in many crops (Höflich et al., 1994; Antoun and Kloepper 2001) and forest trees (Holl and Chanway, 1992) inoculated with different Gram-positive or Gram-negative PGPR.

1. Root Colonization of Nonlegumes by Rhizobia

The ability of a PGPR to colonize roots in the presence of competing indigenous soil microflora is a major key to success in inoculation with beneficial bacteria. The PGPR strain *R. leguminosarum* bv. *trifolii* R39, isolated from red clover nodules, colonized the rhizospheres of pea, maize, and sugar beet better than the PGPR strain *Pseudomonas fluorescens* PsIA12 isolated from a wheat rhizosphere (Höflich et al., 1995). Under field conditions a rifampicin-resistant spontaneous mutant of strain R39 established a stable population (6.3×10^3 cfu per gram of fresh root) in maize roots during the vegetative period (Wiehe and Höflich, 1995). A more elaborate study showed that 4 weeks after inoculation, R39 was present exclusively in the rhizosphere soil and the rhizoplane of maize (Schloter et al., 1997). Fourteen weeks after inoculation the maize root cortex and the intracellular spaces of central root cylinder cells contained microcolonies of strain R39. By using bioluminescent mutants, Chabot et al. (1996) observed that 4 weeks after seeding the two mineral phosphate-solubilizing PGPR strains R1 and P31 of *R. leguminosarum* bv. *phaseoli* were superior maize and lettuce root colonizers compared with other PGPR strains tested. However, the internal root tissues of 4-week-old inoculated maize grown in a silty clay loam contained endophytes, but were free of Lux^+ rhizobia. Maize root colonization by strain $R1Lux^+$ was not affected by P-fertilization (Chabot et al., 1998). In a survey of bacterial endophytes of cotton and sweet corn, McInroy and Kloepper (1995) observed that *B. japonicum* was present exclusively in the roots of both crops.

2. Effect of PGPR Rhizobia on Yields

Yield increases caused by inoculation of nonlegumes with PGPR rhizobia have been reported

in pot and field experiments. *R. leguminosarum* bv. *trifolii* R39 promoted the growth of maize, spring wheat, and spring barley in field trials performed between 1985 and 1993 in a loamy sand soil in Germany (Höflich et al., 1994). Inoculation of these nonleguminous plants with R39 resulted in yields that were significantly ($P<0.05$) increased by 6 to 8%. The two rice endophytes *R. leguminosarum* bv. *trifolii* E11 and E12 enhanced rice grain yield by 46 and 42%, respectively, under field conditions (Yanni et al., 1997). A 20% increase in shoot growth and grain yield of the wild rice *Oryza breviligulata* was obtained under greenhouse conditions by inoculation with photosynthetic endophytic bradyrhizobia (Chaintreuil et al., 2000).

Hilali et al. (2001) studied 100 strains of *R. leguminosarum* bv. *trifolii* isolated from the roots of wheat cultivated in rotation with clover in two different regions of Morocco. Greenhouse wheat inoculation assays performed in two different soils showed that the endophytic strain IAT168 behaved like a PGPR (the 24% increases in shoot dry matter and grain yields were significant at $P<0.1$) in the loamy sand Rabat soil. However, in the silty clay Merchouch soil, six strains had significant ($P<0.05$) deleterious effects, stressing the importance of choosing rhizobial strains that are PGPR for all the plants involved in crop rotation systems. Maize and lettuce growth promotion under field conditions were obtained by inoculation with two mineral phosphate-solubilizing PGPR strains R1 and P31, of *R. leguminosarum* bv. *phaseoli* (Chabot et al., 1996). Interestingly, in a P-depleted loam soil in Quebec, strain P31, significantly ($P<0.05$) increased lettuce shoot dry matter yield when the soil was fertilized with half of the recommended amount of P (35 hg ha^{-1} superphosphate). The yield was equivalent to that obtained with the uninoculated control fertilized with the recommended 70 kg ha^{-1} superphosphate. Greenhouse radish inoculation trials with 266 strains of *Rhizobium* and *Bradyrhizobium* revealed the existence of potential PGPR strains among all the genera and species tested (Antoun et al., 1998). No significant correlation was found between the *in vitro* bacterial characteristics generally associated with PGPR activity (i.e., production of indole 3-acetic acid, siderophores, or HCN and P-solubilization) and radish yield. In fact, strain Soy 213 of *B. japonicum,* which produced the highest increase of radish cultivar Pocker dry matter yield (60% compared with uninoculated control), did not exhibit any of the *in vitro* characteristics tested. Other mechanisms of action such as induced systemic resistance against pathogens (Ramamoorthy et al., 2001) or competition and antagonism toward other deleterious microorganisms might be responsible for the beneficial effect of strain Soy 213. Beneficial genes expressed solely *in planta* might also be present in this strain. However, many strains harboring all these *in vitro* characteristics did not produce plant growth promotion. These observations indicate that plant yield is the outcome of very complex interactions taking place in the rhizosphere between plant roots, soil, and rhizobacteria, and they underline the importance of developing model systems to study the PGPR mechanisms of action *in planta*.

3. Mechanisms of Action of PGPR Rhizobia

PGPR exert their beneficial effect by using one or more direct or indirect mechanisms of action. By using a growth pouch gnotobiotic system, Noel et al. (1996) observed that several strains of *R. leguminosarum* bv. *viciae* promoted the early seedling root growth of canola and lettuce. The observed growth stimulation was associated with the production of the plant growth regulators indole-3-acetic acid (IAA) and cytokinin. The evidence for this was that auxotrophic mutants requiring tryptophan or adenosine (the precursors of hormone synthesis) did not exhibit growth-promoting effects to the same extent as the parent strain did. The growth promotion of wheat and maize inoculated with *R. leguminosarum* bv. *trifolii* R39 in greenhouse and field experiments was mediated by auxine and cytokinine production (Höflich et al., 1994). Similarly, the beneficial effect of the P-solubilizing *R. leguminosarum* bv. *phaseoli* P31 on field grown lettuce cultivated in a soil with low fertility was associated with its ability to produce substantial amounts of IAA (Chabot et al., 1996). The concentration of IAA was increased in the rice root environment when inoculated with rhizobia (Biswas et al., 2000a).

Rhizobial inoculation also significantly increased the uptake of N, P, K, and Fe by rice; however, increased N uptake was not due to biological nitrogen fixation. Rhizobial strains have also been reported to promote growth and vigor of rice seedlings, and this **beneficial effect has led to significantly** increased grain yield at maturity (Biswas et al., 2000b), although the mechanisms of this were not elucidated.

The role of inorganic P-solubilization as a mechanism in maize growth promotion was analyzed by using two Lux^+ mutants of *R. leguminosarum* bv. *phaseoli* R1 with reduced solubilization activity (Chabot et al., 1998). Barley yield and uptake of P, N, K, Ca, and Mg were significantly increased by inoculation with a P-solubilizing strain of *Mesorhizobium mediterraneum* (Peix et al., 2001). Under gnotobiotic conditions, in dual inoculation trials of lettuce, a very significant interaction was observed between *Sinorhizobium meliloti* and the AM fungus *Glomus mosseae*. This translated into a 476% increase in shoot dry matter yield of 40-day-old plants (Galleguillos et al., 2000), and the growth-promoting effect reported was not accompanied by an increase in root colonization by the AM fungus. This observation suggests that the PGPR rhizobial strain did not act as a mycorrhizal helper bacterium (Budi et al., 1999), but that rather that the fungus stimulated the PGPR activity.

The interaction between AM fungi and rhizobia merits further investigation because rhizobial Nod factors have been found to stimulate mycorrhizal colonization in nodulating and nonnodulating soybeans (Xie et al., 1995) as well as *Lablab purpureus* (Xie et al., 1997). Furthermore, the addition of Nod factors to suspension-cultured tomato cells induced a rapid yet transient alkalinization of the culture medium (Staehelin et al., 1994). This reaction is comparable to the one elicited by chitin, and the lipochito-oligosaccharide Nod factors were found to be inactivated by plant chitinases. As chitinases are induced by chitin and are associated with plant defense reactions against bacterial or fungal attack, the hypothesis that rhizobia can induce defense mechanisms in nonlegumes by releasing flavonoid-induced Nod factors needs to be verified. The finding that the arbuscular-mycorrhizal fungus *Gigaspora margarita* harbors endosymbiotic bacteria belonging to the genus *Burkholderia* sp. (Bianciotto et al., 1996a) with a potential P-uptake activity (Ruiz-Lozano and Bonfante, 1999) combined with the knowledge that rhizobia can attach to AM fungal mycelia and spores (Bianciotto et al., 1996b) suggests that the ecology of rhizobia in the mycorrhizosphere should be investigated further. It would be revealing to determine if endophytic rhizobia occur in AM fungi.

The lipopolysaccharides (LPS) produced by *R. etli* strain G12-induced systemic resistance in potato roots against infection by the cyst nematode *Globodera pallida* (Reitz et al., 2000). LPS reduced nematode infection at concentrations as low as 0.1 mg ml^{-1}. G12-induced systemic resistance triggered a signal transduction pathway different from common or chemical induced pathways (Reitz et al., 2001). In fact, the reaction was not accompanied by enhanced accumulation of pathogenesis-related proteins such as chitinase and beta-1,3-glucanase, and the lignin content of the roots was not affected. This indicates that lignified root cells acting as mechanical barriers were absent. A recent study showed that G12 is able to colonize the external and internal roots of potato and *Arabidopsis* (Hallmann et al., 2001). In the presence of *Meloidogyne incognita*, G12 also inhabited the interior of nematode galls in high numbers.

Rhizobial strain YAS34 was selected from the rhizoplane of sunflower based on its ability to produce large amounts of gel-forming exopolysaccharide (EPS) and was identified as a *Rhizobium* sp. (Alami et al., 2000). The inoculation of sunflower seeds and soil with strain YAS34 in pot experiments significantly increased the root-adhering soil in 15- to 16-day-old plantlets. This was accompanied by increases in shoot (up to +50%) and root (up to +70%) dry matter yields under both normal and water stress conditions.

Like other PGPR, rhizobia produce siderophores that are strain specific (Reigh and O'Connell, 1993), and they can utilize a large spectrum of these molecules to overcome iron starvation (Plessner et al., 1993; Carson et al., 2000). Future research will elucidate the importance of rhizobial siderophores in the biological control of pathogens and the possible competitive advantage gained by rhizobia through their ability to utilize siderophores of other organisms (Plessner et al., 1993).

The *in vitro* antagonism of rhizobia against plant pathogenic fungi has been well documented. In field experiments, strains of *S. meliloti*, *R. leguminosarum,* and *B. japonicum* applied either as seed dressing or as soil drench reduced infection by soilborne root fungi such as *Macrophomina phaseolina*, *Rhizoctonia solani,* and *Fusarium* spp. in both leguminous (soybean and mung bean) and nonleguminous (sunflower and okra) plants (Ehteshamul-Haque and Gaffar, 1993). Inoculation with rhizobia resulted in increased shoot length and fresh weight when compared with the untreated controls.

The beneficial effects resulting from the use of legumes in crop rotations or in intercropping systems has conventionally been attributed to their ability to form atmospheric nitrogen fixing symbioses with rhizobia and other rotational benefits relating to disease suppression (Robson, 1990; Pineda et al., 1994; Graham and Vance, 2000). However, considering the growing number of observations of the PGPR activity of rhizobia with nonlegumes, an additional beneficial effect of legumes might emerge, because their ability to supply the inoculum of PGPR rhizobia to the companion or following nonlegume crop becomes more fully understood. In fact, Biederbeck et al. (2000) found that the rhizoplane of wheat cultivated for 20 years in rotation with lentil contained over 10,000 times more rhizobia than that of monoculture wheat. Roots of wheat cultivated in rotation contained 68×10^3 endophytic rhizobia per gram compared with 2 rhizobia per gram in monoculture wheat. Similarly, Lupwayi et al. (2000) estimated that the number of endophytic rhizobia was 100 times greater in roots of barley and canola grown after inoculated or uninoculated peas than in the roots of continuously grown barley or canola.

VII. CONCLUDING REMARKS

Legumes have been an integral part of agriculture for millenia because of their fixation of atmospheric nitrogen and their capacity to minimize the impacts of disease, pests, and soil infertility when used in rotation with crops. Rhizobial inoculants may greatly contribute to N_2-fixation by legumes if appropriate strains that nodulate their host effectively and suit their edaphic environment are applied. Strategies have been developed for the selection and development of elite inoculant strains and interdisciplinary research on environmental and genetic factors that effect nodulation and N_2-fixation will continue to improve our understanding of rhizobial ecology. The establishment of a database recording all pertinent information available and the development of models predicting the benefits of legume cropping and inoculation would advance progress in the exploitation of nitrogen fixation by legumes.

An immense diversity of nodule bacteria and even novel nodulating groups have been discovered recently. It remains to be seen whether these new discoveries contribute to global nitrogen fixation and if these novel bacteria represent potential inoculant strains. Historically, rhizobial diversity has been determined from nodule isolates; however, recent studies indicate that a greater diversity of soil rhizobia actually exists than previously described. Rhizobia are now considered endophytes or rhizobacteria of nonleguminous plants, and many of them show plant growth-promoting effects. However, further research is required to elucidate their diversity, mechanisms of interaction, and contribution to nitrogen fixation and plant growth.

Sequencing efforts have contributed to a rapidly improved understanding of rhizobial genomes, and sequence analysis of whole genomes will enable further comparison of genome structures. Sequence information will also support the application of novel tools such as microarray-based expression analysis to reveal gene function. To gain further insights into the interaction between plants and rhizobia, the use of model organisms that have been fully sequenced will support the identification of genes involved in a given plant or bacterial response.

Nitrogen fixation is one of the most important biological processes on this planet, and a continued improvement in the understanding of the legume/rhizobia interaction will be necessary to sustain a food supply to its inhabitants.

ACKNOWLEDGMENTS

EM acknowledges Julio Martínez and Michael Dunn and to K. Lindström, J. P. W. Young, R. A. Morton, and G. Laguerre for sharing information prior to publication. AS received an APART fellowship funded by the Austrian Academy of Sciences.

REFERENCES

Abaidoo, R.C., Keyser, H.H., Singleton, P.W., and Borthakur, D. 2000. *Bradyrhizobium* spp. (TGx) isolates nodulating the new soybean cultivars in Africa are diverse and distinct from bradyrhizobia that nodulate North American soybeans. *Int. J. Syst. Evol. Microbiol.* **50:** 225–234.

Abril, A., Ryan, A., and Carreras, J. 1997. Inoculation of chickpeas with *Rhizobium* sp. native to the province of Cordoba, Argentina. *Rev. Argent. Microbiol.* **29:**24–31.

Alami, Y., Achouak, W., Marol, C., and Heulin, T. 2000. Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide-producing *Rhizobium* sp. strain isolated from sunflower roots. *Appl. Environ. Microbiol.* **66:**3393–3398.

Amarger, N. 1981. Competition for nodule formation between effective and ineffective strains of *Rhizbium meliloti*. *Soil Biol. Biochem.* **13:**475–80.

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

Allardet-Servent, A., Michaux-Carachon, S., Jumas-Bilak, E., Karayan, L., and Ramuz, M. 1993. Presence of one linear and one circular chromosome in the *Agrobacterium tumefaciens* C58 genome. *J. Bacteriol.* **175:**7869–7874.

Allen, O.N. and Allen, E.K., 1981. *The Leguminosae: a Source Book of Characteristics, Uses and Nodulation.* University of Wisconsin Press, Madison, Wisconsin, and MacMillan Publishers Ltd. London.

Almendras, A.S. and Bottomley, P.J. 1987. Influence of lime and phosphate on nodulation of soil-grown *Trifolium subterraneum* L. by indigenous *Rhizobium trifolii*. *Appl. Environ. Microbiol.* **53:**2090–2097.

Antoun, H., Beauchamp, C.J., Goussard, N., Chabot, R., and Lalande, R. 1998. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativus* L.). *Plant Soil* **204:**57–67.

Antoun, H. and Kloepper, J.W. 2001. Plant growth promoting rhizobacteria (PGPR). *Encyclopedia of Genetics*, Academic Press, http://www.apnet.com/genetics/genopen.htm

App, A.A., Watanabe, I., Ventura, T.S., Bravo, M., and Jurey, C.D. 1986. The effect of cultivated and wild rice varieties on the nitrogen balance of flooded soil. *Soil Sci.* **141:**448–452.

Araujo, R.S., Robleto, E.A., and Handelsman, J. 1994. A hydrophobic mutant of *Rhizobium etli* altered in nodulation competitiveness and growth in the rhizosphere. *Appl. Environ. Microbiol.* **60:**1430–1436.

Bahar, M., de Majnik, J., Wexler, M., Fry, J., Poole, P.S., and Murphy, P.J. 1998. A model for the catabolism of rhizopine in *Rhizobium leguminosarum* involves a ferredoxin oxygenase complex and the inositol degradative pathway. *Mol. Plant-Microbe Interact.* **11:**1057–1068.

Baldani, J.I., Oliveira, A.L.M., Guimaraes, S.L., Baldani, V.L.D., Reis, F.B., Jr., Silva, L.G., Reis, V.M., Teixeira, K.R.S., and Döbereiner, J. 2000. Biological nitrogen fixation (BNF) in non-leguminous plants: the role of endophytic diazotrophs. **In:** *Nitrogen Fixation: From Molecules to Crop Productivity*. pp. 397–400. Pedrosa, F. O., Hungria, M., Yates, G., and Newton, W. E., Eds., Kluwer Academic Publishers, Dordrecht, The Netherlands.

Balkwill, D.L. 2001. *Ensifer*. In: *Bergey's Manual of Systematic Bacteriology, 2nd ed., Volume 2*: *The Proteobacteria*. Garrity, G. M., Ed., Springer-Verlag, New York (in press).

Barnet, Y.M. and Catt, P.C. 1991. Distribution and characteristics of root-nodule bacteria isolated from Australian *Acacia* spp. *Plant Soil* **135:**109–120.

Barnett, M.J., Fisher, R.F., Jones, T., Komp, C., Abola, A.P., Barloy-Hubler, F., Bowser, L, Capela, D., Galibert, F., Gouzy, J., Gurjal, M., Hong, A., Huizar, L., Hyman, R.W., Kahn, D., Kahn, M.L., Kalman, S., Keating, D.H., Palm, C., Peck, M.C., Surzycki, R., Wells, D.H., Yeh, K.-C., Davis, R.W., Federspiel, N.A., and Long, S.R. 2001. Nucleotide sequence and predicted functions of the entire *Sinorhizobium meliloti* pSymA megaplasmid. *Proc. Natl. Acad. Sci. U. S. A.* **98:**9883–9888.

Barraquio, W.L., Revilla, L., and Ladha, J.K., 1997. Isolation of endophytic diazotrophic bacteria from wetland rice. *Plant Soil* **194:**15–24.

Barrera, L.L., Trujillo, M.E., Goodfellow, M., García, F.J., Hernández-Lucas, I., Dávila, G., van Berkum, P., and Martínez-Romero, E. 1997. Biodiversity of bradyrhizobia nodulating *Lupinus* spp. *Int. J. Syst. Bacteriol.* **47:**1086–1091.

Bartosik, D., Wlodarczyk, M., and Thomas, C.M. 1997. Complete nucleotide sequence of the replicator region of *Paracoccus* (*Thiobacillus*) *versutus* pTAV1 plasmid and its correlation to several plasmids of *Agrobacterium* and *Rhizobium* species. *Plasmid* **38:**53–59.

Bauer, W. D., and Caetano-Anollés, G. 1990. Chemotaxis, induced gene expression and competitiveness in the rhizosphere. *Plant Soil* **129:**45–52.

Báscones, E., Imperial, J., Ruiz-Argûeso, T., and Palacios, J.M. 2000. Generation of new hydrogen-recycling *Rhizobiaceae* strains by introduction of a novel *hup* minitransposon. *Appl. Environ. Microbiol.* **66:**4292–4299.

Bianciotto, V., Bandi, C., Minerdi, D., Sironi, M., Tichy, H.V., and Bonfante, P. 1996a. An obligatory endosymbiotic mycorrhizal fungus itself harbors obligatory intracellular bacteria. *Appl. Environ. Microbiol.* **62:**3005–3010.

Bianciotto, V., Minerdi, D., Perotto, S., and Bonfante, P. 1996b. Cellular interactions between arbuscular mycorrhizal fungi and rhizosphere bacteria. *Protoplasma* **193:**123–131.

Biederbeck, V.O., Lupwayi, N.Z., Hanson, K.G., Rice, W.A., and Zentner, R.P. 2000. Effect of long-term rotation with lentil on rhizosphere ecology and on endophytic rhizobia in wheat. **In:** *Proceedings of the 17th North American Conference on Symbiotic Nitrogen Fixation, Laval University, Québec, Canada.* P. 80.

Biswas, J.C., Ladha, J.K., and Dazzo, F.B. 2000a. Rhizobial inoculation improves nutrient uptake and growth of lowland rice. *Soil Sci. Soc. Am. J.* **64:**1644–1650.

Biswas, J.C., Ladha, J.K., Dazzo, F.B., Yanni, Y.G., and Rolfe, B.G. 2000b. Rhizobial inoculation influences seedling vigor and yield of rice. *Agron. J.* **92:**880–886.

Boddey, R.M., Urquiaga, S., Reis, V., and Döbereiner, J., 1991. Biological nitrogen fixation associated with sugar cane. *Plant Soil* **137:**111–117.

Boddey, R.M., de Oliveira, O.C., Urquiaga, S., Reis, V.M., de Olivares, F.L., Baldani, V.L.D., and Döbereiner, J. 1995. Biological nitrogen fixation associated with sugar cane and rice: contributions and prospects for improvement. *Plant Soil* **174:**195–209.

Boddey, L.H. and Hungria, M. 1997. Phenotypic grouping of Brazilian *Bradyrhizobium* strains which nodulate soybean. *Biol. Fert. Soils* **25:**407–415.

Boivin, C., Ndoye, I., Molouba, F., de Lajudie, P., Dupuy, N., and Dreyfus, B. 1997. Stem nodulation in legumes: diversity, mechanisms, and unusual characteristics. *Crit. Rev. Plant Sci.* **16:**1–30.

Boone, C., Olsthoorn, M.M.A., Dakora, F.D., Spaink, H.P., and Thomas-Oates, J. 1999. Structural characterisation of lipo-chitin oligosaccharides isolated from *Bradyrhizobium aspalati*, microsymbionts of commercially important South African legumes. *Carbohydr. Res.* **317:**155–163.

Bosworth, A.H., Breil, B.T., and Triplett, E.W. 1993. Production of the anti-rhizobial peptide, trifolitoxin, in sterile soils by *Rhizobium leguminosarum* bv. *trifolii*. *Soil Biol. Biochem.* **25:**829–832.

Bosworth, A.H., Williams, M.K., Albrecht, K.A., Kwiatkowski, R., Beynon, J., Hankinson, T.R., Ronson, C.W., Cannon, F., Wacek, T.J., and Triplett, E.W. 1994. Alfalfa yield response to inoculation with recombinant strains of *Rhizobium meliloti* with an extra copy of *dctABD* and/or modified *nifA* expression. *Appl. Environ. Microbiol.* **60:** 3815–3832.

Bottomley, P.J. 1992. Ecology of *Rhizobium* and *Bradyrhizobium.* In: *Biological Nitrogen Fixation.* pp. 293–348. (Stacey, G., Burris, R.H., and Evans, H.J. Eds.) Chapman & Hall, New York.

Breil, B.T., Ludden, B.W., and Triplett, E.W. 1993. DNA sequence and mutational analysis of genes involved in the production and resistance of the antibiotic peptide trifolitoxin. *J. Bacteriol.* **175:**3693–3702.

Brockwell, J., Gault, R.R., Zorin, M., and Roberts, M.J. 1982. Effects of environmental variables on the competition between inoculum strains and naturalised populations of *Rhizobium trifolium* for nodulation of *Trifolium subterraneum L* and on rhizobia persistence in the soil. *Aust. J. Agric. Res.* **33:**803–15.

Brockwell, J., and Bottomley, P.J. 1995. Recent advances in inoculant technology and prospects for the future. *Soil Biol. Biochem.* **27:**683–697.

Brockwell, J., Bottomley, P.J., and Thies, J.E. 1995. Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. Plant Soil **174:**143–180.

Brockwell, J. 2001. *Sinorhizobium meliloti* in Australian soils: Population studies of the root-nodule bacteria for species of *Medicago* in soils of the Eyre Peninsula, South Australia. *Aust. J. Exp. Agric.* **41:**753–62.

Brom, S., Garcia de los Santos, A., Stepkowsky, T., Flores, M., Davila, G., Romero, D., and Palacios, R. 1992. Different plasmids of *Rhizobium leguminosarum* bv. *phaseoli* are required for optimal symbiotic performance. *J. Bacteriol.* **174:**5183–5189.

Brom, S., Garcia de los Santos, A., Cervantes, L., Palacios, R., and Romero, D. 2000. In *Rhizobium etli* symbiotic plasmid transfer, nodulation competitivity and cellular growth require interaction among different replicons. *Plasmid* **44:**34–43.

Broughton, W.J., Jabbouri, S., and Perret, X. 2000. Keys to symbiotic harmony. *J. Bacteriol.* **182:**5641–5652.

Budi, S.W., van Tuinen, D., Martinotti, G., and Gianinazzi, S. 1999. Isolation from the *Sorghum bicolor* mycorrhizosphere of a bacterium compatible with arbuscular mycorrhiza development and antagonistic towards soilborne fungal pathogens. *Appl. Environ. Microbiol.* **65:**5148–5150.

Caballero-Mellado, J. and Martínez-Romero, E. 1999. Soil fertilization limits the genetic diversity of *Rhizobium* in bean nodules. *Symbiosis* **26:**111–121.

Capela, D., Barloy-Hubler, F., Gatius, M. -T., Gouzy, J., and Galibert, F. 1999. A high-density physical map of *Sinorhizobium meliloti* 1021 chromosome derived from bacterial artificial chromosome library. *Proc. Natl. Acad. Sci. U.S.A.* **96:**9357–9362.

Capela, D., Barloy-Hubler, F., Gouzy, J., Bothe, G., Ampe, F., Batut, J., Boistard, P., Becker, A., Boutry, M., Cadieu, E., Dréano, S., Gloux, S., Godrie, T., Goffeau, A., Kahn, D., Kiss, E., Lelaure, V., Masuy, D., Pohl, T., Portetelle, D., Pühler, A., Purnelle, B., Ramsperger, U., Renard, C., Thébault, P., Vandenbol, M., Weidner, S., and Galibert, F. 2001. Analysis of the chromosome sequence of the legume symbiont *Sinorhizobium*

meliloti strain 1021. *Proc. Natl. Acad. Sci. U.S.A.* **98:**9877–9882.

Carson, K.C., Meyer, J.-M., and Dilworth, M.J. 2000. Hydroxamate siderophores of root nodule bacteria. *Soil Biol. Biochem.* **32:**11–21.

Carter, J.M., Tiemann, J.S., and Gibson, A.H. 1995. Competitiveness and persistence of strains of rhizobia for faba bean in acid and alkaline soils. *Soil Biol. Biochem.* **27:**617–623.

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

Castillo, M., Flores, M., Mavingui, P., Martínez-Romero, E., Palacios, R., and Hernández, G. 1999. Increase in alfalfa nodulation, nitrogen fixation and, plant growth by specific DNA amplification in *Sinorhizobium meliloti*. *Appl. Environ. Microbiol.* **65:**2716–2722.

Chabot, R., Antoun, H., Kloepper, J.W., and Beauchamp, C.J. 1996. Root colonization of maize and lettuce by bioluminescent *Rhizobium leguminosarum* bv. phaseoli. *Appl. Environ. Microbiol.* **62:**2767–2772.

Chabot, R., Beauchamp, C.J., Kloepper, J.W., and Antoun, H. 1998. Effect of phosphorus on root colonization and growth promotion of maize by bioluminescent mutants of phosphate-solubilizing *Rhizobium leguminosarum* biovar *phaseoli*. *Soil Biol. Biochem.* **30:**1615–1618.

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

Chatel, D.L., Greenwood, R.M, and Parker C.A. 1968. Saprophytic competence as an important character in the selection of *Rhizobium* for inoculation. **In:** *Proceedings of the Ninth International Congress of Soil Science Adelaide*. pp. 65–73, V2.

Chen, L.S., Figueredo, A., Pedrosa, F.O., and Hungria, M. 2000. Genetic characterization of soybean rhizobia in Paraguay. *Appl. Environ. Microbiol.* **66:**5099–5103.

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

Cheng, Y., Watkin, E.L.J., O'Hara, G.W., and Howieson, J.G. 2002. *Medicago sativa* and *Medicago murex* differ in the nodulation response to soil acidity. *Plant Soil* **238:**31–39.

Chun, J.Y. and Stacey, G. 1994. A *Bradyrhizobium japonicum* gene essential for nodulation competitiveness is differently regulated from two promoters. *Mol. Plant-Microbe Interact.* **7:**248–255.

Dammann-Kalinowski, T., Niemann, S., Keller, M., Selbitschka, W., Tebbe, C.C., and Pühler, A. 1996. Characterization of two bioluminescent *Rhizobium meliloti* strains constructed for field releases. *Appl. Microbiol. Biotechnol.* **45:**509–512.

Danso, S.K.A. and Bowen, G.D. 1989. Methods of inoculation and how they influence nodulation pattern and notrogen fixation using two contrasting strains of *Bradyrhizobium japonicum*. *Soil Biol. Biochem.* **8:**1053–1058.

de Lajudie, P., Laurent-Fulele, E., Willems, A., Torck, U., Coopman, R., Collins, M.D., Kersters, K., Dreyfus, B., and Gillis, M. 1998a. *Allorhizobium undicola* gen. nov., sp. nov., nitrogen-fixing bacteria that efficiently nodulate *Neptunia natans* in Senegal. *Int. J. Syst. Bacteriol.* **48:**1277–90.

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

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

Denarié, J., Debellé, F., and Promé, J.-C. 1996. *Rhizobium* lipochitooligosaccharide nodulation factors: signaling molecules mediating recognition and morphogenesis. *Annu. Rev. Biochem.* **65:**503–535.

Deng, W., Gordon, M.P., and Nester, E.W. 1995. Sequence and distribution of IS*1312*: Evidence for horizontal DNA transfer from *Rhizobium meliloti* to *Agrobacterium tumefaciens*. *J. Bacteriol.* **177:**2554–2559.

Denton, M.D., Coventry, D.R., Bellotti, W.D., and Howieson, J.G. 2000. Distribution, abundance and effectiveness of *Rhizobium leguminosarum* bv *trifolii* from alkaline pasture soils in South Australia. *Aust. J. Exp. Agric.* **40:**25–35.

de Oliveira, W.S., Meinhardt, L.W., Sessitsch, A., and Tsai, S.M. 1998. Analysis of *Phaseolus-Rhizobium* interactions in a subsistence farming system. *Plant Soil* **204:**107–115.

Dilworth, M.J., Howieson, J.G., Reeve, W.G., Tiwari, R.P., and Glenn, A.R. 2001 Acid tolerance in root-nodule bacteria and selecting for it. *Aust. J. Exp. Agric.* **41:**435–446.

Dobert, R.C., Breil, B.T., and Triplett, E.W. 1994. DNA sequence od the common nodulation genes of *Bradyrhizobium elkanii* and their phylogenetic relationship to those of other nodulating bacteria. *Mol. Plant Microbe Interact.* **7:**564–572.

Doignon-Bourcier, F., Sy, A., Willems, A., Torck, U., Dreyfus, B., Gillis, M., and de Lajudie, P. 1999. Diversity of bradyrhizobia from 27 tropical *Leguminosae* species native of Senegal. *Syst. Appl. Microbiol.* **22:**647–661.

Doignon-Bourcier, F., Willems, A., Coopman, R., Laguerre, G., Gillis, M., and de Lajudie, P. 2000. Genotypic characterization of *Bradyrhizobium* strains nodulating small Senegalese legumes by 16S-23S rRNA

intergenic gene spacers and amplified length polymorphism fingerprint analysis. *Appl. Environ. Microbiol.* **66:**3987–3997.

Doolittle, W.F. 1999. Phylogenetic classification and the universal tree. *Science* **284:**2124–2129.

Dowling, D.N. and Broughton, W.J. 1986. Competition for nodulation of legumes. *Ann. Rev. Microbiol.* **40:**131–157.

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

Dupuy, N.C. and Dreyfus, B.L. 1992. *Bradyrhizobium* populations occur in deep soil under the leguminous tree *Acacia albida. Appl. Environ. Microbiol.* **58:**2415–2419.

Dupuy, N., Willems, A., Pot, B., Dewettinck, D., Vandenbruaene, I., Maestrojuan, G., Dreyfus, B., Kersters, K., Collins, M. D., and Gillis, M. 1994. Phenotypic and genotypic characterization of bradyrhizobia nodulating the leguminous tree *Acacia albida. Int. J. Syst. Bacteriol.* **44:**461–473.

Duquenne, P., Chenu, C., Richard, G., and Catroux, G. 1999. Effect of carbon source supply and its location on competition between inoculated and established bacterial strains in sterile soil microcosm. *FEMS Microbiol. Ecol.* **29:**331–339.

Dykhuizen, D.E. 1998. Santa Rosalia revisited: why are there so many species of bacteria? *Antonie van Leeuwenhoek* **73:**25–33.

Eardly, B.D., Materon, L.A., Smith, N.H., Johnson, D.A., Rumbaugh, M.D., and Selander, R.K. 1990. Genetic structure of natural populations of the nitrogen-fixing bacterium *Rhizobium meliloti*. *Appl. Environ. Microbiol.* **56:**187–194.

Egener, T., Hurek, T., and Reinhold-Hurek, B., 1998. Use of green fluorescent protein to detect expression of *nif* genes of *Azoarcus* sp. BH72, a grass-associated diazotroph, on rice roots. *Mol. Plant-Microbe Interact.* **11:**71–75.

Engelhard, M., Hurek, T., and Reinhold-Hurek, B., 2000. Preferential occurrence of diazotrophic endophytes, *Azoarcus* spp., in wild rice species and land races of *Oryza sativa* in comparison with modern races. *Environ. Microbiol.* **2:**131–141.

Ehteshamul-Haque, S. and Ghaffar, A. 1993. Use of rhizobia in the control of root diseases of sunflower, okra, soybean and mungbean. *J. Phytopathol.* **138:**157–163.

Finan, T.M., Weidner, S., Wong, K., Buhrmester, J., Chain, P., Vorholter, F.J., Hernandez-Lucas, I., Becker, A., Cowie, A., Gouzy, J., Golding, P., and Pühler, A. 2001. The complete sequence of the 1,683–kb pSymB megaplasmid from the N2–fixing endosymbiont *Sinorhizobium meliloti*. *Proc. Natl. Acad. Sci. U.S.A.* **98:**9889–9894.

Flores, M., Gonzáles, V., Brom, S., Martínez, E., Piñero, D., Romero, D., Dávila, , G., and Palacios, R. 1987. Reiterated DNA sequences in *Rhizobium* and *Agrobacterium* spp. *J. Bacteriol.* **169:**5782–5788.

Flores, M., Mavingui, P., Perret, X., Broughton, W.J., Romero, D., Hernández, G., Dávila, G., and Palacios, R. 2000. Prediction, identification, and artificial selection of DNA rearrangements in *Rhizobium*: toward a natural genomic design. *Proc. Natl. Acad. Sci. U.S.A.* **97:**9138–9143.

Fed, E.B. and Davenport, A. 1918. Influence of reaction on nitrogen assimilating bacteria. *J. Agric. Res. (Washington D.C.)* **14:**317–336.

Freiberg, C., Fellay, R., Bairoch, A., Broughton, W.J., Rosenthal, A., and Perret, X. 1997. Molecular basis of symbiosis between *Rhizobium* and legumes. *Nature* **387:**394–401.

Frioni, L., Malatés, D., Irigoyen, I., and Dodera, R. 1998. Promiscuity for nodulation and effectivity in the N_2–fixing legume tree *Acacia caven* in Uruguay. *Appl. Soil Ecol.* **7:**239–244.

Fry, J., Wood, M., and Poole, P.S. 2001. Investigation of *myo*-inositol catabolism in *Rhizobium leguminosarum* bv. *viciae* and its effect on nodulation competitiveness. *Mol. Plant-Microbe Interact.* **14:**1016–1025.

Fuentes-Ramírez, L.E., Caballero-Mellado, J., Sepúlveda, J., and Martínez-Romero, E. 1999. Colonization of sugarcane by *Acetobacter diazotrophicus* is inhibited by high N-fertilization. *FEMS Microbiol. Ecol.* **29:**117–128.

Galbraith, M.P., Feng, S.F., Borneman, J., Triplett, E.W., de Bruijn, F.J., and Rossbach, S. 1998. A functional *myo*-inositol catabolism pathway is essential for rhizopine utilization by *Sinorhizobium meliloti. Microbiology* **144:**2915–2924.

Galibert, F., Finan, T.M., Long, S.R., Pühler, A., Abola, P., Ampe, F., Barloy-Hubler, F., Barnett, M.J., Becker, A., Boistard, P., Bothe, G., Boutry, M., Bowser, L., Buhrmester, J., Cadieu, E., Capela, D., Chain, P., Cowie, A., Davis, R.W., Dreano, S., Federspiel, N.A., Fisher, R.F., Gloux, S., Godrie, T., Goffeau, A., Golding, B., Gouzy, J., Gurjal, M., Hernandez-Lucas, I., Hong, A., Huizar, L., Hyman, R.W., Jones, T., Kahn, D., Kahn, M.L., Kalman, S., Keating, D.H., Kiss, E., Komp, C., Lelaure, V., Masuy, D., Palm, C., Peck, M.C., Pohl, T.M., Portetelle, D., Purnelle, B., Ramsperger, U., Surzycki, R., Thebault, P., Vandenbol, M., Vorholter, F.J., Weidner, S., Wells, D.H., Wong, K., Yeh, K.C., and Batut, J. 2001. The composite genome of the legume symbiont *Sinorhizobium meliloti. Science* **293:**668–672.

Galleguillos, C., Aguire, C., Barea, J.M., and Azcon, R. 2000. Growth promoting effect of two *Sinorhizobium meliloti* strains (a wild type and its genetically modified derivative) on a non-legume plant species in specific interaction with two arbuscular mycorrhizal fungi. *Plant Sci.* **159:**57–63.

Gándara, B., López Merino, A., Rogel, M.A., and Martínez-Romero, E. 2001. Limited genetic diversity of *Brucella* spp. *J. Clin. Microbiol.* **39:**235–240.

Gao, J., Terefework, Z., Chen, W., and Lindström, K. 2001. Genetic diversity of rhizobia isolated from *Astragalus adsurgens* growing in different geographical regions of China. *J. Biotechnol.* **91:**155–168.

García-de los Santos, A., Brom, S., and Romero, D. 1996. *Rhizobium* plasmids in bacteria-legume interactions. *World J. Microbiol. Biotechnol.* **12:**119–125.

Gaunt, M. W., Turner, S.L., Rigottier-Gois, L., Lloyd-Macgilp, S.A., and Young, J.P.W. 2001. Phylogenies of *atpD* and *recA* support the SSU rRNA-based classification of rhizobia. *Int. J. Syst. Evol. Microbiol.* **51:**2037–2048.

Germida, J.J. and Casida, Jr., L.E. 1983. *Ensifer adhaerens* predatory activity against other soil bacteria in soil, as monitored by indirect phage analysis. *Appl. Environ. Microbiol.* **45:**1380–1388.

Giddings, G., Mytton, L., Taylor, L., Thomas, S., Allen, D., and Skot, L. 2000. A secondary effect of transformation in *Rhizobium leguminosarum* transgenic for *Bacillus thuringiensis* subspecies *tenebrionis* — endotoxin (*cryIIIA*) genes. *Theoret. Appl. Genet.* **100:**820–823.

Gordon, D.M., Ryder, M.H., Heinrich, K., and Murphy, P.J. 1996. An experimental tset of the rhizopine concept in *Rhizobium meliloti*. *Appl. Environ. Microbiol.* **62:**3991–3996.

Göttfert, M., Rothlisberger, S., Kundig, C., Beck, C., Marty, R., and Hennecke, H. 2001. Potential symbiosis-specific genes uncovered by sequencing a 410–kilobase DNA region of the *Bradyrhizobium japonicum* chromosome. *J. Bacteriol.* **183:**1405–12.

Gough, C., Webster, G., Vasse, J., Galera, C., Batchelor, C., O'Callaghan, K., Davey, M., Kothari, S., Dénarié, J., and Cocking, E., 1996. Specific flavonoids stimulate intercellular colonization of non-legumes by *Azorhizobium caulinodans*. **In:** *Biology of Plant-Microbe Interactions*. pp. 409–415. Stacey, G., Mullin, B., and Gresshoff, P. M. Eds., International Society for Molecular Plant-Microbe Interactions, St. Paul, MN.

Gough, C., Vasse, J., Galera, C., Webster, G., Cocking, E., and Dénarié, J. 1997. Interactions between bacterial diazotrophs and non-legume dicots: *Arabidopsis thaliana* as a model plant. *Plant Soil* **194:**123–130.

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

Graham, P.H. 1992. Stress tolerance in *Rhizobium* and *Bradyrhizobium*, and nodulation under adverse soil conditions. *Can. J. Microbiol.* **38:**475–484.

Graham, P.H., Sadowsky, M.J., Tighe, S.W., Thompson, J.A., Date, R.A., Howieson, J.G., and Thomas, R. 1995. Differences among strains of *Bradyrhizobium* in fatty acid-methyl ester analysis. *Can J. Microbiol.* **41:**1038–1042.

Graham, P.H. and Vance, C.P. 2000. Nitrogen fixation in perspective: an overview of research and extension needs. *Field Crops Res.* **65:**93–106.

Guo, X.W., Zhang, X.X., Zhang, Z.M., and Li, F.D. 1999. Characterization of *Astragalus sinicus* rhizobia by restriction fragment length polymorphism analysis of chromosomal and nodulation gene regions. *Curr. Microbiol.* **39:**358–364.

Gutiérrez-Zamora, M.L. and Martínez-Romero, E., 2001. Natural endophytic association between *Rhizobium etli* and maize (*Zea mays* L.). *J. Biotechnol.* **91:**117–126.

Hacker, J. and Carniel, E. 2001. Ecological fitness, genomic islands and bacterial pathogenicity. *EMBO Rep.* **2:**376–381.

Hallmann, J., Quadt-Hallmann, A., Miller, W.G., Sikora, R.A., and Lindow, S. 2001. Endophytic colonization of plants by the biocontrol agent *Rhizobium etli* G12 in relation to *Meloidogyne incognita* infection. *Phytopathol*ogy **91:**415–422.

Hardarson, G., Golbs, M., and Danso, S.K.A. 1989. Nitrogen fixation by soybean [*Glycine max* (L.) Merrill] as affected by nodulation patterns. *Soil Biol. Biochem.* **21:**783–787.

Hartmann, A., Stoffels, M., Eckert, B., Kirchhof, G., and Schloter, M., 2000. Analysis of the presence and diversity of diazotrophic endophytes. In: *Prokaryotic Nitrogen Fixation: A Model System for Analysis of a Biological Process*. pp 727–736. Horizon Scientific Press, Wymondham, UK.

Hartwig, U.A., Joseph, C.M., and Phillips, D.A. 1991. Flavonoids released naturally from alfalfa seeds enhance growth rate of *Rhizobium meliloti*. *Plant Physiol.* **95:**797–803.

Haugland, R. and Verma, D.P.S. 1981. Interspecific plasmid and genomic DNA sequence homologies and localization of *nif* genes in effective and ineffective strains of *Rhizobium japonicum*. *J. Mol. Appl. Genet.* **1:**205–217.

Haukka, K., Lindström, K., and Young, J. P. W. 1998. Three phylogenetic groups of *nodA* and *nifH* genes in *Sinorhizobium* and *Mesorhizobium* isolates from leguminous trees growing in Africa and Latin America. *Appl. Environ. Microbiol.* **64:**419–426.

Heinrich, K., Gordon, D.M., Ryder, M.H., and Murphy, P.J. 1999. A rhizopine strain of *Sinorhizobium meliloti* remains at a competitive nodulation advantage after an extended period in the soil. *Soil Biol. Biochem.* **31:**1063–1065.

Heinrich, K., Ryder, M.H., and Murphy, P.J. 2001. Early production of rhizopine in nodules induced by *S. meliloti* strain L5–30. *Can. J. Microbiol.* **47:**165–171.

Hellriegal, H. 1886. Welche Stickstoffquellen stehen der Pflanze zu Gebote? *Tageblatt der 59. Versammlung deutscher Naturforscher und Ärzte in Berlin* **290.**

Hellriegal, H. and Wilfarth, H. 1888. Untersuchungen über die Stickstoffnahrung der Gramineen und Leguminosen. *Beilagenhefte Zeitschr. Ver. Rübenzucker-Industrie Deutsch. Reichs* **234.**

Herrera-Cervera, J.A., Caballero-Mellado, J., Laguerre, G., Tichy, H.-V., Requena, N., Amarger, N., Martínez-Romero, E., Olivares, J., and Sanjuan, J. 1999. At least five rhizobial species nodulate *Phaseolus vul-*

garis in a Spanish soil. *FEMS Microbiol. Ecol.* **30:**87–97.

Herridge, D. and Rose, I. 2000. Breeding for enhanced nitrogen fixation in crop legumes. *Field Crops Res.* **65:**229–248.

Hilali, A., Prévost, D., Broughton, W.J. and Antoun, H. 2001. Effets de l'inoculation avec des souches de Rhizobium leguminosarum biovar trifolii sur la croissance du blé dans deux sols du Maroc. *Can. J. Microbiol.* **47:**590–593.

Hirsch, P.R. 1979. Plasmid-determined bacteriocin production by *Rhizobium leguminosarum*. *J. Gen. Microbiol.* **113:**219–228.

Hirsch, P.R., Van Montagu, M., Johnston, A.W.B., Brewin, N.J., and Schell, J. 1980. Physical identification of bacteriocinogenic, nodulation, and other plasmids in strains of *Rhizobium leguminosarum*. *J. Gen. Microbiol.* **120:**403–412.

Hochhut, B. and Waldor, M.K. 1999. Site-specific integration of the conjugal *Vibrio cholerae* SXT element into *prfC*. *Mol. Microbiol.* **32:**99–110.

Höflich, G., Wiehe, W., and Kühn, G. 1994. Plant growth stimulation by inoculation with the symbiotic and associative rhizosphere microorganisms. *Experientia* **50:**897–905.

Höflich, G., Wiehe, W., and Hecht-Buchholz, C., 1995. Rhizosphere colonization of different crops with growth promoting *Pseudomonas* and *Rhizobium* bacteria. *Microbiol. Res.* **150:**139–147.

Holl, F.B. and Chanway, C.P. 1992. Rhizosphere colonization and seedling growth promotion of lodgepole pine by *Bacillus polymyxa*. *Can. J. Microbiol.* **38:**303–308.

Honeycutt, R.J., McClelland, M., and Sobral, B.W.S. 1993. Physical map of the genome of *Rhizobium meliloti*. *J. Bacteriol.* **175:**6945–6952.

Howieson, J.G., Abbott, L.K., and Robson, A.D. 1992. Calcium modifies pH effects on acid tolerant and acid sensitive strains of *Rhizobium meliloti*. *Aust. J. Agricult. Res.* **43:**565–572.

Howieson, J.G., Robson, A.D., and Ewing, M.A. 1993. External phosphate and calcium concentrations, but not the products of rhizobial nodulation genes, affect the attachment of *Rhizobium meliloti* to roots of annual medics. *Soil Biol. Biochem.* **25:**567–573.

Howieson J.G., Loi, A., and Carr, S.J. 1995. *Biserrula pelecinus L.* — a legume pasture species with potential for acid, duplex soils which is nodulated by unique root-nodule bacteria. *Aust. J. Agricult. Res.* **46:**997–1007.

Howieson J.G., Malden, J., Yates, R.J., and O'Hara, G.W. 2000a. Techniques for the selection and development of elite inoculant rhizobial strains in southern Australia. *Symbiosis* **28:**33–48.

Howieson J.G., O'Hara, G.W., and Carr, S.J. 2000b. Changing roles for legumes in Mediterranean agriculture: Developments from an Australian perspective. *Field Crops Res.* **65:**107–122.

Hurek, T., Reinhold-Hurek, B., Van Montagu, M., and Kellenberger, E., 1994. Root colonization and systemic spreading of *Azoarcus* sp. strain BH72 in grasses. *J. Bacteriol.* **176:**1913–1923.

Hynes, M.F. 1990. The role of plasmids in competition between strains of *Rhizobium leguminosarum*. **In:** *Nitrogen Fixation: Achievements and Objectives*. p. 262. Gresshof, P., Roth, L.E., Stacey, G., and Newton, W.E., Eds., Chapman & Hall, New York.

Ireland, J.A. and Vincent, J.M. 1968. A quantitative study of competition for nodule formation. *Transactions 9th International Congress of Soil Science Society* **2:**85–93.

Isawa, T., Yuhashi, K., Ichige, H., Suzuki, M., Mikami, T., Itakura, M., and Minamisawa, K. 1997. Genome rearrangements and horizontal gene transfer in *Bradyrhizobium japonicum*. **In:** *Book of Abstracts. 11th International Congress on Nitrogen Fixation*. p. 89. Institut Pasteur, Paris.

James, E.K. Reis, V.M., Olivares, F.L., Baldani, J.I., and Döbereiner, J. 1994. Infection of sugar cane by the nitrogen-fixing bacterium *Acetobacter diazotrophicus*. *J. Exp. Bot.* **45:**757–766.

James, E.K. and Olivares, F.L. 1998. Infection and colonization of sugar cane and other graminaceous plants by endophytic diazotrophs. *Crit. Rev. Plant Sci.* **17:**77–119.

James, E.K. 2000. Nitrogen fixation in endophytic and associative symbiosis. *Field Crops Res.* 65:197–209.

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

Jiang, G., Krishnan, A.H., Kim, Y.-W., Wacek, T.J., and Krishnan, H.B. 2001. A functional *myo*-inositol dehydrogenase gene is required for efficient nitrogen fixation and competitiveness of *Sinorhizobium fredii* USDA191 to nodulate soybean (*Glycine max* [L.] Merr.). *Appl. Environ. Microbiol.* **183:**2595–2604.

Jiménez-Zurdo, J.I., van Dillewijn, P., Soto, M.J., de Felipe, M.R., Olivares, J., and Toro, N. 1995. Characterization of a *Rhizobium meliloti* proline dehydrogenase mutant altered in nodulation efficiency and competitiveness on alfalfa roots. *Mol. Plant-Microbe Interact.* **8:**492–498.

Jiménez-Zurdo, J.I., García-Rodríguez, F.M., and Toro, N. 1997. The *Rhizobium meliloti putA* gene: its role in the establishment of the symbiotic interaction with alfalfa. *Mol. Microbiol.* **23:**85–93.

Jordan, D.C. 1984. Genus II. *Bradyrhizobium*. Jordan 1984, 137VP. **In:** *Bergey's Manual of Systematic Bacteriology*. Vol. I. p. 242. Krieg, N. R. and Holt, J. G., Eds., The Williams and Wilkins Co., Baltimore.

Kamboj, D.V., Sharma, P.K., and Kundu, B.S. 1996. Direct monitoring of *Rhizobium* sp. (*Cicer*) in nodules and soil using *lacZ* fusions. *Biol. Fert. Soils* **21:**309–313.

Kaneko, T., Nakamura, Y., Sato, S., Asamizu, E., Kato, T., Sasamoto, S., Watanabe, A., Idesawa, K., Ishikawa, A., Kawashima, K., Kimura, T., Kishida, Y., Kiyokawa, C., Kohara, M., Matsumoto, M., Matsuno, A., Mochizuki, Y., Nakayama, S., Nakzaki, N., Shimpo, S., Sugimoto, M., Takeuchi, C., Yamada, M., and Tabata, S. 2000. Complete genome structure of the nitrogen-fixing symbiotic bacterium *Mesorhizobium loti*. *DNA Res.* **7:**331–338.

Keister, D.L. 1975. Acetylene reduction by pure cultures of rhizobia. *J. Bacteriol.* **123:**1265–1268.

Kennedy, I.R. and Cocking, E.C. (Eds). 1997. Biological nitrogen fixation; the global challenge and future needs. Rockefeller Foundation Bellagio Conference Proceedings, 83 pp.

Keyser, H.H., Weber, D.F., and Uratsu, S.L. 1984. *Rhizobium japonicum* serogroup and hydrogenase phenotype distribution in twelve states. *Appl. Environ. Microbiol.* **47:**613–615.

Krishnan, H.B. and Pueppke, S.G. 1992. A *nolC-lacZ* gene fusion in *Rhizobium fredii* facilitates direct assessment of competition for nodulation of soybean. *Can. J. Microbiol.* **38:**515–519.

Krishnan, H.B. and Pueppke, S.G. 1998. Genetic characterization of a mutant of *Sinorhizobium fredii* strain USDA208 with enhanced competitive ability for nodulation of soybean, *Glycine max* (L.) Merr. *FEMS Microbiol. Lett.* **165:**215–220.

Kroztky, A. and Werner, D. 1987. Nitrogen fixation in *Pseudomonas stutzeri*. *Arch. Microbiol.* **147:**48–57.

Kündig, C., Hennecke, H., and Göttfert, M. 1993. Correlated physical and genetic map of the *Bradyrhizobium japonicum* 110 genome. *J. Bacteriol.* **175:**613–622.

Kündig, C., Beck, C., Hennecke, H., and Göttfert, M. 1995. A single rRNA gene region in *Bradyrhizobium japonicum*. *J. Bacteriol.* **177:**5151–5154.

Kuykendall, L.D., Young, J.M., Martínez-Romero, E., Kerr, A., and Sawada, H. 2002. Genus *Rhizobium*, a highly divergent genus in a revised family, the Rhizobiaceae. In: *Bergey's Manual of Systematic Bacteriology*. Springer Verlag, New York, in press.

Labes, G., Ulrich, A., and Lentzsch, P. 1996. Influence of bovine slurry deposition on the structure of nodulating *Rhizobium leguminosaum* bv. viciae soil populations in a natural habitat. *Appl. Environ. Microbiol.* **62:**1717–1722.

Ladha, J.K., Garcia, M., Miyan, S., Padre, A., and Watanabe, I. 1989. Survival of *Azorhizobium caulinodans* in the soils and rhizosphere of wetland rice under *Sesbania rostrata*-rice rotation. *Appl. Environ. Microbiol.* **55:**454–460.

Ladha, J. K. and So, R. B. 1994. Numerical taxonomy of photosynthetic rhizobia nodulating *Aeschynomene* species. *Int. J. Syst. Bacteriol.* **44:**62–73.

Lafay, B. and Burdon, J.J. 1998. Molecular diversity of rhizobia occurring on native shrubby legumes in Southeastern Australia. *Appl. Environ. Microbiol.* **64:**3989–3997.

Lagares, A., Caetano-Anollés, G., Niehaus, K., Lorenzen, J., Ljunggren, H.D., Pühler, A., and Favelukes, G. 1992. A *Rhizobium meliloti* lipopolysaccharide mutant altered in competitiveness for nodulation of alfalfa. *J. Bacteriol.* **174:**5941–5952.

Laguerre, G., Geniaux, E., Mazurier, S. I., Rodríguez-Casartelli, R., and Amarger, N. 1993. Conformity and diversity among field isolates of *Rhizobium leguminosarum* bv. *viciae*, bv. *trifolii*, and bv. *phaseoli* revealed by DNA hybridization using chromosome and plasmid probes. *Can. J. Microbiol.* **39:**412–419.

Laguerre, G., Nour, S.M., Macheret, V., Sanjuan, J., Drouin, P., and Amarger, N. 2001. Classification of rhizobia based on *nodC* and *nifH* gene analysis reveals a close phylogenetic relationship among *Phaseolus vulgaris* symbionts. *Microbiol.* **147:**981–993.

Lamrabet, Y., Bellogin, R.A., Cubo, T., Espuny, R., Gil, A., Krishnan, H.B., Megias, M., Ollero, F.J., Pueppke, S.G., Ruiz-Sainz, J., Spaink, H.P., Tejero-Mateo, P., Thomas-Oates, J., and Vinardell, J. 1999. Mutations in GDP-fucose synthesis genes of *Sinorhizobium fredii* alters Nod factors and significantly decreases competitiveness to nodulate soybeans. *Mol. Plant-Microbe Interact.* **12:**207–217.

Loderio, A.R., Lopez-Garcia, S.L., Vazquez, T.E.E., and Favelukes, G. 2000. Stimulation of adhesiveness, infectivity, and competitiveness for nodulation of *Bradyrhizobium japonicum* by its pretreatment with soybean seed lectin. *FEMS Microbiol. Lett.* **188:**177–184.

López-García, S.L., Vázquez, T.E.E., Favelukes, G., and Lodeiro, A.R. 2001. Improved soybean root association of N-starved *Bradyrhizobium japonicum*. *J. Bacteriol.* **183:**7241–7251.

Lortet, G., Méar, N., Lorquin, J., Dreyfus, B., de Lajudie, P., Rosenberg, C., and Boivin, C. 1996. Nod factor thin-layer chromatography profiling as a tool to characterize symbiotic specificity of rhizobial strains: Application to *Sinorhizobium saheli*, *S. teranga*, and *Rhizobium* sp. strains isolated from *Acacia* and *Sesbania*. *Mol. Plant-Microbe Interact.* **9:**736–747.

Louvrier, P., Laguerre, G., and Amarger, N. 1996. Distribution of symbiotic genotypes in *Rhizobium leguminosarum* biovar viciae populations isolated directly from soils. *Appl. Environ. Microbiol.* **62:**4202–4205.

Lupwayi, N.Z., Rice, W.A., and Clayton, G.W. 2000. Endophytic rhizobia in barley and canola in rotation with field peas. **In:** *Proceedings of the 17th North American Conference on Symbiotic Nitrogen Fixation, Laval University, Québec, Canada.* 80.

Malek, W., Inaba, M., Ono, H., Kaneko, Y., and Murooka, Y. 1998. Competition for *Astralagus sinicus* root nodule infection between ist native microsymbiont *Rhizobium huakuii* bv. renge B3 and *Rhizobium* sp. ACMP18

strain, specific for *Astralagus cicer*. *Appl. Microbiol. Biotechnol.* **50:**261–265.

Marr, D.L., Devine, T.E., and Parker, M.A. 1997. Nodulation restrictive genotypes of *Glycine* and *Amphicarpaea*: a comparative analysis. *Plant Soil* **189:**181–188.

Marroquí, S., Zorreguieta, A., Santamaría, C., Temprano, F., Soberón, M., Megías, M., and Downie, J.A. 2001. Enhanced symbiotic performance by *Rhizobium tropici* glycogen synthase mutants. *J. Bacteriol.* **183:**854–864.

Martin, W. 1999. Mosaic bacterial chromosomes: a challenge en route to a tree of genomes. *BioEssays* **21:**99–104.

Martínez, E., Palacios, R., and Sánchez, F. 1987. Nitrogen-fixing nodules induced by *Agrobacterium tumefaciens* harboring *Rhizobium phaseoli* plasmids. *J. Bacteriol.* **169:**2828–2834.

Martínez-Romero, E. and Rosenblueth, M. 1990. Increased bean (*Phaseolus vulgaris* L.) nodulation competitiveness of genetically modified *Rhizobium* strains. *Appl. Environ. Microbiol.* **56:**2384–2388.

Martínez-Romero, E. 1994. Recent developments in *Rhizobium* taxonomy. *Plant Soil* **161:**11–20.

Martínez-Romero, E. 1996. Comments on *Rhizobium* systematics. Lessons from *R. tropici* and *R. etli*. **In:** *Biology of Plant-Microbe Interactions*. pp. 503–508, Stacey, G., Mullin, B., Gresshoff, P. M., Eds., International Society for Molecular Plant-Microbe Interactions, St. Paul, Minnesota.

Martínez-Romero, E., and Caballero-Mellado, J. 1996. *Rhizobium* phylogenies and bacterial genetic diversity. *Crit. Rev. Plant Sci.* **15:**113–140.

Martínez-Romero, E., Wang, E.T., López-Merino, A., Caballero-Mellado, J., Rogel, M.A., Gándara, B., Toledo, I., and Martínez-Romero, J. 2000. Ribosomal gene based phylogenies on trial: The case of *Rhizobium* and related genera. **In:** *Biology of Plant-Microbe Interactions,* Vol. 2, pp. 59–64, de Wit, P. J. G. M., Bisseling, T., Stiekema, W. J., Eds., International Society for Molecular Plant-Microbe Interactions, St. Paul, Minnesota.

Martínez-Romero, E., 2002. Role of nitrogen fixation in common bean based cropping systems in Latin America: an overview of *Phaseolus vulgaris-Rhizobium* symbiosis. *Plant Soil* (accepted for publication).

Mavingui, P., Flores, M., Romero, D., Martínez-Romero, E., and Palacios, R. 1997. Generation of *Rhizobium* strains with improved symbiotic properties by random DNA amplification (RDA). *Nature Biotechnol.* **15:**564–569.

Mavingui, P., Flores, M., Guo, X., Dávila, G., Perret, X., Broughton, W.J., and Palacios, R. 2002. Dynamics of the genome architecture in *Rhizobium* sp. strain NGR234. *J. Bacteriol,* in press.

Mazodier, P. and Davies, J. 1991. Gene transfer between distantly related bacteria. *Annu. Rev. Genet.* **25:**147–171.

McInroy, J.A. and Kloepper, J.W. 1995. Survey of indigenous bacterial endophytes from cotton and sweet corn. *Plant Soil* **173:**337–342.

Miklashevichs, E., Röhrig, H., Schell, J., and Schmidt, J. 2001. Perception and signal transduction of rhizobial NOD factors. *Crit. Rev. Plant Sci.* **20:**373–394.

Minamisawa, K., Seki, T., Onodera, S., Kubota, M., and Asami, T. 1992. Genetic relatedness of *Bradyrhizobium japonicum* field isolates as revealed by repeated sequences and various other characteristics. *Appl. Environ. Microbiol.* **58:**2832–2839.

Moawad, H.A., Ellis, W.R., and Schmidt, E.L. 1984. Rizhosphere response as a factor in competition among three serogroups of indigenous *Rhizobium japonicum* for nodulation of field-grown soybeans. *Appl. Environ. Microbiol.* **47**:607–612.

Moènne-Loccoz, Y. and Weaver, R.W. 1995. Plasmids influence growth of rhizobia in the rhizosphere of clover. *Soil Biol. Biochem.* **27:**1001–1004.

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

Montañez, A., Danso, S.K.A., and Hardarson, G. 1995. The effect of temperature on nodulation and nitrogen fixation by five *Bradyrhizobium japonicum* strains. *Appl. Soil Ecol.* **2**:165–174.

Moreira, F.M.S., Haukka, K., and Young, J.P.W. 1998. Biodiversity of rhizobia isolated from a wide range of forest legumes in Brazil. *Mol. Ecol.* **7:**889–895.

Moreira, F.M.S. 2000. Biodiversity of rhizobia from a wide range of forest Leguminosae species in Brazil. **In:** *Nitrogen Fixation: From Molecules to Crop Productivity*. pp. 181–182. Pedrosa, F. O., Hungria, M., Yates, G., and Newton, W. E., Eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Morton, R.A. 2001. Comparison of chromosomal genes from *M. loti* and *S. meliloti* suggest an ancestral genome. **In:** *13th International Congress on Nitrogen Fixation. Program and Abstract Book*. p. 42, Hamilton, Canada.

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

Mpepereki S., Javaheri F., Davis P., and Giller K.E. 2000. Soybeans and sustainable agriculture. Promiscuous soyabeans in southern Africa. *Field Crops Res.* **65:**137–50.

Murphy, P.J. and Saint, C.P. 1992. Rhizopines in the legume-*Rhizobium* symbiosis. **In:** *Molecular Signals in Plant-Microbe Communications*. pp. 377–390. Verma, D.P.S., Ed., CRC Press, Boca Raton, FL.

Murphy, P.J., Wexler, M., Ggzemski, W., Rao, J.P., and Gordon, D. 1995. Rhizopines: their role in symbiosis and competition. *Soil Biol. Biochem.* **27:**525–529.

Nandasena K.G., O'Hara, G.W., Tiwari, R.P., Yates, R.Y., and Howieson, J.G. 2001. The phylogenetic relationships of three bacteria strains isolated from the pasture legume *Biserrula pelecinus* L. *Int. J. Syst. Evol. Microbiol.* **51:**1983–86.

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

Noel, T.C., Sheng, C., Yost, C.K., Pharis, R.P., and Hynes, M.F. 1996. *Rhizobium leguminosarum* as a plant growth promoting rhizobacterium: direct growth promotion of canola and lettuce. *Can. J. Microbiol.* **42:**279–283.

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

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

O'Callaghan, K.J., Davey, M.R., and Cocking, E.C. 1999. Xylem colonization of *Sesbania rostrata* by *Azorhizobium caulinodans* ORS571. In: *Highlights of Nitrogen Fixation Research.* pp. 145–147, Martínez, E. and Hernández, G. Eds. Kluwer Academic/Plenum Publishers, New York.

Ochman, H., Lawrence, J.G., and Groisman, E.A. 2000. Lateral gene transfer and the nature of bacterial innovation. *Nature* **405:**299–304.

Ogawa, Y. and Mii, M. 2001. Ti and cryptic plasmid-borne virulence of wild-type *Agrobacterium tumefaciens* strain CN15 isolated from chrysanthemum (*Dendranthema grandiflora* Tzvelev). *Arch. Microbiol.* **176:**315–322.

Oresnik, I.J., Pacarynuk, L.A., O'Brien, S.A.P., Yost, C.K., and Hynes, M.F. 1998. Plasmid-encoded catabolic genes in *Rhizobium leguminosarum* bv. *trifolii*: evidence for a plant-inducible rhamnose locus involved in competition for nodulation. *Mol. Plant-Microbe Interact.* **12:**1175–1185.

Oresnik, I.J., Twelker, S., and Hynes, M.F. 1999. Cloning and characterization of a *Rhizobium leguminosarum* gene encoding a bacteriocin with similarities to RTX toxins. *Appl. Environ. Microbiol.* **65:**2833–2940.

Østeras, M., Stanley, J., and Finan, T.M. 1995. Identification of *Rhizobium*-specific intergenic mosaic elements within an essential two-component regulatory system of *Rhizobium* species. *J. Bacteriol.* **177:**5485–5494.

Palanipappan, S.P., Sreedhar, P.S., Loganathan, P., and Thomas, J. 1997. Competitiveness of native *Bradyrhizobium japonicum* strains in two different soil types. *Biol. Fert. Soils* **25:**279–284.

Palmer, K.M. and Young, J.P.W. 2000. Higher diversity of *Rhizobium leguminosarum* biovar viciae populations in arable soils than in grass soils. *Appl. Environ. Microbiol.* **66:**2445–2450.

Parker, C.A. and Oakley, A.E. 1963. Nodule bacteria for two species of serradella — *Ornithopus sativus* and *Ornithopus compressus*. *Austr. J. Exp. Agric. Animal Husbandry* **3:**9–10.

Parker, M.A. 1999. Relationships of bradyrhizobia from the legumes *Apios americana* and *Desmodium glutinosum*. *Appl. Environ. Microbiol.* **65:**4914–4920.

Parker, M.A. and Lunk, A. 2000. Relationships of bradyrhizobia from *Platypodium* and *Machaerium* (Papilionoideae: tribe Dalbergieae) on Barro Colorado Island, Panama. *Int. J. Syst. Evol. Microbiol.* **50:**1179–1186.

Parker, M.A. 2001. Case of localized recombination in 23S rRNA genes from divergent *Bradyrhizobium* lineages associated with neotropical legumes. *Appl. Environ. Microbiol.* **67:**2076–2082.

Parniske, M., Kosch, K., Werner, D., and Müller, P. 1993. ExoB mutants of *Bradyrhizobium japonicum* with reduced competitiveness for nodulation of *Glycine max*. *Mol. Plant-Microbe Interact.* **6:**99–106.

Peick, B., Graumann, P., Schmid, R., Marahiel, M., and Werner, D. 1999. Differential pH-induced proteins in *Rhizobium tropici* CIAT 899 and *Rhizobium etli* CIAT 611. *Soil Biol. Biochem.* **31:**189–194.

Peix, A., Rivas-Boyero, A.A., Mateos, P.F., Rodriguez-Barrueco, C., Martinez-Molina, E., and Velaquez, E. 2001. Growth promotion of chickpea and barley by a phosphate solubilizing strain of *Mesorhizobium mediterraneum* under growth chamber conditions. *Soil Biol. Biochem.* **33:**103–110.

Peoples, M.B., Mosier, A.R., and Freney, J.R. 1994. Minimizing gaseous losses of nitrogen. **In:** *Nitrogen Fertilization and the Environment.* pp. 562–602, Bacon, P.E., Ed., Marcel Dekker, New York.

Peoples, M.B., Herridge, D.F., and Lahda, J.K. 1995. Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production? *Plant Soil* **174:**3–28.

Pérez-Ramírez, N.O., Rogel, M.A., Wang, E.T., Castellanos, J.Z., and Martínez-Romero, E. 1998. Seeds of *Phaseolus vulgaris* bean carry *Rhizobium etli*. *FEMS Microbiol. Ecol.* **26:**289–296.

Perret, X., Viprey, V., Freiberg, C., and Broughton, W.J. 1997. Structure and evolution of NGRRS-1, a complex, repeated element in the genome of *Rhizobium* sp. strain NGR234. *J. Bacteriol.* **179:**7488–7496.

Perret, X. and Broughton, W.J. 1998. Rapid identification of *Rhizobium* strains by targeted PCR fingerprinting. *Plant Soil.* **204:**21–34.

Perret, X., Freiberg, C., Rosenthal, A., Broughton, W.J., and Fellay, R. 1999. High-resolution transcriptional analysis of the symbiotic plasmid of *Rhizobium* sp. NGR234. *Mol. Microbiol.* **32:**415–25.

Perret, X., Staehelin, C., and Broughton, W.J. 2000. Molecular basis of symbiotic promiscuity. *Microbiol. Mol. Biol. Rev.* **64:**180–201.

Perret, X., Parsons, J., Viprey, V., Reichwald, K., and Broughton, W.J. 2001. Repeated sequences of *Rhizobium* sp. NGR234 and *Sinorhizobium meliloti* genomes: a comparative analysis using random sequencing. *Can. J. Microbiol.* **47:**548–558.

Perret, X. 2002. BIME, ERIC, REP, RIME, and other short bacterial repeated elements. **In:** *Encyclopedia of Genetics*. pp. 214–215, Brenner, S. and Miller, J.H., Eds., Academic Press, San Diego.

Phillips, D.A., Wery, J., Joseph, C.M., Jones, A.D., and Teuber, L.R. 1995. Release of flavonoids and betaines from seeds of seven *Medicago* species. *Crop Sci*. **35:**805–808.

Phillips, D.A., Streit, W.R., and Volpin, H. 1996. Applying rhizobia to the future. **In:** *Recent Progress in Research on Symbiotic Nitrogen Fixation*, Pate, J.S., Ed., CLIMA Occasional Publication 14.

Phillips, D.A., Sande, E.S., Vriezen, A.C., de Bruijn, F.J., Le Rudulier, D., and Joseph, C.M. 1998. A new genetic locus in *Sinorhizobium meliloti* is involved in stachydrine utilization. *Appl. Environ. Microbiol.* **64:**3954–3960.

Phillips, D.A. and Martínez-Romero, E., 2000. Biological Nitrogen Fixation. **In:** *Encyclopedia of Microbiology*. Vol. 1., 2nd ed. Academic Press, San Diego.

Phillips, D.A., Martínez-Romero, E., Yang, G.-P., and Joseph, C.M., 2000. Release of nitrogen: a key trait in selecting bacterial endophytes for agronomically useful nitrogen fixation. **In:** *The Quest for Nitrogen Fixation in Rice. Proceedings of the Third Working Group Meeting on Assessing Opportunities for Nitrogen Fixation in Rice, 9–12 Aug. 1999*. pp. 205–217. Ladha, J. K. and Reddy, P. M. Eds. Makati City (Philipines): International Rice Research Institute, Los Baños, Philippines.

Piceno, Y.M. and Lovell, C.R., 2000. Stability in natural bacterial communities. II. Plant resource allocation effects on rhizosphere diazotroph assemblage composition. *Microbiol. Ecol*. **39:**41–48.

Pineda, P., Kipe-Nolt, J.A., and Rojas, E., 1994. *Rhizobium* inoculation increases of bean and maize yields in intercrops on farms in the Peruvian Sierra. *Exp. Agric*. **30:**311–318.

Piñero, D., Martínez, E., and Selander, R.K., 1988. Genetic diversity and relationships among isolates of *Rhizobium leguminosarum* biovar phaseoli. *Appl. Environ. Microbiol.* **54:**2825–2832.

Plessner, O., Klapatch, T., and Guerinot, M.L. 1993. Siderophore utilization by *Bradyrhizobium japonicum*. *Appl. Environ. Microbiol.* **59:**1688–1690.

Poinsot, V., Bélanger, E., Laberge, S., Yang, G.-P., Antoun, H., Cloutier, J., Treilhou, M., Dénarié, J., Promé, J.-C., and Debellé, F. 2001. Unusual methyl-branched α,ß-unsaturated acyl chain substitutions in the Nod factors of an Arctic rhizobium, *Mesorhizobium* sp. strain N33 (*Oxytropis arctobia*). *J. Bacteriol*. **183:**3721–3728.

Pueppke, S.G. and Broughton, W.J. 1999. *Rhizobium* sp. strain NGR234 and *R. fredii* USDA257 share exceptionally broad, nested host ranges. *Mol. Plant-Microbe Interact.* **12:**293–318.

Pulawska, J., Maes, M., Willems, A., and Sobiczewski, P. 2000. Phylogenetic analysis of 23S rRNA gene sequences of *Agrobacterium*, *Rhizobium* and *Sinorhizobium* strains. *Syst. Appl. Microbiol.* **23:**238–244.

Quinto, C., de la Vega, H., Flores, M., Fernández, L., Ballado, T., Soberón, G., and Palacios, R. 1982. Reiteration of nitrogen fixatio gene sequences in *Rhizobium phaseoli*. *Nature* **299:**724–726.

Radeva, G., Jurgens, G., Niemi, M., Nick, G., Suominen, L., and Lindström, K. 2001. Description of two biovars in the *Rhizobium galegae* species: biovar orientalis and biovar officinalis. *Syst. Appl. Microbiol.* **24:**192–205.

Rainey, F.A. and Wiegel, J. 1996. 16S ribosomal DNA sequence analysis confirms the close relationship between the genera *Xanthobacter*, *Azorhizobium*, and *Aquabacter* and reveals a lack of phylogenetic coherence among *Xanthobacter* species. *Int. J. Syst. Bacteriol.* **46:**607–610.

Ramamoorthy, V., Viswanathan, T., Raguchander, T., Prakasam, V., and Samiyappan, R. 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Prot.* **20:**1–11.

Ramírez, M.E., Israel, D.W., and Wollum, A.G. 1998. Using spontaneous antibiotic-resistant mutants to assess competitiveness of bradyrhizobial inoculants for nodulation of soybean. *Can. J. Microbiol.* **44:**753–758.

Ramírez, M., Valderrama, B., Arredondo-Peter, R., Soberón, M., Mora, J., and Hernández, G. 1999. *Rhizobium etli* genetically engineered for the heterologous expression of *Vitreoscilla* sp. hemoglobin: effects on free-living and symbiosis. *Mol. Plant-Microbe Interact.* **12:**1008–1015.

Reanney, D. 1976. Extrachromosomal elements as possible agents of adaptation and development. *Bacteriol. Rev*. **40:**552–590.

Reddy, P.M., Ladha, J.K., So, R.B., Hernández, R.J., Ramos, M.C., Angeles, O.R., Dazzo, F.B., and de Bruijn, F.J., 1997. Rhizobial communication with rice roots: induction of phenotypic changes, mode of invasion and extent of colonization. *Plant Soil* **194:**81–98.

Reeve, T.J., and Ewing, M.A. 1993. Is ley-farming in Mediterranean zones just a passing phase? **In:** *Proc. XVII International Grasslands Congress*. pp. 2169–2177, Baker, M., Ed., New Zealand Grassland Association, Palmerston North.

Reeve, W.G., Tiwari, R.P., Dilworth, M.J., and Glenn, A.R. 1993. Calcium affects the growth and survival of *Rhizobium meliloti*. *Soil Biol. Biochem.* **25:**581–586.

Reeve, W.G., Tiwari, R.P., Wong, C. M., Dilworth, M.J., and Glenn, A.R. 1998. The transcriptional regulator gene *phrR*, in *Sinorhizobium meliloti* is regulated by low pH and other stresses. *Microbiology* **144:**3335–3342.

Reeve, W.G., Tiwari, R.P., Worsely, P.S., Dilworth, M.J., Glenn, A.R. and Howieson, J.G. 1999. Constructs for insertional mutagenesis, transcriptional signal localisation and gene regulation studies in root nodule and other bacteria. *Microbiology* **145:**1307–1316.

Reeve, W.G., Guerreiro, N., Tiwari, R.P., Stubbs, J., Fenner, B. F., Dilworth, M.J., Glenn, A.R., Rolfe, B. G., Djordjevic, M. A., and Howieson, J. G. 2002. Probing for pH-regulated genes and proteins in *Sinorhizobium meliloti* using transcriptional and proteomic analysis. *Mol. Plant Microbe Interact.* (submitted).

Reigh, G. and O'Connell, M. 1993. Siderophore iron transport correlates with the presence of specific iron-regulated proteins in the outer membrane of *Rhizobium meliloti*. *J. Bacteriol*. **175:**94–102.

Reinhold-Hurek, B. and Hurek, T., 1998a. Interactions of gramineous plants with *Azoarcus* spp. and other diazotrophs: identification, localization, and perspectives to study their function. *Crit. Rev. Plant Sci*. **17:**29–54.

Reinhold-Hurek, B. and Hurek, T. 1998b. Life in grasses: diazotrophic endophytes. *Trends Microbiol*. **6:**139–144.

Reitz, M., Rudolph, K., Schröder, I., Hoffmann-Hergarten, S., Hallmann, J., and Sikora, R.A. 2000. Lipopolysaccharides of *Rhizobium etli* strain G12 act in potato roots as an inducing agent of systemic resistance to infection by the cyst nematode *Globodera pallida*. *Appl. Environ. Microbiol*. **66:**3515–3518.

Reitz, M., Hoffmann-Hergaten, S., Hallmann, J., and Sikora, R.A. 2001. Induction of systemic resistance in potato by rhizobaterium *Rhizobium etli* strain G12 is not associated with accumulation of pathogenesis-related proteins and enhanced lignin biosynthesis. *J. Plant Dis. Prot*. **108:**11–20.

Rigottier-Gois, L., Turner, S.L., Young, J.P.W., and Amarger, N. 1998. Distribution of *repC* plasmid-replication sequences among plasmids and isolates of *Rhizobium leguminosarum* bv. *viciae* from field populations. *Microbiology* **144:**771–780.

Robleto, E.A., Scupham, A.J., and Triplett, E.W. 1997. Trifolitoxin production in *Rhizobium etli* strain CE3 increases competitiveness for rhizosphere growth and root nodulation of *Phaseolus vulgaris* in soil. *Mol. Plant-Microbe Interact.* **10:**228–233.

Robleto, E.A., Kmiecik, K., Oplinger, E.S., Nienhuis, J., and Triplett, E.W. 1998. Trifolitoxin production increases nodulation competitiveness of *Rhizobium etli* CE3 under agricultural conditions. *Appl. Environ. Microbiol.* **64:**2630–2633.

Robson, A.D. 1990. The role of self-regenerating pasture in rotation with cereals in Mediterranean areas. **In:** *The Role of Legumes in the Farming Systems of the Mediterranean Areas*. pp. 217–236, Osman, A.E. et al., Eds., ICARDA, The Netherlands.

Rogel, M. A., Hernández-Lucas, I., Kuykendall, L. D., Balkwill, D. L., and Martínez-Romero, E. 2001. Nitrogen-fixing nodules with *Ensifer adhaerens* harboring *Rhizobium tropici* symbiotic plasmids. *Appl. Environ. Microbiol.* **67:**3264–3268.

Romero, D. and Palacios, R. 1997. Gene amplification and genomic placticity in prokaryotes. *Annu. Rev. Genet*. **31:**91–111.

Romero, D., Dávila, and Palacios, R. 1997. The dynamic genome of *Rhizobium*. **In:** *Bacterial Genomes: Physical Structure and Analysis*. pp. 153–161, de Bruijn, F.J., Lupski, J.R., and Weinstock, G., Eds., Chapman & Hall, New York.

Rome, S., Brunel, B., Normand, P., Fernandez, M., and Cleyet-Marel, J.-C. 1996a. Evidence that two genomic species of *Rhizobium* are associated with *Medicago truncatula*. *Arch. Microbiol*. **165:**285–288.

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

Ronson, C., Sullivan, J., Wijkstra, G., Carlton, T., Muirhead, K., Trzebiatowski, J., Gouzy, J., and de Bruijn, F. 2001. Genome diversity at the phe-tRNA locus in a field population of mesorhizobia. **In:** *13th International Congress on Nitrogen Fixation. Program and Abstract Book*. p.21, Hamilton, Canada.

Rosenblueth, M., Hynes, M. F., and Martínez-Romero, E. 1998. *Rhizobium tropici teu* genes involved in specific uptake of *Phaseolus vulgaris* bean-exudate compounds. *Mol. Gen. Genet*. **258:**587–598.

Rovira, A.D. 1961. *Rhizobium* numbers in the rhizospheres of red clover and paspalum in relation to soil treatment and the numbers of bacteria and fungi. *Aust. J. Agric. Res.* **12:**77–83.

Ruiz-Argûeso, T., Imperial J., and Palacios, J.M. 1999. Uptake hydrogenases in root nodule bacteria. **In:** *Prokaryotic Nitrogen Fixation: A Model System for Analysis of a Biological Process*. pp. 489–507, Triplett, E.W., Ed., Horizon Scientific Press, Wymondham, United Kingdom.

Ruiz-Lozano, J.M. and Bonfante, P. 1999. Identification of a putative P-transporter operon in the genome of a *Burkholderia* strain living inside the arbuscular mycorrhizal fungus *Gigaspora margarita*. *J. Bacteriol.* **181:**4106–4109.

Rumjanek, N.G., Dobert, R.C., Van Berkum, P., and Triplett, E.W. 1993. Common soybean inoculant strains in Brazil are members of *Bradyrhizobium elkanii*. Appl. Environ. Microbiol. **59:**4371–4373.

Ruschel, A.P., Henis, Y., and Salati, E., 1975. Nitrogen^{15} tracing of N-fixation with soil-grown sugarcane seedlings. *Soil Biol. Biochem*. **7:**181–182.

Rynne, F.G., Glenn, A.R., and Dilworth, M.J. 1994. Effect of mutations in aromatic catabolism on the persistence and competitiveness of *Rhizobium leguminosarum* bv. *trifolii*. *Soil Biol. Biochem.* **26:**703–710.

Sadowsky, M.J. and Graham, P.H. 1999–2001. Root and Stem Nodule Bacteria of Legumes. **In:** *The Prokaryotes:* Dworkin, M. Ed., Springer-Verlag, New York.

Sanjuan, J. and Olivares, J. 1991. NifA-NtrA regulatory system activates transcription of *nfe*, a gene locus involved in nodulation competitiveness of *Rhizobium meliloti*. *Arch. Microbiology* **155:**543–548.

Sawada, H., Ieki, H., Oyaizu, H., and Matsumoto, S. 1993. Proposal for rejection of *Agrobacterium tumefaciens*

and revised descriptions for the genus *Agrobacterium* and for *Agrobacterium radiobacter* and *Agrobacterium rhizogenes*. *Int. J. Syst. Bacteriol.* **43:**694–702.

Schloter, M., Wiehe, W., Assmus, B., Steindl, H., Becke, H., Höflich, G., and Hartmann, A. 1997. Root colonization of different plants by plant-growth-promoting *Rhizobium leguminosarum* bv. *trifolii* R39 studied with monospecific polyclonal antisera. *Appl. Environ. Microbiol.* **63:**2038–2046.

Schofield, P.R., Gibson, A.H., Dudman, W.F., and Watson, J.M. 1987. Evidence for genetic exchange and recombination of *Rhizobium* symbiotic plasmids in a soil population. *Appl. Environ. Microbiol.* **53:**2942–2947.

Schwieger, F., Willke, B., Munch, J.C., and Tebbe, C.C. 1997. Ecological pre-release risk assessment of two genetically engineered bioluminescent *Rhizobium meliloti* strains in soil column model systems. *Biol. Fert. Soils* **25:**340–348.

Schwieger, F. and Tebbe, C.C. 2000. Effect of field inoculation with *Sinorhizobium meloloti* L33 on the composition of bacterial communities in rhizospheres of a target plant (*Medicago sativa*) and a non-target plant (*Chenopodium album*) — linking of 16S rRNA gene-based single-strand conformation polymorphism community profiles to the diversity of cultivated bacteria. *Appl. Environ. Microbiol.* **66:**3556–3565.

Schwinghamer, E.A. and Brockwell, J. 1978. Competitive advantage of bacteriocin and phage-producing strains of *Rhizobium trifolii* in mixed culture. *Soil Biol. Biochem.* **10:**383–387.

Segovia, L., Piñero, D., Palacios, R., and Martinez-Romero, E. 1991. Genetic structure of a soil population of nonsymbiotic *Rhizobium leguminosarum Appl. Environ. Microbiol.* **57:**426–433.

Selenska-Pobell, S., Doring, H., and Evguenieva-Hackenberg, E. 1997. Unusual organization of the 23S rRNA genes in the rhizobiaceae. *Soil Biol. Biochem.* **29:**905–909.

Sessitsch, A., Wilson, K.J., Akkermans, A.D.L., and de Vos, W.M. 1996. Simultaneous detection of different *Rhizobium* strains marked with either the *Escherichia coli gusA* gene or the *Pyrococcus furiosus celB* gene. *Appl. Environ. Microbiol.* **62:**4191–4194.

Sessitsch, A., Ramírez-Saad, H., Hardarson, G., Akkermans, A. D. L., and de Vos, W. M. 1997a. Classification of Austrian rhizobia and the Mexican isolate FL27 obtained from *Phaseolus vulgaris* L. as *Rhizobium gallicum*. *Int. J. Syst. Bacteriol.* **47:**1097–1101.

Sessitsch, A., Hardarson, G., Akkermans, A.D.L., and de Vos, W.M. 1997b. Characterization of *Rhizobium etli* and other *Rhizobium* spp. that nodulate *Phaseolus vulgaris* L. in an Austrian soil. *Mol. Ecol.* **6:**601–608.

Sessitsch, A., Jjemba, P.J., Hardarson, G., Akkermans, A.D.L., and Wilson, K.J. 1997c. Measurement of the competitiveness index of *Rhizobium tropici* strain CIAT899 derivatives marked with the *gusA* gene. *Soil Biol. Biochem.* **29:**1099–1110.

Sessitsch, A., Hardarson, G., de Vos, W.M., and Wilson, K.J. 1998. Use of marker genes in competition studies of *Rhizobium*. *Plant Soil* **204:**35–45.

Siciliano, S. D., Fortin, N., Mihoc, A, Wisse, G., Labelle, S., Beaumier, D., Ouellette, D., Roy, R., Whyte, L.G., Banks, M. K., Schwab, P., Lee, K., and Greer, C.W. 2001. Selection of specific endophytic bacterial genotypes by plants in response to soil contamination. *Appl. Environ. Microbiol.* **67:**2469–2475.

Smit, G., Swart, S., Lugtenberg, B.J.J., and Kijne, J.W. 1992. Molecular mechanisms of attachment of *Rhizobium* bacteria to plant roots. *Mol. Microbiol.* **6:**2897–2903.

So, R. B., Ladha, J. K., and Young, J. P. W. 1994. Photosynthetic symbionts of *Aeschynomene* spp. form a cluster with bradyrhizobia on the basis of fatty acid and rRNA analysis. *Int. J. Syst. Bacteriol.* **44:**392–403.

Soberón, M., López, O., Morera, C., de Lourdes Girard, M., Tabche, M.L., and Miranda, J. 1999. Enhanced nitrogen fixation in a *Rhizobium etli ntrC* mutant that overproduces the *Bradyrhizobium japonicum* symbiotic terminal oxidase *cbb3*. *Appl. Environ. Microbiol.* **65:**2015–2019.

Soedarjo, M. and Borthakur, D. 1998. Mimosine, a toxin produced by the tree-legume *Leucaena* provides a nodulation competition advantage to mimosine-degrading *Rhizobium* strains. *Soil Biol. Biochem.* **30:**1605–1613.

Soto, M.J., Zorzano, A., Mercado-Blanco, J., Lepek, V., Olivares, J., and Toro, N. 1993. Nucleotide sequence and characterization of *Rhizobium meliloti* nodulation competitiveness genes *nfe*. *J. Mol. Biol.* **229:**570–576.

Souza, V. and Eguiarte, L.E., 1997. Bacteria gone native vs. bacteria gone awry?: Plasmidic transfer and bacterial evolution. *Proc. Natl. Acad. Sci. U.S.A.* **94:**5501–5503.

Souza, V., Bain, J., Silva, C., Bouchet, V., Valera, A., Marquez, E., and Eguiarte, L.E. 1997. Ethnomicrobiology: do agricultural practices modify the population structure of the nitrogen fixing bacteria *Rhizobium etli* biovar phaseoli. *J. Ethnobiol.* **17:**249–266.

Spaink, H.P. 2000. Root nodulation and infection factors produced by rhizobial bacteria. *Annu. Rev. Microbiol.* **54:**257–288.

Spiers, A.J., Buckling, A., and Rainey, P.B. 2000. The causes of *Pseudomonas* diversity. *Microbiol*ogy **146:**2345–2350.

Spoerke, J.M., Wilkinson, H.H., and Parker, M.A. 1996. Non random genotypic associations in a legume-*Bradyrhizobium* mutualism. *Evolution* **50:**146–154.

Sprent, J.I., 2001. *Nodulation in Legumes*. Royal Botanical Gardens, Kew, U. K.

Sprent, J.I. and James, E.K., 1995. N_2–fixation by endophytic bacteria: questions of entry and operation. In: *Azospirillum VI and related microorganims*. pp. 15–30. del Gallo, F. I., Vanderleyden, J., and de Zamaroczy, M., Eds., Springer-Verlag, Heidelberg, Germany.

Staehelin, C., Granado, J., Müller, J., Wiemken, A., Mellor, R.B., Felix, G., Regenass, M., Broughton, W.J., and Boller, T. 1994. Perception of *Rhizobium* nodulation

factors by tomato cells and inactivation by root chitinases. *Proc. Natl. Acad. Sci. U.S.A.* **91:**2196–2200.

Sterner, J.P. and Parker, M.A. 1999. Diversity and relationships of bradyrhizobia from *Amphicarpaea bracteata* based on partial *nod* and ribosomal sequences. *Syst. Appl. Microbiol.* **22:**387–392.

Streeter, J.G. 1994. Failure of inoculant rhizobia to overcome the dominance of indigenous strains for nodule formation. *Can. J. Microbiol.* **40:**513–522.

Streit, W., Botero, L., Werner, D., and Beck, D. 1995. Competition for nodule occupancy on *Phaseolus vulgaris* by *Rhizobium etli* and *Rhizobium tropici* strains can be efficiently monitored in an ultisol during the early stages of growth using a constitutive gus gene fusion. *Soil Biol. Biochem.* **27:**1075–1081.

Streit, W.R., Joseph, C.M., and Phillips, D.A. 1996. Biotin and other water-soluble vitamins are key growth factors for alfalfa rhizosphere colonization by *Rhizobium meliloti* 1021. *Mol. Plant-Microbe Interact.* **5:**331–338.

Streit, W.R. and Phillips, D.A. 1996. Recombinant *Rhizobium meliloti* strains with extra biosynthesis capability. *Appl. Environ. Microbiol.* **62:**3333–3338.

Sturz, A.W. and Christie, B.R., 1996. Endophytic bacteria of red clover as agents of allelopathic clover-maize syndromes. *Soil Biol. Biochem.* **28:**583–588.

Sturz, A.W., Christie, B.R., Matheson, B.G., and Nowak, J. 1997. Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. *Biol. Fert. Soils* **25:**13–19.

Sullivan, J.T., Patrick, H.N., Lowther, W.L., Scott, D.B., and Ronson, C.W. 1995. Nodulating strains of *Rhizobium loti* arise through chromosomal symbiotic gene transfer in the environment. *Proc. Natl. Acad. Sci. U.S.A.* **92:**8985–8989.

Sullivan, J.T., Eardly, B.D., van Berkum, P., and Ronson, C.W. 1996. Four unnamed species of nonsymbiotic rhizobia isolated from the rhizosphere of *Lotus corniculatus*. *Appl. Environ. Microbiol*ogy **62:**2818–2825.

Sullivan, J.T. and Ronson, C.W. 1998. Evolution of rhizobia by aquisition of a 500–kb symbiosis island that integrates into a *phe*-tRNA gene. *Proc. Natl. Acad. Sci. U.S.A.* **95:**5145–5149.

Sullivan, J.T., Brown, S.D., Yocum, R.R., and Ronson, C.W. 2001. The bio operon on the acquired symbiosis island of *Mesorhizobium* sp. strain R7A includes a novel gene involved in pimeloyl-CoA synthesis. *Microbiol.* **147:**1315–1322.

Sy, A., Giraud, E., Jourand, P., Garcia, N., Willems, A., de Lajudie, P., Prin, Y., Neyra, M., Gillis, M., Boivin-Masson, C., and Dreyfus, B. 2001. Methylotrophic *Methylobacterium* bacteria nodulate and fix nitrogen in symbiosis with legumes. *J. Bacteriol.* **183:**214–220.

Tan, Z., Hurek, T., Vinuesa, P., Müller, P., Ladha, J. K., and Reinhold-Hurek, B., 2001a. Specific detection of *Bradyrhizobium* and *Rhizobium* strains colonizing rice (*Oryza sativa*) roots by 16S-23S ribosomal DNA intergenic spacer-targeted PCR. *Appl. Environ. Microbiol.* **67:**3655–3664.

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

Tas, E., Leinonen, P., Saano, A., Räsänen, L.A., Kaijalainen, S., Piippola, S., Hakola, S., and Lindström, K. 1996. Assessment of competitiveness of rhizobia infecting *Galega orientalis* on the basis of plant yield, nodulation, and strain identification by antibiotic resistance and PCR. *Appl. Environ. Microbiol.* **62:**529–535.

Terefework, Z., Nick, G., Suomalainen, S., Paulin, L., and Lindström, K. 1998. Phylogeny of *Rhizobium galegae* with respect to other rhizobia and agrobacteria. *Int. J. Syst. Bacteriol.* **48:**349–356.

Terefework, Z., Lortet, G., Suominen, L., and Lindström, K. 2000. Molecular evolution of interactions between rhizobia and their legume hosts. **In:** *Prokaryotic Nitrogen Fixation: A Model System for Analysis of a Biological Process*. pp. 187–206, Triplett, E. W., Ed., Horizon Scientific Press, Wymondham, UK.

Thies, J.E., Cook, S.E., and Corner, R.J. 1991. Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. *Appl. Environ. Microbiol.* **57:**19–28.

Thrall, P.H., Burdon, J.J., and Woods, M.J. 2000. Variation in the effectiveness of symbiotic associations between native rhizobia and temperate Australian legumes: interactions within and between genera. *J. Appl. Ecol.* **37:**52–65.

Timmers A.C.J. Soupène, E., Auriac, M.-C., de Billy, F., Vasse, J., Boistard, P., and Truchet, G., 2000. Saprophytic intracellular rhizobia in alfalfa nodules. *Mol. Plant-Microbe Interact.* **13:**1204–1213.

Tiwari, R.P., Reeve, W.G., Dilworth, M.J., and Glenn, A.R. 1993. Characterisation of *act206*–a gene involved in intracellular pH maintenance in *Rhizobium meliloti* WSM419. **In:** *New Horizons in Nitrogen Fixation.* p. 658, Palacios, R., Mora, J., and Newton, W.E., Eds., Kluwer.

Tiwari, R.P., Reeve, W.G., Dilworth, M.J., and Glenn, A.R. 1996a. An essential role for *actA* in acid-tolerance of *Rhizobium meliloti*. *Microbiol.* **142:**601–610.

Tiwari, R.P., Reeve, W.G., Dilworth, M.J., and Glenn, A.R. 1996b. Acid tolerance in *Rhizobium meliloti* strain WSM419 involves a two component sensor-regulator system. *Microbiology* **142:**1693–1704.

Toro, N. 1996. Nodulation competitiveness in the *Rhizobium*-legume symbiosis. *World J. Microbiol. Biotechnol.* **12:**157–162.

Triplett, E.W. and Barta, T.M. 1987. Trifolitoxin production and nodulation are necessary for the expression of superior nodulation competitiveness by *Rhizobium*

leguminosarum bv. trifolii T24. *Plant Physiol.* **85:**335–342.

Triplett, E.W. 1988. Isolation of genes involved in nodulation competitiveness from *Rhizobium leguminosarum* bv. *trifolii* T24. *Proc. Natl. Acad. Sci. USA* **85:**3810–3814.

Triplett, E.W. 1990. Construction of a symbiotically effective strain of *Rhizobium leguminosarum* bv. *trifolii* with increased nodulation competitiveness. *Appl. Environ. Microbiol.* **56:**98–103.

Triplett, E.W., Breil, B.T., and Splitter, G.A. 1994. Expression of *tfx* and sensitivity to the rhizobial antipeptide trifolitoxin in a taxonomically distinct group of α-proteobacteria including the animal pathogen *Brucella abortus*. *Appl. Environ. Microbiology* **60:**4163–4166.

Turner, S.L., Rigottier-Gois, L., Power, R.S., Amarger, N., and Young, J.P.W. 1996. Diversity of *repC* plasmid-replication sequences in *Rhizobium leguminosarum*. *Microbiol.* **142:**1705–1713.

Turner, S.L. and Young, J.P.W. 2000. The glutamine synthetases of rhizobia: phylogenetics and evolutionary implications. *Mol. Biol. Evol.* **17:**309– 319.

Ueda, T., Suga, Y., Yahiro, N., and Matsuguchi, T., 1995. Remarkable N_2–fixing bacterial diversity detected in rice roots by molecular evolutionary analysis of *nifH* gene sequences. *J. Bacteriol.* **177:**1414–1417.

Urquiaga, S., Botteon, P.B.L., and Boddey, R.M., 1989. Selection of sugar cane cultivars for associated biological nitrogen fixation using ^{15}N-labelled soil. **In:** *Nitrogen Fixation with Non-Legumes*. pp. 311–319. Skinner, A. Ed. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Urquiaga, S., Cruz, K.H.S., and Boddey, R.M., 1992. Contribution of nitrogen fixation to sugar cane: nitrogen-^{15} and nitrogen-balance estimates. *Soil Sci. Soc. Am. J.* **56:**105–114.

van Berkum, P., Kotob, S.I., Basit, H.A., Salem, S., Gewaily, E.M., and Angle, J.S. 1993. Genotypic diversity among strains of *Bradyrhizobium japonicum* belonging to serogroup 110. *Appl. Environ. Microbiol.* **59:**3130–3133.

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

van Berkum, P., Ruihua, F., Campbell, T.A., and Eardly, B.D. 1999. Some issues of relevance in the taxonomy of rhizobia. **In:** *Highlights of Nitrogen Fixation Research*. pp. 267–269, Martínez, E. and Hernández, G., Eds., Kluwer Academic/Plenum Publishers, New York.

van Berkum, P. and Fuhrmann, J.J. 2000. Evolutionary relationships among the soybean bradyrhizobia reconstructed from 16S rRNA gene and internally transcribed spacer region sequence divergence. *Int. J. Syst. Evol. Microbiol.* **50:**2165–2172.

van Dillewijn, P., Soto, M.J., Villadas, P.J., and Toro, N. 2001. Construction and environmental release of a *Sinorhizobium meliloti* strain genetically modified to be more competitive for alfalfa nodulation. *Appl. Environ. Microbiol.* **67:**3860–3865.

van Rossum, D., Schuurmans, F.P., Gillis, M., Muyotcha, A., van Verseveld, H.W., Stouthamer, A.H., and Boogerd, F.C. 1995. Genetic and phenetic analyses of *Bradyrhizobium* strains nodulating peanut (*Arachis hypogaea* L.) roots. *Appl. Environ. Microbiol.* **61:**1599–1609.

Vásquez-Arroyo, J., Sessitsch, A., Martínez, E., and Peña-Cabriales, J.J. 1998. Nitrogen fixation and nodule occupancy by native strains of *Rhizobium* on different cultivars of common bean (*Phaseolus vulgaris* L.). *Plant Soil* **204:**147–154.

Velázquez, E., Cruz-Sánchez, J. M., Mateos, P. F., and Martínez-Molina, E. 1998. Analysis of stable low-molecular-weight RNA profiles of members of the family *Rhizobiaceae*. *Appl. Environ. Microbiol.* **64:**1555–1559.

Velázquez, E., Martínez-Romero, E., Rodríguez-Navarro, D.N., Trujillo, M.E., Daza, A., Mateos, P.F., Martínez-Molina, E., and van Berkum, P. 2001a. Characterization of rhizobial isolates of *Phaseolus vulgaris* by staircase electrophoresis of low-molecular-weight RNA. *Appl. Environ. Microbiol.* **67:**1008–1010.

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

Vermeiren, H., Vanderleyden, J., and Hai, W. 1998. Colonization and *nifH* expression on rice roots by *Alcaligenes faecalis* A15. In: *Nitrogen Fixation with Non-Legumes*. pp. 287–305, Malik, K. A., Mirza, M. A., and Ladha, J. K., Eds., Kluwer Academic Publishers. Dordrecht, The Netherlands.

Vinuesa, P., Rademaker, J.L.W., de Bruijn, F.J., and Werner, D. 1998. Genotypic characterization of *Bradyhizobium* strains nodulating endemic woody legumes of the Canary Islands by PCR-restriction fragment length polymorphism analysis of genes encoding 16S rRNA (16S rDNA) and 16S-23S rDNA intergenic spacers, repetitive extragenic palindromic PCR genomic fingerprinting, and partial 16S rDNA sequencing. *Appl. Environ. Microbiol.* **64:**2096–2104.

Viprey, V., Rosenthal, A., Broughton, W.J., and Perret, X. 2000. Genetic snapshots of the *Rhizobium* species NGR234 genome. *Genome Biol.* **1:** research/0014.1–17.

Wadisirisuk, P., Danso, S.K.A., Hardarson, G., and Bowen, G.D. 1989. Influence of *Bradyrhizobium japonicum* location and movement on nodulation and nitrogen fixation in soybean. *Appl. Environ. Microbiol.* **55:**1711–1716.

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

Wang, E.T., Martínez-Romero, J., and Martínez-Romero, E. 1999a. Genetic diversity of rhizobia from *Leucaena leucocephala* nodules in Mexican soils. *Mol. Ecol.* **8:**711–724.

Wang, E. T., Rogel, M. A., García-de los Santos, A., Martínez-Romero, J., Cevallos, M. A., and Martínez-Romero, E. 1999b. *Rhizobium etli* bv. mimosae, a novel biovar isolated from *Mimosa affinis*. *Int. J. Syst. Bacteriol.* **49:**1479–1491.

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

Wang, E.T. and Martínez-Romero, E. 2000. Phylogeny of root- and stem-nodule bacteria associated with legumes. **In:** *Prokaryotic Nitrogen Fixation: A Model System for Analysis of a Biological Process*. pp. 177–186, Triplett, E. W., Ed., Horizon Scientific Press, Wymondham, UK.

Wang, E.T., Tan, Z.Y., Willems, A., Fernández-López, M., Reinhold-Hurek, B., and Martínez-Romero, E. 2002. *Sinorhizobium morelense* sp. nov., isolated from *Leucaena leucocephala* that is highly resistant to multiple antibiotics. *Int. J. Syst. Evol. Microbiol.* submitted.

Watanabe, I., Barraquio, W.L., DeGuzman, M.R., and Cabrera, D.A. 1979. Nitrogen-fixing (acetylene-reducing) activity and population of aerobic heterotrophic nitrogen-fixing bacteria associated with wetland rice. *Appl. Environ. Microbiol.* **39:**813–819.

Watanabe, I., Yoneyama, T., Padre, B., and Ladha, J.K., 1987. Difference in natural abundance of ^{15}N in several rice (*Oryza sativa* L.) varieties: Applications for evaluating N_2 fixation. *Soil Sci. Plant Nutr.* **33:**407–415.

Webster, G., Jain, V., Davey, M. R., Gough, C., Vasse, J., Dénarié, J., and Cocking, E.C. 1998. The flavonoid naringenin stimulates the intercellular colonization of wheat roots by *Azorhizobium caulinodans*. *Plant Cell Environ.* **21:**373–383.

Wernegreen, J.J. and Riley, M. A. 1999. Comparison of the evolutionary dynamics of symbiotic and housekeeping loci: a case for the genetic coherence of rhizobial lineages. *Mol. Biol. Evol.* **16:**98–113.

West, P.M. and Wilson, P.W. 1939. Growth factor requirements of the root nodule bacteria. *J. Bacteriol.* **37:**161–185.

Wexler, M., Gordon, D., and Murphy, P.J. 1995. The distribution of inositol rhizopine genes in *Rhizobium* populations. *Soil Biol. Biochem.* **27:**531–537.

Wiehe, W. and Höflich, G. 1995. Survival of plant growth promoting rhizosphere bacteria in the rhizosphere of different crops and migration to noninoculated plants under field conditions in northeast Germany. *Microbiol. Res.* **150:**201–206.

Wilkinson, H.H., Spoerke, J.M., and Parker, M.A. 1996. Divergence in symbiotic compatibility in a legume-*Bradyrhizobium* mutualism. *Evolution* **50:**1470–1477.

Willems, A. and Collins, M.D. 1993. Phylogenetic analysis of rhizobia and agrobacteria based on 16S rRNA gene sequences. *Int. J. Syst. Bacteriol.* **43:**305–313.

Willems, A., Doignon-Bourcier, F., Coopman, R., Hoste, B., de Lajudie, P., and Gillis, M. 2000. AFLP fingerprint analysis of *Bradyrhizobium* strains isolated from *Faidherbia albida* and *Aeschynomene* species. *Syst. Appl. Microbiol.* **23:**137–147.

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

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

Wilson, K.J., Sessitsch, A., and Akkermans, A.D.L. 1994. Molecular markers as tools to study the ecology of microorganisms. **In:** *Beyond the Biomass: Compositional and Functional Analysis of Soil Microbial Communities*. pp. 149–156, Ritz, K., Dighton, J., and Giller, K.E., Eds., John Wiley, Chichester, UK.

Wilson, K.J. 1995. Molecular techniques for the study of rhizobial ecology in the field. *Soil Biol. Biochem.* **27:**501–514.

Wilson, K.J., Sessitsch, A., Corbo, J., Giller, K.E., Akkermans, A.D.L., and Jefferson, R.A. 1995. β-glucuronidase (GUS) transposons for ecological and genetic studies of rhizobia and other Gram-negative bacteria. *Microbiol.* **141:**1691–1705.

Wood, M. and Stanway, A.P. 2001. *myo*-inositol catabolism by *Rhizobium* in soil: HPLC and enzymatic studies. *Soil Biol. Biochem.* **33:**375–399.

Xie, Z-P., Staehelin, C., Vierheilig, H., Wiemken, A., Jabbouri, S., Broughton, W.J., Vögeli-Lange, R., and Boller, T. 1995. Rhizobial nodulation factors stimulate mycorrhizal colonization of nodulating and nonnodulating soybeans. *Plant Physiol.* **108:**1519–1525.

Xie, Z-P., Müller, J., Wiemken, A., Broughton, W.J., and Boller, T. 1997. Nod factors and tri-iodobenzoic acid stimulate mycorrhizal colonization and affect carbohydrate partitioning in mycorrhizal roots of *Lablab purpureus*. *New Phytol.* **139:**361–366.

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

Yan, A.M., Wang, E.T., Kan, F.L., Tan, Z.Y., Sui, X.H., Reinhold-Hurek, B., and Chen, W.X. 2000. *Sinorhizobium meliloti* associated with *Medicago sativa* and *Melilotus* spp. in arid

saline soils in Xinjiang, China. *Int. J. Syst. Evol. Microbiol.* **50:**1887–1891.

Yanni, Y.G., Rizk, R.Y., Corich, V., Squartini, A., Ninke, K., Philip-Hollingsworth, S., Orgambide, G., De Bruijn, F., Stoltzfus, J., Buckley, D., Schmidt, T.M., Mateos, P.F., Ladha, J.K., and Dazzo, F.B., 1997. Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. *Plant Soil* **194:**99–114.

Yanni, Y.G., Rizk, R.Y., Abd El-Fattah, F.K., Squartini, A., Corich, V., Giacomini, A., de Bruijn, F., Rademaker, J., Maya-Flores, J., Ostrom, P., Vega-Hernández, M., Hollinsworth, R. I., Martínez-Molina, E., Mateos, P., Velázquez, E., Wopereis, J., Triplett, E., Umali-García, M., Anarna, J.A., Rolfe, B.G., Ladha, J.K., Hill, J., Mujoo, R., Ng., P.K., and Dazzo, F.B., 2001. The beneficial plant growth-promoting association of *Rhizobium leguminosarum* bv. *trifolii* with rice roots. *Aust. J. Plant. Physiol.* **28:**1–26.

Yates, R.J., di Mattia, E., O'Hara, G., Real, D., and Howieson, J.G. 2002. The role of *Rhizobium leguminosarum* bv. *trifolii* in extending (or restricting) the adaptation of *Trifolium* spp. in natural and managed ecosystems. **In:** *Survival of Perennial Legumes in Dry Mediterranean Areas.* Bennett, S.J., Ed., UWA Press, Perth, in press.

Yoneyama, T., Muraoka, T., Kim, T.H., Dacanay, E., and Nakanishi, Y, 1997. The natural ^{15}N abundance of sugarcane and neighbouring plants in Brazil, the Philippines and Miyako (Japan). *Plant Soil* **189:** 239–244.

You, Z., Gao, X., Ho, M.M., and Borthakur, D. 1998. A stomatin-like protein encoded by the *slp* gene of *Rhizobium etli* is required for nodulation competitiveness on the common bean. *Microbiology* **144:**2619–2627.

Young, J. M., Kuykendall, L. D., Martínez-Romero, E., Kerr, A., and Sawada, H. 2001. A revision of *Rhizobium* Frank 1889, with an emended description of the genus, and the inclusion of all species of *Agrobacterium* Conn 1942 and *Allorhizobium undicola* de Lajudie et al. 1998 as new combinations: *Rhizobium radiobacter*, *R. rhizogenes*, *R. rubi*, *R. undicola* and *R. vitis*. *Int. J. Syst. Evol. Microbiol.* **51:**89–103.

Young, J.P.W. and K.E. Haukka. 1996. Diversity and phylogeny of rhizobia. *New Phytol.* **133:**87–94.

Zézé, A., Mutch, L.A., and Young, J.P.W. 2001. Direct amplification of *nodD* from community DNA reveals the genetic diversity of *Rhizobium leguminosarum* in soil. *Environ. Microbiol.* **3:**363–370.

Zhang, X., Nick, G., Kaijalainen, S., Lonnqvist, C., Tas, E., Tighe, S.W., Graham, P.H., and Lindström, K. 1997. Diversity of peanut *Rhizobium* isolated from Sichuan, China. **In:** *Book of Abstracts. 11th International Congress on Nitrogen Fixation.* p. 49, Institut Pasteur. Paris.

Zhang, X.-X., Turner, S.L., Guo, X.-W., Yang, H.-J., Debellé, F., Yang, G.-P., Dénarié, J., Young, J.P.W., and Li, F.-D. 2000. The common nodulation genes of *Astragalus sinicus* rhizobia are conserved despite chromosomal diversity. *Appl. Environ. Microbiol.* **66:**2988–2995.