Critical Reviews in Plant Sciences, 15(2):113–140 (1996)

Rhizobium Phylogenies and Bacterial Genetic Diversity

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ABSTRACT: The Leguminosae is one of the largest families of plants. It has a broad geographical distribution. The principal legume species have defined sites of origin and these coincide with the diversification centers for their "specific" symbiotic bacteria. These nitrogen-fixing bacteria, which form nodules in the roots or stems of the plants, belong to different bacterial lineages (*Rhizobium, Bradyrhizobium, and Azorhizobium*) related to other nonsymbiotic bacteria. A remarkable characteristic of these bacteria is their large genetic diversity. The genetic relationships among the different bacterial groups are being defined based mainly on the analysis of the sequences of the ribosomal genes. Recent results point out the need to have a broader genomic scope. Gene maps, genome sizes, and sequence of metabolic genes would serve to validate the present *Rhizobium* and *Bradyrhizobium* phylogenies. More realistic phylogenies should perhaps consider lateral transfer between clusters of bacteria.

A compilation of records of bacterial genetic diversity, including enterobacteria and pathogens, is presented and compared with *Rhizobium* diversity. It is proposed that human activities are having important effects on microbe diversity.

KEY WORDS: *Rhizobium*, taxonomy, phylogenies, diversity, pathogens, enterobacteria.

I. *RHIZOBIUM* PHYLOGENIES

A. Introduction

The Leguminosae comprise one of the largest families of plants with over 17,000 to 19,000 described species.[4] It is estimated that the different species were already diverging by the time the continental masses were separating approximately 65 million years ago.[86,88,170] Different legumes evolved and diversified in various regions of the world. For example, the soybean developed in Asia;[107] *Phaseolus* species flourished in both Mesoamerica and in the Andean region of South America;[72] *Trifolium*, *Medicago*, and *Pisum* species in the Middle East;[122,123] and *Galega officinalis* and *G. orientalis* probably in the Caucasus.[125] Africa is the only site of origin of bambara groundnut (*Vigna subterranea*), cowpea (*V. unguiculata*), and others.[39,151] In addition to these main crops, perhaps hundreds of legumes also have specific diversification sites.[200] Other, perhaps older legumes, such as *Acacia*, with about 1500 species, are distributed worldwide.[151]

0735-2689/96/$.50

It is generally considered that the Leguminosae family has both a tropical and subtropical origin and that colonization and adaptation to template regions occurred later.[88]

Legumes are remarkable because they establish a symbiotic interaction with nitrogen-fixing bacteria from the genera *Rhizobium*, *Bradyrhizobium*, and *Azorhizobium*, which form nodules on the roots or stems of the plants. The already identified species and their principal host legumes are shown in Table 1. The symbiotic bacteria seem to have evolved locally, coinciding with the legume distribution. Thus, we encounter native *Bradyrhizobium japonicum* and *Rhizobium fredii* (nodulating soybean) in China.[107] There is a large diversity of *R. etli* (nodulating *Phaseolus* bean) in Mexico[135,167,179] and Colombia.[54] There are native populations of *R. leguminosarum* bv. viciae (nodulating pea) in Afghanistan[122,123] and of *R. galegae* in the Caucasus.[125] We may suppose that while *Rhizobium* chromosomal lineages radiated and diversified, different types of symbiotic relationships may have evolved. In some cases, some bacterial lineages became highly specialized for some plant hosts. A great deal of research has been devoted to *Rhizobium*, *Bradyrhizobium*, and *Azor-*

TABLE 1
***Rhizobium*, *Bradyrhizobium*, and *Azorhizobium* Species[a]**

Species	Principal associated host legumes
R. meliloti	*Medicago, Melilotus, Trigonella*
R. fredii	*Glycine max* and *G. soja*
S. saheli[b]	*Acacia* and *Sesbania*
S. teranga[b]	*Acacia* and *Sesbania*
R. leguminosarum	
bv. viciae	*Vicia* and *Pisum*
bv. trifolii	*Trifolium*
bv. phaseoli	*Phaseolus vulgaris*
R. tropici	*Phaseolus vulgaris and Leucaena*
R. etli	*Phaseolus vulgaris*
R. galegae	*Galega officinalis, G. orientalis*
R. loti[c]	*Lotus* spp.
R. huakuii[c]	*Astragalus sinicus*
B. japonicum	*Glycine max*
B. elkanii	*G. max*
A. caulinodans	*Sesbania rostrata*

[a] Other published species are *R. ciceri* for *Cicer arietinum* and *R. tianshanense* for *Glycine max*.

[b] A new genera has been proposed to encompass *R. meliloti* and *R. fredii*, and the new species *S. teranga* and *S. saheli*. Due to the taxonomic rules, the new genera name adopted must be the last one that was proposed and rejected, and corresponds to *Sinorhizobium*.

[c] It has been proposed that *R. loti* and *R. huakuii* be assigned to another genera.

hizobium and several reviews on their interaction with plants have been published,[18,24,46,61,62,91,127,169,178,194] as well as other reviews concerning *Rhizobium* taxonomy[136,138,224] and evolution.[195,196,228]

B. *Rhizobium* Distribution

It is a puzzle as to what the effects of agricultural practices have been on promoting *Rhizobium* populations and it is tempting to speculate that large densities of single species in crops have been a driving force in selecting rhizobia with narrow host ranges and higher specificities for some host plants. There is evidence that man has contributed to the spread of rhizobia around the world. Supposedly, the Romans helped to spread *R. leguminosarum* bv. viciae in Europe by carrying the soil as well as the pea plants from Middle Asia in historical times. *Phaseolus vulgaris* bean was introduced from Mexico to Europe through Spain in the sixteenth century.[73] In Spain, there are abundant native populations of *R. etli*[159] (whereas no *R. etli* is found in France[7,70,117] nor in England[220]). It is known that *B. japonicum* was introduced into the U.S. and Brazil as bacterial inoculants when soybean was adopted as a main crop in those countries.[81] *B. japonicum* was also introduced into Mexico as a soybean inoculant in the northwestern agricultural areas approximately 30 years ago, and in Poland, Italy, and India within the last 10 or 15 years.[106,132]

The introduction of crops into other regions may have an impact on native bacterial populations, but the extent of these effects has not been evaluated. In some cases, the plants may recruit local "opportunistic bacteria" that have cross specificity to nodulate these plants. This seems to be the case with the peanuts introduced into China[230] and with the *Leucaena* trees introduced into different regions.[95,162] The *Leucaena* plants introduced into Brazil are nodulated by the broad host range bacteria *R. tropici*[139] and by *Rhizobium* spp. related to *R. meliloti*.[87] Native leucaenas, in contrast, are nodulated by strains related to *R. etli* in Mexico,[87] which together with other countries of Central America is the site of origin of *Leucaena* species.[172]

It is sometimes difficult to determine whether the *Rhizobium* populations are native to an area or were introduced to it. This is the case with *R. tropici*[7] and with *Rhizobium* spp. related to *R. etli*[117] in French soils. It has been pointed out "how ignorant we are about the mechanisms by which prokaryotes are distributed across the Earth and the factors which influence their establishment and success in a new environment,"[199] and different possibilities for *Rhizobium* spread have been proposed.[199] However, it still seems reasonable to suppose that there has been a natural and successful distribution of *Rhizobium* and *Bradyrhizobium* lineages independent of man.

In South Africa, *R. tropici* strains, identified by total protein profiles, have been recovered from *Bolusanthus* and *Spartium* nodules.[38] "*R. hainanensis*" (still not recognized as a *bona fide Rhizobium* species)[126] has been isolated from China,[30] and it is clearly related to *R. tropici* by the 16S gene sequence and DNA-DNA homology. Originally, *R. tropici* was isolated from *P. vulgaris* (common bean) and from *Leucaena* trees from acid soils in South America.[139] Both *P. vulgaris* and *Leucaena* trees are introduced plants in these regions, so it was proposed that *R. tropici* was the natural symbiont of some other legume. *R. tropici* was also found in bean nodules from plants grown in acid soils in Kenya.[74] Possibly, the Portuguese introduced *R. tropici* to these areas as well as the *P. vulgaris* bean plants. Also intriguing is the fact that *Agrobacterium* isolates from tumors of kiwi and cherry plants from Japan,[173] as well as *Agrobacterium* biovar 2

strains, are among the closest relatives to *R. tropici* strains, as determined by the sequence of 16S ribosomal genes.[174,214] Other similarities among these agrobacteria and *R. tropici* have been found as well.[138] The genetic differences that determine whether a bacterium is a pathogen or a symbiont are discussed later.

There are more examples of a wide geographic distribution of other *Rhizobium* lineages. The same types of *R. leguminosarum* have been encountered both in the U.S. and in the U.K.[199] Some isolates from *Medicago ruthenica* from Mongolia are related to *R. etli* (by sequence of 16S ribosomal genes) and the majority of *M. ruthenica* isolates are a lineage between *R. etli* and *R. tropici*.[207] In South Africa, *R. etli* (identified by protein profiles) has been isolated from *Desmodium*, *Memolobium*, and *Indigofera* and from the tree legumes *Acacia melanoxylon* and *Chamaecrista stricta*.[38] It would be interesting to analyze the South African isolates to determine if they are similar to the Mesoamerican *R. etli* isolates in other respects. *R. etli*-like symbionts also have been identified in Argentina from bean nodules.[2] Originally, *R. etli* was found nodulating *P. vulgaris* plants in Mesoamerica.[180] Bacteria related to *R. etli* have been isolated from different native Mesoamerican legumes[87] and from bean nodules in France.[117] These bacteria are diverging lineages that, we suppose, had a common ancestor with *R. etli*.

As mentioned previously, *B. japonicum* diversified in Asia. There has been a successful *B. japonicum* inoculation response in soybean crops in other regions of the world. The response to inoculation has been linked, in the majority of the cases, to the absence of native bacteria with the ability to nodulate the introduced crop.[81] In Mexico, there are no native populations of *B. japonicum*; however, native *Bradyrhizobium* have been recovered from native *Lupinus* plants. These bacteria are closely related to *B. japonicum*, and we estimate that they have been geographically isolated from *B. japonicum* for many millions of years. Yet, they differ by only one nucleotide in a fragment of the 16S ribosomal genes (our own unpublished results). *Lupinus* isolates from other regions are clearly related to *Bradyrhizobium* as well.[104]

C. *Rhizobium* Genetic Diversity

The wide geographical distribution of *Rhizobium* and *Bradyrhizobium* bacteria may be related to the large genetic diversity encountered in rhizobia, which is a common finding when analyzing these populations[16,38,45,53,117,208,209,229] (see Section II). Their large biodiversity, as well as their broad geographical distribution may be interpreted as evidence that *Rhizobium* and *Bradyrhizobium* are ancient lineages of bacteria with a long evolutionary history.[167]

A single legume, for example, *Acacia*, soybean, or *Leucaena*, can pick up genetically dissimilar symbionts. Therefore, with a few legume species, we have samples of large bacterial heterogeneity. *Acacia* is nodulated by *Bradyrhizobium* spp.,[10,50,51] by *Rhizobium* related to *R. loti* (cluster U),[42] by *Rhizobium* related to *R. meliloti* (the recently proposed *Sinorhizobium saheli* and *teranga*[42]), and by *R. huakuii*. Soybean is nodulated by widely divergent bacteria, *B. japonicum*,[104] *B. elkanii*,[113] "*B. liaoningense*,"[217] "*B. tianshanense*"[31] (the last two not yet recognized as *bona fide* species),[126] and by *R. fredii*.[96,107] *Leucaena* is nodulated by a wide range of rhizobia.[71,87,95,139,162] The diversity of *Rhizobium* nodulating *P. vulgaris* has not been completely described and, in addition to *R. tropici*,[139] *R. etli*,[180] and *R. leguminosarum* bv. phaseoli,[103] there are new findings of lineages nodulating bean.[54,117,206]

High genetic diversity is not only a prevalent characteristic of tropical legume symbionts, but it is also a characteristic of sym-

bionts from temperate legumes,[157] such as *Astragalus* spp., *Oxytropis campanulata*, *Hedysarum alpinum*, *Ononis arvensis*, *Glycyrrhiza* spp., *Coronilla varia*, and *Lupinus* species. *R. huakuii* was identified from *Astragalus sinicus* from China[29] and it is related to *R. loti*[97] (from *Lotus* spp.) by the sequence of ribosomal genes. More recently, *R. ciceri*,[155] related to this cluster but not yet officially recognized as a species,[126] was proposed to encompass part of the symbionts obtained from *Cicer arietinum* (chickpea). As more strains from *Astragalus* have been considered for their ribosomal sequences, it has been found that *Astragalus* and chickpea isolates are not really separated, but that they overlap.[206] A recently described group of rhizobia ("*R. tianshanense*"[31]), not yet recognized as a species,[126] originating in an arid and saline environment in northwestern China, was found to be related to the *R.loti–R.huakuii* cluster. It remains to be established if the *Ononis* isolates, related to the *R.huakuii–R.ciceri* cluster,[156] constitute a distinct branch. By other criteria, such as phage typing, *R. loti* is related to several *Astragalus*, *Hedysarum*, and *Ononis* rhizobia as well as to *R. huakuii* strains.[156] Furthermore, *R. loti* strains are able to nodulate *O. arvensis*.[156] *Astragalus*, *Hedysarum*, *Glycyrrhiza*, and *Ononis* plants seem to belong to a single cross-nodulation group.

Another example of various distinct host legumes nodulated by a cluster of related bacteria is the case of the Mesoamerican legumes *Dalea leporina*, *Clitoria ternatea*, *Crotalaria* spp., *Macroptilium gibbosifolium*, and *Leucaena* spp., whose symbionts are a group of genetically intermingled rhizobia closely related to *R. etli*.[87]

Heterogeneity inside *B. japonicum* served as a basis to propose *B. elkanii*.[112,113] *Bradyrhizobium* with specificity for *Vigna*, *P. lunatus*, *Arachis*, and *Macroptilium* are a highly heterogeneous group of bacteria.[202] Photosynthetic rhizobia are included within the *Bradyrhizobium* cluster. Some of them perhaps constitute an independent branch.[190] A large number of native legumes from Amazonia are nodulated by *Bradyrhizobium* strains that do not correspond to the described *Bradyrhizobium* groups.[145] Based on the complete sequences of 16S rRNA genes, a distinct branch of *Bradyrhizobium* has been distinguished, including *B. elkanii* and isolates from *Astragalus* and from a Manchurian tree.[206]

D. Grouping *Rhizobium*

At present, *Rhizobium* and *Bradyrhizobium* lineages look quite complicated, yet only a small percentage of the nodulating legumes have been sampled for their rhizobia. Furthermore, it has been claimed that by looking only at the isolates from nodules we may be overlooking diversity, as perhaps only a small proportion of the whole population, the one with the highest capacity to nodulate, is analyzed. Actually, this is the case; it has been shown that the *R. meliloti* population obtained from plant nodules is not representative of the total population present in soil.[19] We must expect that in the near future, we will have to deal with an overwhelmingly diverse collection of rhizobia. It seems that many of the new species will be isolated from the tropics and subtropics.[162]

Microbial biodiversity has been considered as one of the most valuable resources for mankind. Yet, for people working with *Rhizobium* and *Bradyrhizobium*, it is very difficult to handle such diversity, and proper and simpler methods to identify and classify *Rhizobium* bacteria need to be developed. At present, researchers have more tools and criteria to accommodate *Rhizobium* strains in biologically meaningful groups,[36,41,77,98,115,130] yet the general principle is the same that was used about a century ago to define phylogenies among

living organisms:[80,216] the more characteristics they share, the more similar they are. Now it is agreed that *Rhizobium* lineages should be established based on stable (chromosomal) properties.[77,138] The present trends are considering more genetic traits in addition to phenotypic characteristics, but in several cases, phenotypic- and genetic-based groupings do not seem to match,[139,190,210] raising controversy as to the usefulness of the polyphasic approach to define rhizobia species.[190,210]

From the present knowledge of bacterial genetics, we predict that even a few genetic changes may cause profound (pleiotropic) effects in phenotype. For this reason, systematics based solely on phenotypes may lead to unclear[66,114,124,229] or distorted relationships,[32,96,190] and diverging, but otherwise closely related organisms, may be placed far apart. On the other hand, we should recognize that the present genetic approaches are based, in many cases, on a limited sample of the vast genetic information from an organism. To make the scope more complicated, even if bacteria share a very large part of their genetic information, the differences may determine outstanding characteristics strong enough to place them apart. At any rate, only by analyzing the information in macromolecules can the evolutionary histories of bacteria be traced.[160,176,231]

Related organisms should share not only DNA-sequence similarities in the majority of their genes, but also they should have their genes organized in a similar array. This means that genomic maps should be conserved among related organisms. Chromosome sizes should be similar as well. A common evolutionary origin of *R. meliloti* and *R. leguminosarum* chromosomes may be postulated based on the similarities of the arrangement of their genes. By genetic crosses between *R. leguminosarum* and *R. meliloti* to analyze chromosome recombination, the alleles for particular nutritional requirements comprising 16 genes were mapped in similar positions in the tested strains.[12,101,102,108] Unfortunately, these experiments were performed when only a limited number of *Rhizobium* species were known. It would be interesting to have this type of information (gene maps) from the other new species, and these would be still more valuable if more genetic markers were considered, as well as the frequencies of chromosomal recombination among different species. It would also be useful to know if chromosome sizes are equivalent in different lineages as an additional support to *Rhizobium* systematics.

Bacterial genomes are small compared to eukaryotic genomes. The genome size of *Rhizobium* spp. has been estimated to be 5300 kb[37] and that of *Agrobacterium*, 5500 kb.[43] More recently, the size of the *R. meliloti* chromosome was calculated to be 3500 kb[92] and that of *B. japonicum*, 8700 kb,[111] based on physical maps. In theory, there may be more than 3000 genes in *Rhizobium*. With present DNA sequencing facilities, it is not possible to have the complete genomic DNA sequence from a large number of organisms. At most, we anticipate that the chromosome from one or two *Rhizobium* or *Bradyrhizobium* strains will be completely sequenced in the near future. However, it seems more feasible to sequence single genes from a number of representative strains. However, this is an enormous task, and perhaps there will always be the possibility that the gene sample is not big enough to represent the whole genome. It would be ideal to identify "clue" chromosomal genes to represent the majority or core genetic information from the bacteria. Ribosomal genes, being highly conserved, have proven to be very useful to infer phylogenies among organisms and they may be preferable to metabolic genes, especially for distantly related organisms. With approximately 1500 to 2000 16S rRNA gene sequences reported for bacteria, this

gene is becoming a universal reference code,[131,160,216,224] and it is undoubtedly at present a yardstick to compare *Rhizobium* and *Bradyrhizobium* lineages even to outgroups.[162,174,214,219] One interesting finding is that *Zoogloea ramigera* strain, a sewage-borne bacterium, is a member of the *Rhizobium* and *Bradyrhizobium* cluster by the sequence of the 16S rRNA[171] (see also Reference 224). *Rhizobium* phylogenies based on 16S ribosomal genes are generally in agreement with those derived from the analysis of other gene sequences.[223,224]

An example of a phylogenetic tree encompassing most of the described *Rhizobium* groups is shown in Figure 1, based on 16S gene sequences. It is a probabilistic tree that is in agreement with others described.[162,174,214,219] Some differences in trees may arise depending on the methodology used to construct them (reviewed in Ludwig and Schleifer[131]). The results derived from this type of analysis are the following: *Rhizobium* and *Bradyrhizobium* species are clearly separated, as has been recognized for a long time using other criteria. In addition, symbionts and pathogens are closely related, confirming previous data that showed that *Rhizobium* and *Agrobacterium* were close relatives. Now the relationship among many

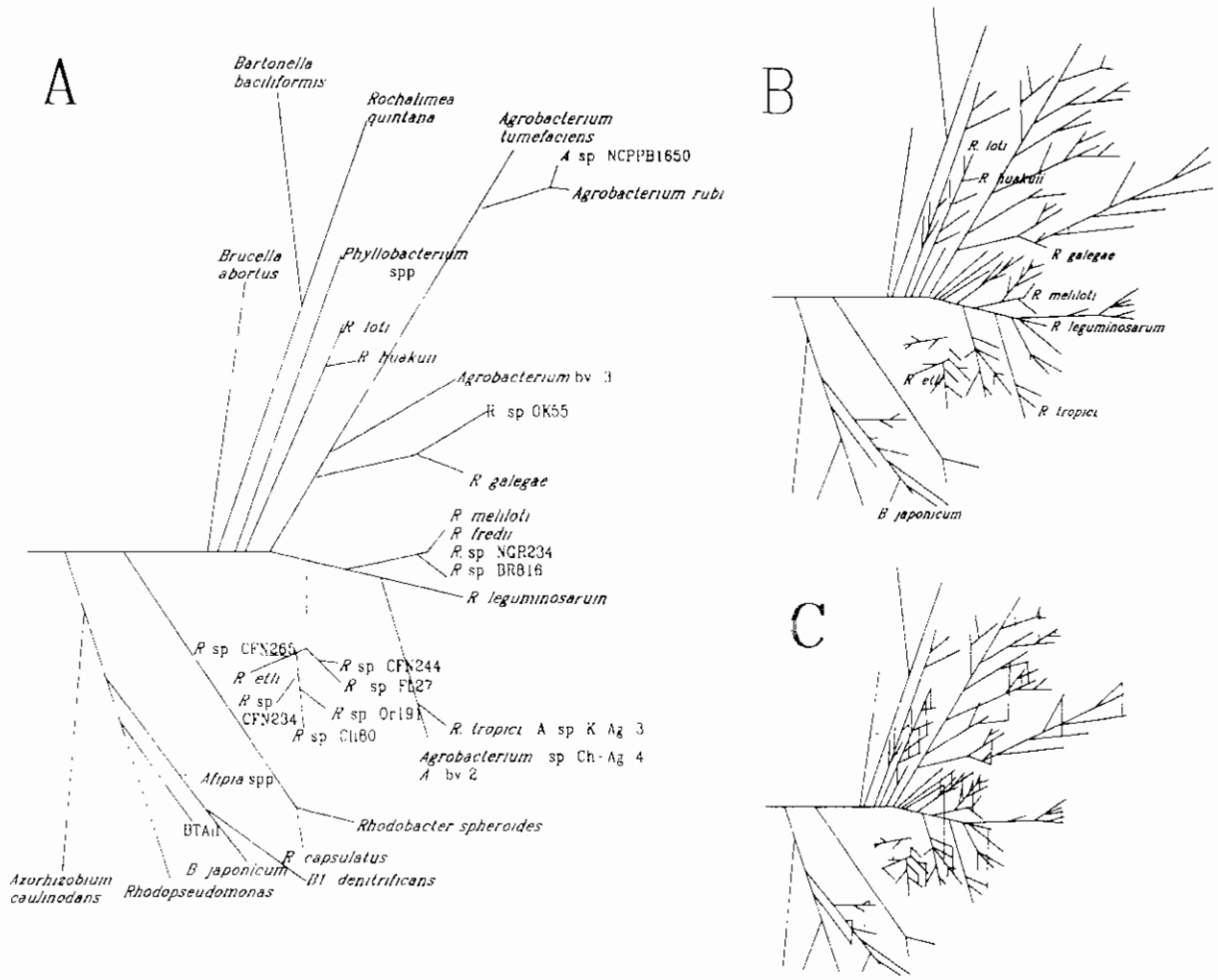

FIGURE 1. **(A)** Phylogenetic tree based on the analysis of 16S RNA gene sequences. **(B)** Theoretical future tree considering more bacterial groups and close relationships between some of them. **(C)** Theoretical tree considering lateral transfer among some lineages.

different species may be finally defined and a close relationship to other genera (*Bartonella*, *Rochalimea*, *Brucella*, *Afipia*, *Rhodopseudomonas*, and *Blastobacter*) is evident. It is possible that if more strains were analyzed for the sequence of their ribosomal genes, the differences among species would be eliminated or largely diminished. Sequence variability within *R. tropici*[139] and *Bradyrhizobium* 16S rRNA has been observed[162,206,226] and there are some indications that there could be a continuum of strains between closely related *Rhizobium* species.[227] A theoretical tree considering this possibility, as well as new branches that could be obtained with other new *Rhizobium* and *Bradyrhizobium* isolates, is shown in Figure 1B. A more branched phylogenetic tree derived from the analysis of a wide number of strains has already been published.[162]

There are some node positions (origins of the branches) in the phylogenetic trees that cannot be determined with certainty using 16S ribosomal genes.[138] The advantages of using 23S gene sequences have been reviewed.[131] However, perhaps for related species, only DNA sequences from intergenic regions or from genes changing faster than ribosomal genes would clarify their genetic relationships. Some housekeeping genes could be good choices to use as molecular clocks to trace *Rhizobium* phylogenies.[110,153,163,165]

Although intervening sequences have been reported in ribosomal genes,[131] the implications for taxonomy and their biological significance are not understood. For example, one such sequence has been found in the 16S rRNA gene in many *R. tropici* type A strains but not in *R. tropici* type B strains.[7,214] Intervening sequences are common in the 23S rRNA genes and the small fragments derived from processing the ribosomal RNA, highly variable in length and sequence, have been proposed to serve as markers to distinguish *Rhizobium* groups.[56]

E. Genetic Recombination

Different types of phylogenetic trees have been obtained when, instead of considering the complete 16S gene, only internal fragments from it are analyzed. For example, *R. galegae* comes close to the *R. loti–R. huakuii* cluster when phylogenies are constructed using only the Y1-Y2 fragment[226] (300 bp in the first part of the gene).[223] The position of *R. etli* also changes depending upon whether the whole gene is considered or only a fragment.[206] The significance of these discrepancies is not understood at present. However, in other cases, when phylogenies derived from fragments of genes do not reflect the whole gene, it has been speculated that there is a mosaic behavior inside the gene that may be caused by intragenic recombination.[34] We must remember that ribosomal genes are multicopy in the genome and that genetic mechanisms to conserve or recombine multicopy genes in the genome have been identified.[8,90,166] Phylogenies based on multicopy genes sometimes are difficult to interpret.[211] Moreover, evidence of some unexpected behavior of ribosomal genes has been reported by Eardly et al.[54] They found that some *R. etli* strains (identified by their pattern of metabolic enzymes) harbor an *R. leguminosarum* allele of 16S ribosomal genes. One interpretation of their results is that there was a lateral transfer of ribosomal genes from *R. leguminosarum* to *R. etli*. In *Aeromonas*, recombination of ribosomal genes has been proposed based on discrepancies between clusters derived from DNA-DNA homologies and from ribosomal sequences[137] and by identifying mosaic patterns in the genes.[189]

There are other reports that support lateral transfer of chromosomal markers in *Rhizobium*.[44,193] Recently, genetic recombination to generate biodiversity has been directly explored in agricultural fields. *Lotus corniculatus* plants were inoculated

with an *R. loti* strain in soils devoid of *R. loti* in New Zealand. The majority of rhizobia recovered from *L. corniculatus* nodules seemed to be local bacteria that acquired the symbiotic genetic information from the inoculant strain. In *R. loti,* the genetic information for symbiosis is located on the chromosome and it seems that the transferred DNA (at least 105 kb) is integrated in the chromosome of the recipients as well.[201]

Recombination of genes occurs in bacteria,[34,141,142] which has been solidly documented from the comparative analysis of sequences from metabolic genes derived from different *Escherichia coli* and *Salmonella* strains.[13,17] It looks like the position or functionality of the genes could play a role in determining their recombination rate. Flagellin genes, determining serovars, are highly recombinant in *Salmonella.*[188] The proximity to recombination hot spots (e.g., antigenic determinants) or the proximity to the transfer initiation sites for conjugation may increase intragenic recombination rates. Recombination will bring sequences from different lineages together, thus altering their relationship to the whole genome. Highly recombinant genes are unsuitable to trace phylogenies. Moreover, bacteria with high recombination rates[142] may be considered "evolutionary chimeras," a collection of genes, each with its own history.[216] On the other hand, in clonal populations where recombination is not enough to affect the genetic structure, there is a linkage among different markers on the chromosome. Within clonal populations, the sampling of small parts of the genome reflects the overall genetic information from the bacteria. There are means to evaluate the clonality of populations;[34,142,193,199] these estimates may be derived from the same type of studies in which genetic diversity is analyzed (see Section II for a broader scope of bacterial genetic diversity and genetic structure of populations). *Rhizobium* seems to be rather clonal with some recombination among geographically related strains.[44,193,199] Real recombination rates should be determined,[34] and perhaps more realistic *Rhizobium* phylogenies would have to consider bars linking different lineages, as has been proposed for other bacteria[9] (illustrated in Figure 1C).

F. *Rhizobium* Plasmids

Up to this point, we have mainly reviewed the genetic diversity that is dictated by the chromosome, and phylogenies that reflect chromosomal characteristics have been considered. In *Rhizobium*, extra chromosomal elements are an important proportion of the genome.[138] Plasmids ranging from 150 to 1500 kb have been reported.[136] There seems to be more genetic diversity in plasmids than in chromosomes. There are data that indicate that the same type of chromosome may harbor different types of plasmids.[118] For closely related bacteria, the difference between symbiotic or pathogenic behavior may be determined by a different plasmid content. Perhaps the phylogenetic relationships of the different plasmids should also be considered, and most probably they would be independent of those from the chromosome. In *Agrobacterium*, the molecular systematics of the tumorigenic Ti plasmids seems to be more useful than other approaches to understand the phytopathological behavior of strains.[164]

In *Rhizobium* spp., with the exception of *R. loti*, the genetic information for nodulation and nitrogen fixation is on the plasmids, and these may be transferred from one *Rhizobium* bacterium to another, and even to other genera of bacteria both in the laboratory and in the field.[138] *Agrobacterium* with sym(biotic) plasmids may nodulate and even fix nitrogen in the corresponding host legumes.[134] The acquisition of *R. trifolii* sym

plasmids enables a new type of bacteria from a branch of the *Proteobacteria*, not related to any of the reference *Rhizobium* strains, to nodulate clover.[58,99]

Bacteria may lose the sym plasmids concomitantly with the ability to nodulate and fix nitrogen. Nonsymbiotic bacteria that are otherwise identical to *R. etli* have been isolated from the *P. vulgaris* bean rhizosphere.[179] Nonsymbiotic *R. leguminosarum* also have been isolated from soil.[116] Plasmids seem to be changing faster that the whole genome, except perhaps for the very large plasmids (megaplasmids, >1000 kb).[69,84] To address whether chromosomal phylogenies are in agreement with the phylogenies from the different symbiotic genes, different authors have compared trees derived from DNA sequences of *nod* (nodulation) and *nif* (nitrogen fixation) genes to 16S rRNA-based phylogenies.[48,125,205,223] The *nif* gene phylogeny agrees with the chromosomal lineages because, despite some exceptions, the different trees are coherent.[85,223,224] The *nifH* gene (partial sequence) from *Rhizobium* spp. OR191 (related to *R. etli*) seems to be substantially different from those of other *Rhizobium* spp.,[55] perhaps as a result of recombination.

Phylogenies based on *nod* genes show that *Azorhizobium caulinodans nod* genes are the most distantly related to all others,[125] and *A. caulinodans* is the farthest branch from *Rhizobium*.[49] It has been proposed that *Azorhizobium nod* genes have been acquired in *Azorhizobium* from some external origin because the nucleotide composition content (GC content) of the *nod* genes do not correspond to the general GC content from the bacteria.[68] The *nod* genes from *R. etli* are anomalous inasmuch as they have different phylogenetic positions, depending on the gene or gene fragment considered,[125] perhaps as a result of some recombination event. The *nod* phylogenies are based on analyses of a small number of strains, and in several cases, the positions of the branches are not definitive.[48,125,205] Some of the conclusions derived from these reports are that phylogenies of *nod* genes are related to the taxonomy of the host legumes instead of being related to the bacterial chromosome,[48,205] but in other cases, as that of *R. galegae*, *nod* genes and chromosome seem to agree.[125] Other types of analysis show that *nod* genes involved in host specificity are found scattered in the different *Rhizobium* lineages bearing no correlation to chromosomal phylogenies.[133] This is further supported by the *S. saheli* or *S. teranga* strains, which, with the same genotype, may have alternative sets of host specificity nodulation genes, determining either *Sesbania* or *Acacia* nodulation.[15]

Lateral transfer of *nod* genes is probable among *Rhizobium* lineages.[105,125] In many instances, people have relied on host range to identify or group *Rhizobium* bacteria. The weakness of this type of approach is now beginning to be understood, as *nod* genes correspond undoubtedly to a hyper-variable part of the genome. Furthermore, host range may change depending on the plant growth conditions to assay nodulation.[169] Being broad-host seems to be more prevalent among *Rhizobium* and *Bradyrhizobium* than was previously thought. *R. meliloti* may nodulate *Neptunia oleracea*,[40] *R. fredii* has a wide host-range,[169] and *R. etli* nodulates many more legumes in addition to bean.[87] A majority of *Bradyrhizobium* from Amazonia[145] are promiscuous and have no clear specificity for legumes.

G. Conclusions and Perspectives

As more *Rhizobium* and *Bradyrhizobium* isolates are analyzed, more complications seem to appear.[162] However, the new approaches open the possibility of understanding bacterial evolutionary mechanisms. In order to evaluate the influence of the host

plant on the bacterial populations, we need to have confident legume phylogenies, which may be derived from molecular approaches. The analysis of DNA sequences from one chloroplast gene showed that representative plants of all ten families with nitrogen-fixing symbiosis were clustered together, indicating that the capacity to form nitrogen-fixing nodules evolved in only one lineage of closely related taxa. Taxonomy derived from morphologically based characters, on the other hand, suggested that the nitrogen-fixing families were only distantly related.[191] We should wait to see if the one-gene phylogeny remains valid after more plant genetic information is analyzed. In regard to the *Rhizobium–Bradyrhizobium–*legume interaction, it would be interesting to analyze the internal genetic diversity of *Acacia* and of other legumes to help explain the large genetic diversity of their symbionts. It would be useful to know if plants belonging to the same cross-inoculation group (with affinities to related symbionts) are genetically similar even though classical taxonomy would classify them separately.

Defining solid *Rhizobium* and *Bradyrhizobium* phylogenies is the basis for establishing a biologically meaningful taxonomy. The advances in *Rhizobium* systematics are remarkable, and the need to consider a genetic approach when analyzing bacterial population should be emphasized. Characterizations based only on the phenotype have proved to be misleading in many cases, not only in *Rhizobium*, but also in other bacteria.[5] At present, there are clear definitions to distinguish bacterial species and genera. Distinct species should have <70% DNA-DNA homology[197] and distinct genera <95% conservation in the ribosomal gene sequences,[75] among other criteria. However, the usefulness of the bacterial species and genera concepts and the taxonomies derived from them remain to be evaluated in the future.

II. BACTERIAL GENETIC DIVERSITY

It is estimated that bacteria have a very long history on Earth, over 3 billion years. The unimaginable number of generations over this period and the selection of lineages to fit an enormous diversity of habitats warrant and explain their large genetic diversity, which constitutes, in the case of non-pathogenic bacteria, an invaluable biological reserve for humanity. *Rhizobium* genetic diversity, just reviewed, would represent only a small part of the overall natural bacterial diversity. Recently, the scope of natural bacterial variability has been enlarged even more due to the recognition of the existence of a large proportion of organisms that are unculturable under present laboratory conditions.[59,204] The analysis of these populations has become possible mainly because of the development of new molecular biology techniques.

Recently, there have been many studies on genetic variation in natural bacterial populations taking advantage of the standard approaches developed to analyze population genetics of plants and animals. We present here a compilation of records of bacterial diversity together with a general interpretation of these values and speculation on the possible effects of human activity on bacterial diversity.

A. Genetic Diversity

The most widely used method to measure genotypic diversity and genetic structure in a great variety of bacterial species has been multilocus enzyme electrophoresis (MLEE). The data obtained with this approach in many different species are summarized in Table 2. Historic and conceptual aspects,[221] laboratory procedures, and data analysis of MLEE have been reviewed before.[183]

It is important to mention that estimates of genetic relatedness of bacterial

TABLE 2
Genetic Diversity in Bacterial Species Based on Multilocus Enzyme Electrophoresis

Species	No. of ETs[a]	Mean genetic diversity	Genetic structure	Ref.
Acetobacter diazotrophicus				
Recovered from sugarcane	4	0.064	Clonal	22
Recovered from different hosts	6	0.056	Clonal	21
Actinobacillus actinomycetemcomitans	11	0.369	Clonal	28
Bacillus spp.	27	0.556	N.A.	23
B. cereus	18	0.529		
B. thuringiensis	10	0.550		
Bacillus subtilis	55	0.440	No clonal[b]	94
Bordetella spp.	14	0.284	Clonal	148
B. bronchiseptica	10	0.277		
B. parapertussis	1	0.000		
B. pertussis	3	0.133		
Bordetella bronchiseptica	21	0.248	Clonal	146
Bradyrhizobium sp.	17	0.690	No clonal[b]	16
Burkholderia (Pseudomonas) cepacia	79	0.574	No clonal[b]	215
Escherichia coli				
From human and animal sources	98	0.471	Clonal	186
From feces and urine	302	0.520	Clonal	212
Neonatal septicemia and meningitis	39	0.369	Clonal	185
From a human host	53	0.390	Clonal	27
Haemophilus aphrophilus	5	0.400	Clonal	28
H. influenzae				
Serotype b	32	0.342	Clonal	147
Diverse serotypes	280	0.467	Clonal	149
Haemophilus pleuropneumoniae	32	0.428	Clonal	150
Klebsiella oxytoca	18	0.486	Clonal	93
Recovered from fruit flies				
Klebsiella spp. (clinical sources)	57	0.605	Clonal	35
K. pneumoniae	34	0.559		
K. oxytoca	23	0.673		
Legionella pneumophila	62	0.413	Clonal	187
L. pneumophila (sensu stricto)	50	0.312		
"Species 1"	9	0.182		
"Species 2"	3	0.061		
Listeria monocytogenes				
245 strains from different sources	33	0.303	Clonal	154
181 strains from different sources	50	0.415	Clonal	14
Neisseria gonorrhoeae	89	0.410	No clonal[b]	161
N. meningitidis				
460 strains	192	0.536	Clonal	26
152 strains	55	0.615	N.A.[c]	25
Porphyromonas gingivalis	78	0.384	Clonal	128
P. gingivalis (sensu stricto)	71	0.321		
"Division II"	4	0.275		
"Divison III"	3	0.356		
Pseudomonas cepacia	—	0.54-0.70	N.A.	143
P. syringae	10	0.683	N.A.	47
P.s. pv. syringae	6	0.479		

TABLE 2 (CONTINUED)
Genetic Diversity in Bacterial Species Based on Multilocus Enzyme Electrophoresis

Species	No. of ETs[a]	Mean genetic diversity	Genetic structure	Ref.
P.s. pv. tomato	4	0.179		
Rhizobium etli	—	0.487	No clonal[b]	192
R. leguminosarum				
Strains of three biovars	15	0.500	Clonal	220
R. leguminosarum bv. phaseoli				
Symbiotic strains	38	0.615	Clonal	167
Nonsymbiotic strains	38	0.504	Clonal	179
R. leguminosarum bv. trifolii	11	0.500	Clonal	82
R. leguminosarum bv. trifolii	16	0.580	N.A.	45
R. leguminosarum bv. trifolii			Clonal	120
From one location	53	0.460	No clonal[b]	
From culture collections	29	0.570		
R. leguminosarum			No clonal[b]	198
bv. trifolii	54	0.470		
bv. viciae	42	0.510		
R. leguminosarum bv. viciae				
Fava bean-nodulating strains	24	0.660	Clonal	208
R. leguminosarum bv. viciae				
Pea-nodulating strains	15	0.592	Clonal	225
R. meliloti			Clonal	53
"Division A"	35	0.254		
"Division B"	15	0.249		
R. tropici			N.A.	139
Type IIA strains	14	0.289		
Type IIB strains	18	0.363		
Rhizobium sp.				
Bean-nodulating strains	75	0.669	N.A.	54
Salmonella paratyphi B	14	0.167	Clonal	181
Salmonella spp.				
From different species and hosts	71	0.255	Clonal	11
Salmonella spp.			Clonal	
7 serotypes (human adapted)	54	0.093		
9 serotypes (nonhost adapted)	83	0.176		
Serratia marcescens	33	0.376	Clonal	67
Nonchromogenic strains	23	0.294		
Chromogenic strains	10	0.293		
Shigella spp.				
From different species	23	0.290	Clonal	212
Streptococcus spp.	40	0.857	N.A.[c]	76
Mutans complex	18	0.849		
Sanguis complex	22	0.726		
Yersinia ruckeri	4	0.014	Clonal	175

Note: N.A. = Not analyzed.

[a] ETs = Electrophoretic types. Distinctive profiles of electromorphs or allozymes.
[b] Variable levels of linkage disequilibrium.

strains[21,76,120,158,179,187,218] obtained by MLEE are strongly correlated with estimates of divergence obtained from DNA-DNA hybridization experiments and with the phylogenetic trees derived from nucleotide sequence analysis.[144,153] However, by using MLEE, the genetic diversity may be underestimated compared with DNA sequence analysis.[17,153]

The levels of genetic diversity differ greatly among species (Table 2). The studied bacterial species include human,[26,35,147,182] animal,[11,148,150,175] and plant pathogens bacteria,[47] as well as soil bacterial species,[23,94,143] including *Rhizobium* spp.,[53,54,120,139,167,192,208] *Bradyrhizobium* spp.,[16] and endophytic nitrogen-fixing bacteria.[21,22]

In addition to MLEE, the method of restriction fragment length polymorphisms (RFLPs) is being used with increasing frequency for population genetic studies on bacterial species.[152]

Considering the results derived from any of these approaches, it is observed that the bacterial populations with the lowest levels of genetic variability are encountered among the intracellular bacterial species of the rickettsiae group,[26] *Mycobacterium leprae* and other mycobacteria species.[33,79] In contrast, the highest levels of genetic diversity frequently are recorded among the soil bacterial species.[16,23,45,143,167,179,208] Many other well-known bacteria are between these extremes; however, most of the 5000 species of bacteria validly described[6] have not been analyzed.

Bacterial genetic diversity has mainly been related to effective population size,[148,150,182,187] length of time since the evolutionary origin of the species,[21,33,146,148] along with ecological factors,[1,65,187] niche specialization,[21,47,119,148,177] and horizontal gene transfer.[16,54,94,192,193]

It seems that variability of a bacterial group is dependent upon the habitat(s) from which individuals were recovered; groups with little genetic variability probably occupy very specific habitats.[89] This seems to be true for *Xanthomonas campestris* pathovars,[119] and for different *Pseudomonas syringae* pathovars,[47,177] wherein a high level of genetic homogeneity correlated with habitat specialization is observed. The same idea seems to explain, alternatively to the other suggested hypothesis,[33,64] the limited genetic diversity of the intracellular pathogenic bacteria of the genera *Rickettsia* and *Mycobacterium*, which inhabit homeostatic organisms and live under constant environmental conditions. Conversely, large genetic diversity could be expected in bacterial populations that occupy several niches,[89] or that live in more variable environments subject to wide fluctuations of factors (e.g., pH, temperature), which act as selective forces on the genes controlling enzyme systems.[143]

Bacterial species of the genera *Bacillus*[23] and *Azospirillum*[57,168] show large genetic diversity. They are inhabitants from a highly variable environment, the soil, where fluctuations are recorded in nutrient concentration, quality and availability of nutrients (particularly in the rhizosphere zone), as well as changes in moisture content, temperature, pH, among other factors. In *Burkholderia cepacia*, formerly *Pseudomonas cepacia*, the largest genetic diversity was correlated with the highest soil environment variability across a landscape gradient.[143] Similarly, the amount of genetic variation in populations of *R. leguminosarum* bv. trifolli was dependent on the pH of the soil.[82] It was also observed that the diversity of strains of *Bacillus polymyxa*, a common soil bacterium, is in relation to their isolation origin. Diversity within a population of these bacteria isolated from rhizosphere and nonrhizospheric soil is higher than that of *B. polymyxa* isolated from the rhizoplane. The latter is a genetically homogeneous population.[140]

Genetic diversity levels estimated (by MLEE method) in *Bradyrhizobium* and *Rhizobium* spp. are generally near or higher than 0.500 (see Table 2). As mentioned pre-

viously, *Rhizobium* and *Bradyrizobium* are very ancient lineages of bacteria. This fact could explain their large genetic biodiversity. However, is not possible to exclude that host specialization, as well as selective faetors from soil, perhaps connected with genetic recombination as described beforc, could influence the biodiversity of these bacterial species.

Rhizobium and *Bradyrhizobium* are genetically related to other bacterial genera (reviewed in the Section I) such as the intracellular pathogen *Brucella abortus*. It would be interesting to have a quantitative estimate (by MLEE) of *B. abortus* genetic diversity to elucidate if it bchaves as other intracellular pathogens (with low genetic diversity) or as the highly heterogeneous *Rhizobium* spp. By other criteria, there are some indications that there is a limited genetic diversity in *Brucella* spp.[3,78] The information about the genetic diversity levels of these closely related bacterial species will help explain the influence of environmental conditions and their fluctuations.

B. Genetic Structure of Bacterial Populations

Out of the studies analyzing genetic diversity presented in Table 2, the genetic structure of the population may be inferred. "Genetic structure refers to the genetic composition of a population, including the frequencies of alleles at individual loci and the frcquencies of genotypes."[184] On this basis it has been observed that a majority of the bacterial populations are essentially clonal,[65,109] as shown in Table 2. From thesc results, the paradigm of the bacterial clonality has arisen. However, studies in the last 3 years have demostrated that not all bacterial populations are strictly clonal.[16,94,120,161,192,215]

Clonal structure has been deduced by the prcsence of an overrepresented particular genotype, especially when the same genotype was recovered from different localities, or in samples taken years apart.[203] A clonal structure has been characterized by the existence of strong linkage disequilibrium[20,27] or nonrandom association between the alleles of different loci,[83] implying that chromosomal recombination is a very infrequent event in natural populations.[213] The fact that bacterial reproduction is asexual necessarily leads to a clonal structure, unless there exists a high rate of genetic recombination.[94] Such recombination can be achieved through the well-known processes of conjugation, transduction, and transformation.[129] Localized recombination in bacteria has been suggested based on a comparison of nucleotide sequences.[52,141,144] In some cases, genetic transfer was not sufficient to break the clonal structure[121,184,213] because the exchange often involved only a few hundred base pairs.[141]

Genetic diversity levels from MLEE data indicate that naturally transformable bacteria, such as the Gram-positive *Streptococcus-Bacillus* and the Gram-negative *Haemophilus-Neisseria* exhibit higher genetic variation than other analyzed species.[184] However, it has been indicated[142] that transformability per se does not necessarily imply extensive recombination because *Haemophilus influenzae* is transformable as well, but shows little evidence of frequent recombination in nature.

Recently, it was observed that linkage disequilibrium may be minimal in some local populations of bacterial species, including *Bacillus subtilis*,[94] *Rhizobium etli* bv. phaseoli,[192,193] *R. leguminosarum* bv. trifolii,[120] *Bradyrhizobium* sp.[16] and *Burkholderia cepacia*,[215] as well as in global populations of *Neisseria gonorrhoeae*.[161] These results would imply extensive genetic recombination in the analyzed species, under some conditions. The population structures in bacterial species are likely to range from strictly elonal to panmictic,[94,142] challenging the paradigm of bacterial clonality.

C. Final Remarks

It is not possible to explain the genetic diversity of a particular bacterial species using a single hypothesis. It is more probable that such diversity could reflect the influence of alternating conditions that a particular population has undergone through evolutionary times.

Perhaps, human activity through the modern practices in the industry (with the input of pollutants in the environment), in medicine (by the widespread use of antibiotics and vaccines), and in agriculture (by the pesticides and fertilizers used) may be influencing the genetic diversity levels of bacterial populations. For instance, the restricted levels of genetic diversity recorded in the species *Bordetella parapertussis* and *B. pertussis* (see Table 2) could suggest that the clones of these species have evolved rather recently. However, there is the possibility that there has been a significant reduction of the effective population size of clones due to widespread vaccination in recent times.[148] This could also be the case for other pathogenic bacterial populations controlled through vaccination.

We have suggested that isolation frequencies[63] and genetic diversity in *Acetobacter diazotrophicus*, living endophytically in sugarcane, may be diminished by the high rates of nitrogen fertilization used in the crops.[21] In this case, it may not be a direct effect of the nitrogen on the bacteria, but through the plant. It has been reported that nitrogen induces many drastic changes in plant physiology,[60] consequently inducing changes in the natural populations of endophytic bacteria. We should ask if the high rates of nitrogen fertilizer frequently applied to field crops in modern agricultural practices could be influencing or even extinguishing the genetic diversity of many other presently unknown endophytic bacteria. There are indications that endophytic bacteria are more prevalent in nature than we estimate. For instance, we recently isolated *A. diazotrophicus* and some other diazotrophs from the internal tissues of rarely fertilized *Coffea arabica* L.,[100] unknown at present as a host plant for this bacterium. It could be that the ancestor plants from which modern cultivars developed had a great diversity of endophytic populations. It is imperative to preserve this diversity (both from the plant and from the microbes) and to direct research projects to analyze it.

ACKNOWLEDGMENTS

We acknowledge Rafael Palacios for discussing the work and Julio Martínez Romero for technical support. We are grateful to Linda Werner and Michael Dunn for reviewing the paper. Partial financial support came from a VLIR-ABOS grant from Belgium.

REFERENCES

1. **Adhikari, T. B., Vera Cruz, C. M., Zhang, Q., Nelson, R. J., Skinner, D. Z., Mew, T. W., and Leach, J. E.,** Genetic diversity of *Xanthomonas oryzae* pv. oryzae in Asia, *Appl. Environ. Microbiol.*, 61, 966, 1995.
2. **Aguilar, M.,** personal communication.
3. **Allardet-Servent, A., Bourg, G., Ramuz, M., Pages, M., Bellis, M., and Roizes, G.** DNA polymorphism in strains of the genus *Brucella*, *J. Bacteriol.*, 170, 4603, 1988.
4. **Allen, O. N. and Allen, E. K.,** *The Leguminosae: A Source Book of Characteristics, Uses, and Nodulation*, The University Wisconsin Press, Madison, WI, 1981.
5. **Amann, R. I., Lin, C., Key, B., Montgomery, L., and Stalh, D. A.,** Diversity among *Fibrobacter* isolates: towards a phylogenetic classification, *Syst. Appl. Microbiol.*, 15, 23, 1992.
6. **Am**
K.-I
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7. **Am**
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14. **Boerli**
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6. **Amann, R., Ludwig, W., and Schleifer, K.-H.,** Identification of uncultured bacteria: a challenging task for molecular taxonomists, *ASM News*, 60, 360, 1994.

7. **Amarger, N., Bours, M., Revoy, F., Allard, M. R., and Laguerre, G.,** *Rhizobium tropici* nodulates field-grown *Phaseolus vulgaris* in France, *Plant Soil*, 161, 147, 1994.

8. **Anderson, P. and Roth, J. R.,** Spontaneous tandem genetic duplications in *Salmonella typhimurium* arise by unequal recombination between rRNA (rrn) cistrons, *Proc. Natl. Acad. Sci. U.S.A.*, 78, 3113, 1981.

9. **Arber, W.,** Elements in microbial evolution, *J. Mol. Evol.*, 33, 4, 1991.

10. **Barnet, Y., Catt, P. C., Jenjarcontham, R., and Mann, K.,** Fast-growing root-nodule bacteria from Australian *Acacia* spp., in *New Horizons in Nitrogen Fixation*, Palacios, R. et al., Eds., Kluwer Academic Publishers, Dordrecht, The Netherlands, 1993, 594.

11. **Beltran, P., Musser, J. M., Helmuth, R., Farmer, J. J., III, Frerichs, W. M., Wachsmuth, I. K., Ferris, K., McWhorter, A. C., Wells, J. G., Cravioto, A., and Selander, R. K.,** Toward a population genetic analysis of *Salmonella*: genetic diversity and relationships among strains of serotypes *S. choleraesuis*, *S. derby*, *S. dublin*, *S. enteritidis*, *S. heidelberg*, *S. infantis*, *S. newport*, and *S. typhimurium*, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 7753, 1988.

12. **Beringer, J. E., Johnston, A. W. B., and Kondorosi, A.,** Genetic maps of *Rhizobium meliloti* and *R. leguminosarum* biovars *phaseoli*, *trifolii* and *viciae*, in *Genetic Maps*, Vol. 4, O'Brien, S. J., Ed., Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1987.

13. **Bisercic, M., Feutrier, J. Y., and Reeves, P. R.,** Nucleotide sequences of the *gnd* genes from nine natural isolates of *Escherichia coli*: evidence of intragenic recombination as a contributing factor in the evolution of the polymorphic *gnd* locus, *J. Bacteriol.*, 173, 3894, 1991.

14. **Boerlin, P. and Piffaretti, J. C.,** Typing of human, animal, food, and environmental isolates of *Listeria monocytogenes* by multilocus enzyme electrophoresis, *Appl. Environ. Microbiol.*, 57, 1624, 1991.

15. **Boivin, C.,** personal communication.

16. **Bottomley, P. J., Cheng, H., and Strain, S. R.,** Genetic structure and symbiotic characteristics of a *Bradyrhizobium* population recovered from a pasture soil, *Appl. Environ. Microbiol.*, 60, 1754, 1994.

17. **Boyd, E. F., Nelson, K., Wang, F., Whittam, T. S., and Selander, R. K.,** Molecular genetic basis of allelic polymorphism in malate dehydrogenase (*mdh*) in natural populations of *Escherichia coli* and *Salmonella enterica*, *Proc. Natl. Acad. Sci. U.S.A.*, 91, 1280, 1994.

18. **Brewin, N. J.,** Development of the legume root nodule, *Annu. Rev. Cell Biol.*, 7, 191, 1991.

19. **Bromfield, E. S. P., Barran, L. R., and Wheatcroft, R.,** Relative genetic structure of a population of *Rhizobium meliloti* isolated directly from soil and from nodules of alfalfa (*Medicago sativa*) and sweet clover (*Melilotus alba*), *Mol. Ecol.*, 4, 183, 1995.

20. **Brown, A. H. D. and Feldman, M. W.,** Population structure of multilocus associations, *Proc. Natl. Acad. Sci. U.S.A.*, 78, 5913, 1981.

21. **Caballero-Mellado, J., Fuentes-Ramírez, L. E., Reis, V. M., and Martínez-Romero, E.,** Genetic structure of *Acetobacter diazotrophicus* populations and identification of a new genetically distant group, *Appl. Environ. Microbiol.*, 61, 3008, 1995.

22. **Caballero-Mellado, J. and Martínez-Romero, E.,** Limited genetic diversity in the endophytic sugarcane bacterium *Acetobacter diazotrophicus*, *Appl. Environ. Microbiol.*, 60, 1532, 1994.

23. **Carlson, C. R., Caugant, D. A., and Kolsto, A.-B.,** Genotypic diversity among *Bacillus cereus* and *Bacillus thuringiensis* strains, *Appl. Environ. Microbiol.*, 60, 1719, 1994.

24. **Carlson, R. W., Price, N. P. J., and Stacey, G.,** The biosynthesis of rhizobial lipo-oligosaccharide nodulation signal molecules, *Mol. Plant–Microbe Interact.*, 7, 684, 1994.

25. **Caugant, D. A., Bovre, K., Gaustad, P., Bryn, K., Holten, E., Hoiby, E. A., and Froholm, L. O.,** Multilocus genotypes determined by enzyme electrophoresis of *Neisseria meningitidis* isolated from patients with systemic disease and from healthy carriers, *J. Gen. Microbiol.*, 132, 641, 1986.

26. **Caugant, D. A., Froholm, L. O., Bovre, K., Holten, E., Frasch, C. E., Mocca, L. F., Zollinger, W. D., and Selander, R. K.,** Intercontinental spread of a genetically distinctive complex of clones of *Neisseria meningitidis* causing epidemic disease, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 4927, 1986.

27. **Caugant, D. A., Levin, B. R., and Selander, R. K.,** Genetic diversity and temporal variation in the *E. coli* population of a human host, *Genetics*, 98, 467, 1981.

28. **Caugant, D. A., Selander, R. K., and Olsen, I.,** Differentiation between *Actinobacillus (Haemophilus) actinomycetemcomitans, Haemophilus aprophilus* and *Haemophilus paraphrophilus* by multilocus enzyme electrophoresis, *J. Gen. Microbiol.*, 136, 2135, 1990.

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

30. **Chen, W. X., Wang, E. T., Li, Y. B., Li, Y., and Gao, J. L.,** *Rhizobium hainanensis*. A new species of root nodule bacteria isolated from tropical region, in *Proc. Int. Symp. Diversity and Taxonomy of Rhizobia*, Wuban, Hubei, China, 1994, 19.

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

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

33. **Clark-Curtiss, J. E. and Walsh, G. P.,** Conservation of genomic sequences among isolates of *Mycobacterium leprae*, *J. Bacteriol.*, 171, 4844, 1989.

34. **Cohan, F. M.,** Genetic exchange and evolutionary divergence in prokaryotes, *Trends Ecol. Evol.*, 9, 175, 1994.

35. **Combe, M.-L., Pons, J.-L., Sesboue, R., and Martin, J.-P.,** Electrophoretic transfer from polyacrylamide gel to nitrocellulose sheets, a new method to characterize multilocus enzyme genotypes of *Klebsiella* strains, *Appl. Environ. Microbiol.*, 60, 26, 1994.

36. **Cooper, J. E., Bjourson, A. J., and Thompson, J. K.,** Identification of *Lotus* rhizobia by direct DNA hybridization of crushed root nodules, *Appl. Environ. Microbiol.*, 53, 1705, 1987.

37. **Crow, V. L., Jarvis, B. D. W., and Greenwood, R. M.,** Deoxyribonucleic acid homologies among acid-producing strains of *Rhizobium*, *Int. J. Syst. Bacteriol.*, 31, 152, 1981.

38. **Dagutat, H. and Steyn, P. L.,** Taxonomy and distribution of rhizobia indigenous to South African soils, in *Nitrogen Fixation: Fundamentals and Applications*, Tikhonovich, I. A. et al., Eds., Kluwer, The Netherlands, 1995, 683.

39. **Dakora, F. D.** Plant flavonoids: biological molecules for useful exploitation, *Aust. J. Plant Physiol.*, 22, 87, 1995.

40. **Debellé, F., Rosenberg, C., Vasse, J., Maillet, F., Martínez, E., Denarié, J., and Truchet, G.,** Assignment of symbiotic development phenotypes to common and specific nodulation (*nod*) genetic loci of *Rhizobium meliloti*, *J. Bacteriol.*, 168, 1075, 1986.

41. **De Bruijn, F. J.,** Use of repetitive (repetitive extragenic palindromic and enterobacterial repetitive intergenerie consensus) sequences and the polymerase chain reaction to fingerprint the genomes of *Rhizobium meliloti* isolates and other soil bacteria, *Appl. Environ. Microbiol.*, 58, 2180, 1992.

42. **De Lajudie, P., Willems, A., Pot, B., Dewettinck, D., Maestrojuan, G., Neyra, M., Collins, M. D., Dreyfus, B., Kersters, K., and Gillis, M.,** 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, 1994.

43. **De Ley, J.**, Phylogeny of prokariotes, *Taxon*, 23, 291, 1974.

44. **Demezas, D. H., Reardon, T. B., Strain, S. R., Watson, J. M., and Gibson, A. H.**, Diversity and genetic structure of a natural population of *Rhizobium leguminosarum* bv. *trifolii* isolated from *Trifolium subterraneum* L., *Mol. Ecol.*, 4, 209, 1995.

45. **Demezas, D. H., Reardon, T. B., Watson, J. M., and Gibson, A. H.**, Genetic diversity among *Rhizobium leguminosarum* bv. trifolii strains revealed by allozyme and restriction fragment length polymorphism analyses, *Appl. Environ. Microbiol.*, 57, 3489, 1991.

46. **Dénarié, J., Debellé, F., and Rosenberg, C.**, Signaling and host range variation in nodulation, *Annu. Rev. Microbiol.*, 46, 497, 1992.

47. **Denny, T. P., Gilmour, M. N., and Selander, R. K.**, Genetic diversity and relationships of two pathovars of *Pseudomonas syringae*, *J. Gen. Microbiol.*, 134, 1949, 1988.

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

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

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

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

52. **Dykhuizen, D. E. and Green, L.**, Recombination in *Escherichia coli* and the definition of a biological species, *J. Bacteriol.*, 173, 7257, 1991.

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

54. **Eardly, B. D., Wang, F.-S, Whittam, T. S., and Selander, R. K.**, Species limits in *Rhizobium* populations that nodulate the common bean (*Phaseolus vulgaris*), *Appl. Environ. Microbiol.*, 61, 507, 1995.

55. **Eardly, B. D., Young, J. P. W., and Selander, R. K.**, Phylogenetic position of *Rhizobium* sp. strain Or 191, a symbiont of both *Medicago sativa* and *Phaseolus vulgaris*, based on partial sequences of the 16S rRNA and *nifH* genes, *Appl. Environ. Microbiol.*, 58, 1809, 1992.

56. **Evguenieva-Hackenberg, E. and Selenska-Pobell, S.**, Variability of the 5'-end of the large subunit rDNA and presence of a new short class of rRNA in *Rhizobiaceae*, *Lett. Appl. Microbiol.*, in press.

57. **Fani, R., Bazzicalupo, M., Gallori, E., Giovannetti, L., Ventura, S., and Polsinelli, M.**, Restriction fragment length polymorphism of *Azospirillum* strains, *FEMS Microbiol. Lett.*, 83, 225, 1991.

58. **Fenton, M. and Jarvis, B. D. W.**, Expression of the symbiotic plasmid from *Rhizobium leguminosarum* biovar *trifolii* in *Sphingobacterium multivorum*, *Can. J. Microbiol.*, 40, 873, 1994.

59. **Ferguson, R. L., Buckley, E. N., and McPerson, S.**, Response of marine bacterioplankton to differential centrifugation and confinement, *Appl. Environ. Microbiol.*, 47, 49, 1984.

60. **Fernandes, M. S. and Rossiello, R. O. P.**, Mineral nitrogen in plant physiology and plant nutrition, *Crit. Rev. Plant Sci.*, 14, 111, 1995.

61. **Fisher, R. F. and Long, S. R.**, *Rhizobium*-plant signal exchange, *Nature*, 357, 655, 1992.

62. **Franssen, H. J., Vijn, I., Yang, W. C., and Bisseling T.**, Developmental aspects of the *Rhizobium*-legume symbiosis, *Plant Mol. Biol.*, 19, 89, 1992.

63. **Fuentes-Ramírez, L. E., Jiménez-Salgado, T., Abarca-Ocampo, I. R., and Caballero-Mellado, J.**, *Acetobacter diazotrophicus*, an indoleacetic acid producing bacterium isolated from sugarcane cultivars of Mexico, *Plant Soil*, 154, 145, 1993.

64. **Fuerst, P. A., Poetter, K. P., Pretzman, C., and Perlman, P. S.**, Molecular genetics of populations of intracellular bacteria: the spotted fever group Rickettsiae, *Ann. N.Y. Acad. Sci.*, 590, 430, 1990.

65. **Gabriel, D. W., Hunter, J. E., Kingsley, M. T., Miller, J. W., and Lazo, G. R.**, Clonal population structure of *Xanthomonas campestris* and genetic diversity among citrus canker strains, *Mol. Plant–Microbe Interact.*, 1, 59, 1988.

66. **Gao, J. L., Sun, J. G., Li, Y., Wang, E. T., and Chen, W. X.**, Numerical taxonomy and DNA relatedness of tropical rhizobia isolated from Hainan province, China, *Int. J. Syst. Bacteriol.*, 44, 151, 1994.

67. **Gargallo-Viola, D.**, Enzyme polymorphism, prodigiosin production, and plasmid fingerprints in clinical and naturally occurring isolates of *Serratia marcescens*, *J. Clin. Microbiol.*, 27, 860, 1989.

68. **Geelen, D., Mergaert, P., Geremia, R. A., Goormachtig, S., Van Montagu, M., and Holsters, M.**, Identification of *nodSUIJ* genes in Nod locus *1* of *Azorhizobium caulinodans*: evidence that *nodS* encodes a methyltransferase involved in Nod factor modification, *Mol. Microbiol.*, 9, 145, 1993.

69. **Geniaux, E., Flores, M., Palacios, R., and Martínez, E.**, Presence of megaplasmids in *Rhizobium tropici* and further evidence of differences between the two *R. tropici* subtypes, *Int. J. Syst. Bacteriol.*, 45, 392, 1995.

70. **Geniaux, E., Laguerre, G., and Amarger, N.**, Comparison of geographically distant populations of *Rhizobium* isolated from root nodules of *Phaseolus vulgaris*, *Mol. Ecol.*, 2, 295, 1993.

71. **George, M. L. C., Young, J. P. W., and Borthakur, D.**, Genetic characterization of *Rhizobium* sp. strain TAL 1145 that nodulates tree legumes, *Can. J. Microbiol.*, 40, 208, 1994.

72. **Gepts, P.**, Biochemical evidence bearing on the domestication of *Phaseolus* (Fabaceae) beans, *Econ. Bot.*, 44, 28, 1990.

73. **Gepts, P. and Bliss, F. A.**, Dissemination pathways of common bean (*Phaseolus vulgaris*) deduced from phaseolin electrophoretic variability. II. Europe and Africa, *Econ. Bot.*, 42, 86, 1988.

74. **Giller, K. E., Anyango, B., Beynon, J. L., and Wilson, K. J.**, The origin and diversity of rhizobia nodulating *Phaseolus vulgaris* L. in African soils, in *Advances in Legume Systematics 5: The Nitrogen Factor*, Sprent, J. I. and McKey, D., Eds., Royal Botanic Gardens, Kew, 1994, 57.

75. **Gillis, M.**, personal communication.

76. **Gilmour, M. N., Whittam, T. S., Kilian, M., and Selander, R. K.**, Genetic relationships among the oral streptococci, *J. Bacteriol.*, 169, 5247, 1987.

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

78. **Grimont, F., Verger, J. M., Cornelis, P., Limet, J., Lefèvre, M., Grayon, M., Régnault, B., Van Broeck, J., and Grimont, P. A. D.**, Molecular typing of *Brucella* with cloned DNA probes, *Res. Microbiol. (Inst. Pasteur)*, 143, 55, 1992.

79. **Grosskinsky, C. M., Jacobs, W. R., Jr., Clark-Curtiss, J. E., and Bloom, B. R.**, Genetic relationships among *Mycobacterium leprae*, *Mycobacterium tuberculosis*, and candidate leprosy vaccine strains determined by DNA hybridization: identification of an *M. leprae*-specific repetitive sequence, *Infect. Immun.*, 57, 1535, 1989.

80. **Haeckel, E.**, *Generelle Morphologie der Organismen*, Verlag Georg Reimer, Berlin, 1866.

81. **Ham, G. E., Cardwell, V. B., and Johnson, H. W.**, Evaluation of *Rhizobium japonicum* inoculants in soils containing naturalized populations of rhizobia, *Agron. J.*, 63, 301, 1971.

82. **Harrison, S. P., Jones, D. G., and Young, J. P. W.**, *Rhizobium* population genetics: genetic variation within and between populations from diverse locations, *J. Gen. Microbiol.*, 135, 1061, 1989.

83. **Hartl, D. L., Medhora, M., Green, L., and Dykbuizen, D. E.**, The evolution of DNA sequences in *Escherichia coli*, *Philos. Trans. R. Soc. London*, B 312, 191, 1986.

84. **Hartmann, A. and Amarger, N.**, Genotypic diversity of an indigenous *Rhizobium meliloti* field population assessed by plasmid profiles, DNA fingerprinting, and insertion sequence typing, *Can. J. Microbiol.*, 37, 600, 1991.

85. **Hennecke, H., Kaluza, K., Thöny, B., Fubrmann, M., Ludwig, W., and Stackebrandt, E.**, Concurrent evolution of nitrogenase genes and 16*S* rRNA in *Rhizobium* species and other nitrogen fixing bacteria, *Arch. Microbiol.* 142, 342, 1985.

86. **Herendeen, P. S. and Dilcher, D. L., Eds.**, *Advances in Legume Systematics 4: The Fossil Record*, Royal Botanic Gardens, Kew, 1992.

87. **Hernández-Lucas, I., Segovia, L., Martínez-Romero, E., and Pueppke, S. G.**, Phylogenetic relationships and host range of *Rhizobium* spp. that nodulate *Phaseolus vulgaris* L., *Appl. Environ. Microbiol.*, 61, 2775, 1995.

88. **Heywood, V. H.**, The Leguminosae. A systematic purview, in *Chemotaxonomy of the Leguminosae*, Harborne, J. B. et al., Eds., Academic Press, New York, 1971, 1.

89. **Hildebrand, D. C., Schroth, M. N., and Huisman, O. C.**, The DNA homology matrix and non-random variation concepts as the basis for the taxonomic treatment of plant pathogenic and other bacteria, *Annu. Rev. Phytopathol.*, 20, 235, 1982.

90. **Hill, C. W., Grafstrom, R. H., Harnish, B. W., and Hillman, B. S.**, Tandem duplications resulting from recombination between ribosomal RNA genes in *Escherichia coli*, *J. Mol. Biol.*, 116, 407, 1977.

91. **Hirsch, A. M.**, Developmental biology of legume nodulation, *New Phytol.*, 122, 211, 1992.

92. **Honeycutt, R. J., McClelland, M., and Sobral, B. W. S.**, Physical map of the genome of *Rhizobium meliloti* 1021, *J. Bacteriol.*, 175, 6945, 1993.

93. **Howard, D. J., Bush, G. L., and Breznak, J. A.**, The evolutionary significance of bacteria associated with *Rhagoletis*, *Evolution*, 39, 405, 1985.

94. **Istock, C. A., Duncan, K. E., Ferguson, N., and Zhou, X.**, Sexuality in a natural population of bacteria–*Bacillus subtilis* challenges the clonal paradigm, *Mol. Ecol.*, 1, 95, 1992.

95. **Jarvis, B. D. W.**, Genetic diversity of *Rhizobium* strains which nodulate *Leucaena leucocephala*, *Curr. Microbiol.*, 8, 153, 1983.

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

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

98. **Jarvis, B. D. W. and Tighe, S. W.**, Rapid identification of *Rhizobium* species based on cellular fatty acid analysis, *Plant Soil*, 161, 31, 1994.

99. **Jarvis, B. D. W., Ward, L. J. H., and Slade, E. A.**, Expression by soil bacteria of nodulation genes from *Rhizobium leguminosarum* biovar *trifolii*, *Appl. Environ. Microbiol.*, 55, 1426, 1989.

100. **Jiménez-Salgado, T., Fuentes-Ramírez, L. E., Tapia-Hernández, A., Mascarúa-Esparza, M. A., Martínez-Romero, E., and Caballero-Mellado, J.**, unpublished results.

101. **Johnston, A. W. B. and Beringer, J. E.**, Chromosomal recombination between *Rhizobium* species, *Nature*, 267, 611, 1977.

102. **Johnston, A. W. B., Setchell, S. M., and Beringer, J. E.**, Interspecific crosses between *Rhizobium leguminosarum* and *R. meliloti*:

formation of haploid recombinants and of R-primes, *J. Gen. Microbiol.*, 104, 209, 1978.

103. **Jordan, D. C.**, *Rhizobiaceae* Conn 1938, 321[AL], in *Bergey's Manual of Systematics Bacteriology, Vol. 1*, Krieg, N. R. and Holt, J. G., Eds., William & Wilkins, Baltimore, 1984, 234.

104. **Jordan, D. C.**, Genus II. *Bradyrhizobium* Jordan 1982, 137[AL], in *Bergey's Manual of Systematics Bacteriology, Vol. 1*, Krieg, N. R. and Holt, J. G., Eds., William & Wilkins, Baltimore, 1984, 242.

105. **Kaijalainen, S. and Lindström, K.**, Restriction fragment length polymorphism analysis of *Rhizobium galegae* strains, *J. Bacteriol.*, 171, 5561, 1989.

106. **Kay, H. E., Coutinho, H. L. C., Fattori, M., Manfio, G. P., Goodacre, R., Nuti, M. P., Basaglia, M., and Beringer, J. E.**, The identification of *Bradyrhizobium japonicum* strains isolated from Italian soils, *Microbiol*ogy, 140, 2333, 1994.

107. **Keyser, H. H., Bohlool, B. B., Hu, T. S., and Weber, D. F.**, Fast-growing rhizobia isolated from root nodules of soybean, *Science*, 215, 1631, 1982.

108. **Kondorosi, A., Vincze, E., Johnston, A. W. B., and Beringer, J. E.**, A comparison of three *Rhizobium* linkage maps, *Mol. Gen. Genet.*, 178, 403, 1980.

109. **Kubicek, Q. B., Civerolo, E. L., Bonde, M. R., Hartung, J. S., and Peterson, G. L.**, Isozyme analysis of *Xanthomonas campestris* pv. *citri*, *Phytopathology*, 79, 297, 1989.

110. **Kumada, Y., Benson, D. R., Hillemann, D., Hosted, T. J., Rochefort, D. A., Thompson, C. J., Wohlleben, W., and Tateno, Y.**, Evolution of the glutamine synthetase gene, one of the oldest existing and functioning genes, *Proc. Natl. Acad. Sci. U.S.A.*, 90, 3009, 1993.

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

112. **Kuykendall, L. D., Roy, M. A., O'Neill, J. J., and Devine, T. E.** Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*, *Int. J. Syst. Bacteriol.*, 38, 358, 1988.

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

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

115. **Laguerre, G., Allard, M., Revoy, F., and Amarger, N.**, Rapid identification of rhizobia by restriction fragment length polymorphism analysis of PCR-amplified 16S rRNA genes, *Appl. Environ. Microbiol.*, 60, 56, 1994.

116. **Laguerre, G., Bardin, M., and Amarger, N.**, Isolation from soil of symbiotic and nonsymbiotic *Rhizobium leguminosarum* by DNA hybridization, *Can. J. Microbiol.*, 39, 1142, 1993.

117. **Laguerre, G., Fernandez, M. P., Edel, V., Normand, P., and Amarger, N.**, Genomic heterogeneity among French *Rhizobium* strains isolated from *Phaseolus vulgaris* L., *Int. J. Syst. Bacteriol.*, 43, 761, 1993.

118. **Laguerre, G., Geniaux, E., Mazurier, S. I., Rodríguez Casartelli, R., and Amarger, N.**, 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, 1993.

119. **Lazo, G. R., Roffey, R., and Gabriel, D. W.**, Pathovars of *Xanthomonas campestris* are distinguishable by restriction fragment-length polymorphism, *Int. J. Syst. Bacteriol.*, 37, 214, 1987.

120. **Leung, K., Strain, S. R., De Bruijn, F. J., and Bottomley, P. J.**, Genotypic and phenotypic comparisons of chromosomal types within an indigenous soil population of *Rhizobium leguminosarum* bv. trifolii, *Appl. Environ. Microbiol.*, 60, 416, 1994.

121. **Levin, B. R.**, Periodic selection, infectious gene exchange and the genetic structure of *E. coli* populations, *Genetics*, 99, 1, 1981

122. **Lie, T. A.**, Symbiotic specialisation in pea plants: the requirement of specific *Rhizobium* strains for peas from Afghanistan, *Ann. Appl. Biol.*, 88, 462, 1978.

123. **Lie, T. A., Göktan, D., Engin, M., Pijnenborg, J., and Anlarsal, E.**, Co-evolution of the legume-*Rhizobium* association, *Plant Soil*, 100, 171, 1987.

124. **Limeschenko, E., Vorobyov, N., and Novikova, N.**, Diversity of bacteria isolated from root nodules of *Onobrychis* spp. plants, in *Nitrogen Fixation: Fundamentals and Applications*, Tikhonovich, I. A. et al., Eds., Kluwer, The Netherlands, 1995, 707.

125. **Lindström, K., Paulin, L., Roos, C., and Suominen, L.**, Nodulation genes of *Rhizobium galegae*, in *Nitrogen Fixation: Fundamentals and Applications*, Tikhonovich, I. A. et al., Eds., Kluwer, The Netherlands, 1995.

126. **Lindström, K., van Berkum, P., Gillis, M., Martínez, E., Novikova, N., and Jarvis, B.**, Report from the roundtable on *Rhizobium* taxonomy, in *Nitrogen Fixation: Fundamentals and Applications*, Tikhonovich, I. A. et al. Eds., Kluwer, The Netherlands, 1995, 807.

127. **Long, S. R.**, *Rhizobium*-legume nodulation: life together in the underground, *Cell*, 56, 203, 1989.

128. **Loos, B. G., Dyer, D. W., Whittaun, T. S., and Selander, R. K.**, Genetic structure of populations of *Porphyromonas gingivalis* associated with periodontitis and other oral infections, *Infect. Immun.*, 61, 204, 1993.

129. **Lorenz, M.G. and Wackernagel, W.**, Bacterial gene transfer by natural genetic transformaation in the environment, *Microbiol. Rev.*, 58, 563, 1994.

130. **Ludwig W., Dorn, S., Springer, N., Kirchhof, G., and Schleifer, K.-H.**, PCR-based preparation of 23S rRNA-targeted group-specific polynucleotide probes, *Appl. Environ. Microbiol.*, 60, 3236, 1994.

131. **Ludwig, W. and Schleifer, K. H.**, Bacterial phylogeny based on 16S and 23S rRNA sequence analysis, *FEMS Microbiol. Rev.*, 15, 155, 1994.

132. **Madrzak, C. J., Golinska, B., Króliczak, J., Pudelko, K., Lazewska, D., Lampka, B., and Sadowsky, M. J.**, Diversity among field populations of *Bradyrhizobium japonicum* in Poland, *Appl. Environ. Microbiol.*, 61, 1194, 1995, 281

133. **Martínez, E., Laeremans, T., Poupot, R., Rogel, M. A., López, L., García, F., Vanderleyden, J., Promé, J. C., and Lara, F.**, Nod metabolites and other compounds excreted by *Rhizobium* spp., in *Nitrogen Fixation: Fundamentals and Applications*, Tikhonovich, I. A. et al., Eds. Kluwer, The Netherlands, 1995.

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

135. **Martínez, E., Pardo., M. A., Palacios, R., and Cevallos, M. A.**, Reiteration of nitrogen fixation gene sequences and specificity of *Rhizobium* in nodulation and nitrogen fixation in *Phaseolus vulgaris*, *J. Gen. Microbiol.*, 131, 1779, 1985.

136. **Martínez, E., Romero, D., and Palacios, R.**, The *Rhizobium* genome, *Crit. Rev. Plant Sci.*, 9, 59, 1990.

137. **Martínez-Murcia, A. J., Benlloch, S., and Collins, M. D.**, Phylogenetic interrelationships of members of the genera *Aeromonas* and *Plesiomonas* as determined by 16S ribosomal DNA sequencing: lack of congruence with results of DNA-DNA hybridization, *Int. J. Syst. Bacteriol.*, 42, 412, 1992.

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

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

140. **Mavingui, P., Laguerre, G., Berge, O., and Heulin, T.**, Genetic and phenotypic diversity of *Bacillus polymyxa* in soil and in the wheat rhizosphere, *Appl. Environ. Microbiol.*, 58, 1894, 1992.

141. **Maynard Smith, J., Dowson, C. G., and Spratt, B. G.**, Localized sex in bacteria, *Nature*, 349, 29, 1991.

142. **Maynard Smith, J., Smith, N. H., O'Rourke, M., and Spratt, B. G.**, How clonal are bacteria? *Proc. Natl. Acad. Sci. U.S.A.*, 90, 4384, 1993.

143. **McArthur, J. V., Kovacic, D. A., and Smith, M. H.**, Genetic diversity in natural populations of a soil bacterium across a landscape gradient, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 9621, 1988.

144. **Milkman, R. and Bridges, M. M.**, Molecular evolution of the *Escherichia coli* chromosome. III. Clonal frames, *Genetics*, 126, 505, 1990.

145. **Moreira, F., Gillis, M., Pot, B., Kersters, K., and Franco, A. A.** Characterization of rhizobia isolated from different divergence groups of tropical *Leguminosae* by comparative polyacrylamide gel electrophoresis of their total proteins, *Syst. Appl. Microbiol.*, 16, 135, 1993.

146. **Musser, J. M., Bemis, D. A., Ishikawa, H., and Selander, R. K.**, Clonal diversity and host distribution in *Bordetella bronchiseptica*, *J. Bacteriol.*, 169, 2793, 1987.

147. **Musser, J. M., Granoff, D. M., Pattison, P. E., and Selander, R. K.**, A population genetic framework for the study of invasive diseases caused by serotype b strains of *Haemophilus influenzae*, *Proc. Natl. Acad. Sci. U.S.A.*, 82, 5078, 1985.

148. **Musser, J. M., Hewlett, E. L., Peppler, M. S., and Selander, R. K.**, Genetic diversity and relationships in populations of *Bordetella* spp., *J. Bacteriol.*, 166, 230, 1986.

149. **Musser, J. M., Kroll, J. S., Moxon, E. R., and Selander, R. K.**, Clonal population structure of encapsulated *Haemophilus influenzae*, *Infect. Immun.*, 56, 1837, 1988.

150. **Musser, J. M., Rapp, V. J., and Selander, R. K.**, Clonal diversity in *Haemophilus pleuropneumoniae*, *Infect. Immun.*, 55, 1207, 1987.

151. **National Academy of Sciences**, *Tropical Legumes: Resources for the Future*, Washington, D. C., 1979.

152. **Nei, M.**, *Molecular Evolutionary Genetics*, Columbia University Press, New York, 1987.

153. **Nelson, K., Whittam, T. S., and Selander, R. K.**, Nucleotide polymorphism and evolution in the glyceraldehyde-3-phosphate dehydrogenase gene (*gapA*) in natural populations of *Salmonella* and *Escherichia coli*, *Proc. Natl. Acad. Sci. U.S.A.*, 88, 6667, 1991.

154. **Norrung, B. and Skovgaard, N.**, Application of multilocus enzyme electrophoresis in studies of the epidemiology of *Listeria monocytogenes* in Denmark, *Appl. Environ. Microbiol.*, 59, 2817, 1993.

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

156. **Novikova, N., Limeschenko, E., Bustos, P., Rodríguez, O., Davila, G., and Vorobyov, N.**, Taxonomy of *Rhizobium* strains from legumes of the temperate zone, in *Nitrogen Fixation: Fundamentals and Applications*, Tikhonovich, I. A. et al., Eds., Kluwer, The Netherlands, 1995, 716.

157. **Novikova, N. I., Pavlova E. A., Vorobjev, N. I., and Limeshchenko, E. V.**, Numerical taxonomy of *Rhizobium* strains from legumes of the temperate zone, *Int. J. Syst. Bacteriol.*, 44, 734, 1994.

158. **Ochman, H., Whittam, T. S., Caugant, D. A., and Selander, R. K.**, Enzyme polymorphism and genetic population structure in *Escherichia coli* and *Shigella*, *J. Gen. Microbiol.*, 129, 2715, 1983.

159. **Olivares, J. and Sanjuan, J.**, personal communication.

160. **Olsen, G. J., Woese, C. R., and Overbeek, R.**, The winds of (evolutionary) change: breathing new life into microbiology, *J. Bacteriol.*, 176, 1, 1994.

161. **O'Rourke, M. and Stevens, E.**, Genetic structure of *Neisseria gonorrhoeae* populations: a non-clonal pathogen, *J. Gen. Microbiol.*, 139, 2603, 1993.

162. **Oyaizu, H., Matsumoto, S., Minamisawa, K., and Gamou, T.**, Distribution of rhizobia in leguminous plants surveyed by phylogenetic identification, *J. Gen. Appl. Microbiol.*, 39, 339, 1993.

163. **Pardo, M. A., Lagúnez, J., Miranda, J., and Martínez, E.**, Nodulating ability of *Rhizobium tropici* is conditioned by a plasmid-encoded citrate synthase, *Mol. Microbiol.*, 11, 315, 1994.

164. **Paulus, F., Huss, B., Bonnard, G., Ride, M., Szegedi, E., Tempe, J., Petit, A., and Otten, L.**, Molecular systematics of biotype III Ti plasmids of *Agrobacterium tumefaciens*, *Mol. Plant–Microbe Interact.*, 2, 64, 1989.

165. **Pesole, G., Bozzetti, M. P., Lanave, C., Preparata, G., and Saccone, C.** Glutamine synthetase gene evolution: a good molecular clock, *Proc. Natl. Acad. Sci. U.S.A.*, 88, 522, 1991.

166. **Petes, T. D. and Hill, C. W.**, Recombination between repeated genes in microorganisms, *Annu. Rev. Genet.*, 22, 147, 1988.

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

168. **Plazinski, J., Dart, P. J., and Rolfe, B. G.**, Plasmid visualization and *nif* gene location in nitrogen-fixing *Azospirillum* strains, *J. Bacteriol.*, 155, 1429, 1983.

169. **Pueppke, S. G.**, The genetic and biochemical basis for nodulation of legumes by rhizobia, *Crit. Rev. Biotechnol.*, in press.

170. **Raven, P. H. and Polhill, R. M.**, Biogeography of the Leguminosae, in *Advances in Legume Systematics VI*, Polhill, R. M. and Raven, P. H., Eds., 1981.

171. **Rosselló-Mora, R. A., Wagner, M., Amann, R., Ludwig, W., and Schleifer, K. H.**, Based on phylogenetical studies strains of *Zoogloea ramigera* belong either to alpha or beta subclass of *Protobacteria*, in *FEMS Meet. Identification of Bacteria Present Trends — Future Prospects*, University of Granada, Granada, 1993, 132.

172. **Ruskin, F. R.**, *Leucaena, Promising Forage and Tree Crop for the Tropics*, National Academy of Sciences, Washington, D.C., 1977.

173. **Sawada, H. and Ieki, H.**, Phenotypic characteristics of the genus *Agrobacterium*, *Ann. Phytopathol. Soc. Jpn.*, 58, 37, 1992.

174. **Sawada, H., Ieki, H., Oyaizu, H., and Matsumoto, S.**, 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, 1993.

175. **Schill, W. B., Phelps, S. R., aud Pyle, S. W.**, Multilocus electrophoretic assessment of the genetic structure and diversity of *Yersinia ruckeri*, *Appl. Environ. Microbiol.*, 48, 975, 1984.

176. **Schleifer, K. H. and Stackebrandt, E.**, Molecular systematics of prokaryotes, *Annu. Rev. Microbiol.*, 37, 143, 1983.

177. **Scholz, B. K., Jakobek, J. L., and Lindgren, P. B.**, Restriction fragment length polymorphism evidence for genetic homology within a pathovar of *Pseudomonas syringae*, *Appl. Environ. Microbiol.*, 60, 1093, 1994.

178. **Schultze, M., Kondorosi, E., Ratet, P., Buire, M., and Kondorosi, A.**, Cell and molecular biology of *Rhizobium*-plant interactions, *Int. Rev. Cytol.*, 156, 1, 1994.

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

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

181. **Selander, R. K., Beltran, P., Smith, N. H., Barker, R. M., Crichton, P. B., Old, D. C., Musser, J. M., and Whittam, T. S.**, Genetic population structure, clonal phylogeny, and pathogenicity of *Salmonella paratyphi* B, *Infect. Immun.*, 58, 1891, 1990.

182. **Selander, R. K., Beltran, P., Smith, N. H., Helmuth, R., Rubin, F. A., Kopecko, D. J., Ferris, K., Tall, B. D., Cravioto, A., and Musser, J. M.**, Evolutionary genetic relationships of clones of *Salmonella* serovars that cause human typhoid and other enteric fevers, *Infect. Immun.*, 58, 2262, 1990.

183. **Selander, R. K., Caugant, D. A., Ochman, H., Musser, J. M., Gilmour, M. N., and Whittam, T. S.**, Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics, *Appl. Environ. Microbiol.*, 51, 873, 1986.

184. **Selander, R. K., Caugant, D. A., and Whittam, T. S.**, Genetic structure and variation in natural populations of *Escherichia coli*, in Cellular and Molecular Biology, Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, H. E., Eds., American Society for Microbiology, Washington, D.C., 1987, 1625.

185. **Selander, R. K., Korhonen, T. K., Vaisanen-Rhen, V., Williams, P. H., Pattison, P. E., and Caugant, D. A.**, Genetic relationships and clonal structure of strains of *Escherichia coli* causing neonatal septicemia and meningitis, *Infect. Immun.*, 52, 213, 1986.

186. **Selander, R. K. and Levin, B. R.**, Genetic diversity and structure in *Escherichia coli* populations, *Science*, 210, 545, 1980.

187. **Selander, R. K., McKinney, R. M., Whittam, T. S., Bibb, W. F., Brenner, D. J., Nolte, F. S., and Pattison, P. E.**, Genetic structure of populations of *Legionella pneumophila*, *J. Bacteriol.*, 163, 1021, 1985.

188. **Smith, N. H., Beltrán, P., and Selander, R. K.**, Recombination of *Salmonella* phase 1 flagellin genes generates new serovars, *J. Bacteriol.*, 172, 2209, 1990.

189. **Sneath, P. H. A.**, Evidence from *Aeromonas* for genetic crossing-over in ribosomal sequences, *Int. J. Syst. Bacteriol.*, 43, 626, 1993.

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

191. **Soltis, D. E., Soltis, P. S., Morgan, D. R., Swensen, S. M., Mullin, B. C., Dowd, J. M., and Martin, P. G.**, Chloroplast gene sequence data suggest a single origin of the predisposition for symbiotic nitrogen fixation in angiosperms, *Proc. Natl. Acad. Sci. U.S.A.*, 92, 2647, 1995.

192. **Souza, V., Eguiarte, L., Avila, G., Cappello, R., Gallardo, C., Montoya, J., and Piñero, D.**, Genetic structure of *Rhizobium etli* biovar phaseoli associated with wild and cultivated bean plants (*Phaseolus vulgaris* and *Phaseolus coccineus*) in Morelos, Mexico, *Appl. Environ. Microbiol.*, 60, 1260, 1994.

193. **Souza, V., Nguyen, T. T., Hudson, R. R., Piñero, D., and Lenski, R. E.**, Hierarchical analysis of linkage disequilibrium in *Rhizobium* populations: evidence for sex?, *Proc. Natl. Acad. Sci. U.S.A.*, 89, 8389, 1992.

194. **Spaink, H. P.**, The molecular basis of the host specificity of the *Rhizobium* bacteria, *Antonie van Leeuwenhoek*, 65, 81, 1994.

195. **Sprent, J. I.**, Evolution and diversity in the legume-*Rhizobium* symbiosis: chaos theory?, *Plant Soil*, 161, 1, 1994.

196. **Sprent, J. I., Minchin, F. R., and Parsons, R.**, Evolution since Knoxville: were nitrogen-fixing organisms wise to inhabit land plants?, in *New Horizons in Nitrogen Fixation*, Palacios, R. et al., Eds., Kluwer, The Netherlands, 1993, 65.

197. **Stackebrandt, E. and Goebel, B. M.**, Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology, *Int. J. Syst. Bacteriol.*, 44, 846, 1994.

198. **Strain, S. R., Leung, K., Whittam, T. S., De Bruijn, F. J., and Bottomley, P. J.**, Genetic structure of *Rhizobium leguminosarum* biovar trifolii and viciae populations found in two Oregon soils under different plant communities, *Appl. Environ. Microbiol.*, 60, 2772, 1994.

199. **Strain, S. R., Whittam, T. S., and Bottomley, P. J.**, Analysis of genetic structure in soil populations of *Rhizobium leguminosarum* recovered from the USA and the UK, *Mol. Ecol.*, 4, 105, 1995.

200. **Subramaniam, B. and Babu, C. R.**, New nodulating legumes of potential agricultural and forestry value from subtropical Himalayan ecosystems, *Biol. Agric. Hortic.*, 10, 297, 1994.

201. **Sullivan, J. T., Patrick, H. N., Lowther, W. L., Scott, D. B., and Ronson, C. W.**,

Nodulating strains of *Rhizobium loti* arise through chromosomal symbiotic gene transfer in the environment, *Proc. Natl. Acad. Sci. U.S.A.*, 92, 8985, 1995.

202. **Thies, J. E., Bohlool, B. B., and Singleton, P. W.**, Subgroups of the cowpea miscellany: symbiotic specificity within *Bradyrhizobium* spp. for *Vigna unguiculata*, *Phaseolus lunatus*, *Arachis hypogaea*, and *Macroptilium atropurpureum*, *Appl. Environ. Microbiol.*, 57, 1540, 1991.

203. **Tibayrenc, M., Kjellberg, F., Arnaud, J., Oury, B., Brenière, S. F., Dardé, M.-L., and Ayala, F. J.**, Are eukaryotic microorganisms clonal or sexual? A population genetics vantage, *Proc. Natl. Acad. Sci. U.S.A.*, 88, 5129, 1991.

204. **Torsvik, V., Goksoyr, J., and Daae, F. L.**, High diversity in DNA of soil bacteria, *Appl. Environ. Microbiol.*, 56, 782, 1990.

205. **Ueda, T., Suga, Y., Yahiro, N., and Matsuguchi, T.**, Phylogeny of sym plasmids of rhizobia by PCR-based sequencing of a *nodC* segment, *J. Bacteriol.*, 177, 468, 1995.

206. **van Berkum, P.**, personal communication.

207. **van Berkum, P., Beyene, D., Bao, G., Campbell, T. A., and Eardly, B. D.**, Genetic diversity and phylogeny of rhizobia nodulating *Medicago ruthenica* (L.) Ledeb. in *Nitrogen Fixation: Fundamentals and Applications*, Tikhonovich, I. A. et al., Eds., Kluwer, The Netherlands, 1995, 724.

208. **van Berkum, P., Beyene, D., Temprano Vera, F., and Keyser, H. H.**, Variability among *Rhizobium* strains originating from nodules of *Vicia faba*, *Appl. Environ. Microbiol.*, 61, 2649, 1995.

209. **van Berkum, P., Navarro, R. B., and Vargas, A. A. T.**, Classification of the uptake hydrogenase-positive (Hup$^+$) bean rhizobia as *Rhizobium tropici*, *Appl. Environ. Microbiol.*, 60, 554, 1994.

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

211. **Viale, A. M., Arakaki, A. K., Soncini, F. C., and Ferreyra, R. G.**, Evolutionary relationships among eubacterial groups as inferred from GroEL (chaperonin) sequence comparisons, *Int. J. Syst. Bacteriol.*, 44, 527, 1994.

212. **Whittam, T. S., Ochman, H., and Selander, R. K.**, Multilocus genetic structure in natural populations of *Escherichia coli*, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 1751, 1983.

213. **Whittam, T. S., Ochman, H., and Selander, R. K.**, Geographic components of linkage disequilibrium in natural populations of *Escherichia coli*, *Mol. Biol. Evol.*, 1, 67, 1983.

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

215. **Wise, M. G., Shimkets, L. J., and McArthur, J. V.**, Genetic structure of a lotic population of *Burkholderia* (*Pseudomonas*) *cepacia*, *Appl. Environ. Microbiol.*, 61, 1791, 1995.

216. **Woese, C. R.**, Bacterial evolution, *Microbiol. Rev.*, 51, 221, 1987.

217. **Xu, L. M., Ge, C., Cui, Z., Li, J., and Fan, H.**, *Bradyrhizobium liaoningenesis* sp. nov. isolated from the root nodules of soybean, in *Int. J. Syst. Bacteriol.*, 45, 706, 1995.

218. **Yamada, Y. and Akita, M.**, An electrophoretic comparison of enzymes in strains of *Gluconobacter* species, *J. Gen. Appl. Microbiol.*, 30, 115, 1984.

219. **Yanagi, M. and Yamasato, K.**, Phylogenetic analysis of the family Rhizobiaceae and related bacteria by sequencing of 16S rRNA gene using PCR and DNA sequencer, *FEMS Microbiol. Lett.*, 107, 115, 1993.

220. **Young, J. P. W.**, *Rhizobium* population genetics: enzyme polymorphism in isolates from peas, clover, beans and lucerne grown at the same site, *J. Gen. Microbiol.*, 131, 2399, 1985.

221. **Young, J. P. W.**, The population genetics of bacteria, in *Genetics of Bacterial Diversity*, Hopwood, D. A. and Chater, K. F., Eds., Academic Press, London, 1989, 417.

222. **Young, J. P. W.**, Phylogenetic classification of nitrogen-fixing organisms, in *Biological Nitrogen Fixation*, Stacey, G. et al., Eds., Chapman and Hall, New York, 1992, 43.

223. **Young, J. P. W.**, Molecular phylogeny of rhizobia and their relatives, in *New Horizons in Nitrogen Fixation*, Palacios, R. et al., Eds., Kluwer, The Netherlands, 1993, 587.

224. **Young, J. P. W.**, All those new names: an overview of the molecular phylogeny of plant-associated bacteria, in *Advances in Molecular Genetics of Plant-Microbe Interactions*, Daniels, M. J., et al., Eds., Kluwer, The Netherlands, 1994, 73.

225. **Young, J. P. W., Demetriou, L., and Apte, R. G.**, *Rhizobium* population genetics: enzyme polymorphism in *Rhizobium leguminosarum* from plants and soil in a pea crop, *Appl. Environ. Microbiol.*, 53, 397, 1987.

226. **Young, J. P. W., Downer, H. L., and Eardly, B. D.**, Phylogeny of the phototrophic *Rhizobium* strain BTAi1 by polymerase chain reaction-based sequencing of a 16S rRNA gene segment, *J. Bacteriol.*, 173, 2271, 1991.

227. **Young, J. P. W. and Haukka, K.**, personal communication.

228. **Young, J. P. W. and Johnston, A. W. B.**, The evolution of specificity in the legume-*Rhizobium* symbiosis, *Trends Ecol. Evol.*, 4, 341, 1989.

229. **Zhang, X., Harper, R., Karsisto, M., and Lindström, K.**, Diversity of *Rhizobium* bacteria isolated from the root nodules of leguminous trees, *Int. J. Syst. Bacteriol.*, 41, 104, 1991.

230. **Zhang, X., Nick, G., Kaijalainen, S., Lönnqvist, C., Tas, E., Tighe, S. W., Graham, P. H., and Lindström, K.**, Diversity of peanut bradyrhizobia from China, in 10th Int. Congress Nitrogen Fixation, St. Petersburg, Russia, 1995, 445.

231. **Zuckerkandl, E. and Pauling, L.**, Molecules as documents of evolutionary history, *J. Theor. Biol.*, 8, 357, 1965.