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GUEST COMMENTARY

Roses by Other Names: Taxonomy of the *Rhizobiaceae*

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When Shakespeare wrote (46), "What's in a name? that which we call a rose / By any other name would smell as sweet," he implied that phenotypes (scent in this case) take precedence over nomenclature. In popular usage, they usually do. Cartoonists classify politicians by their ears or noses. Scientists use physical characteristics to delimit everything from species (e.g., cranium size in the genus *Homo*) to kingdoms. Throughout much of taxonomic history, macroscopic characters have been preferred for obvious reasons.

EPOCHS IN TAXONOMY

As individual bacteria are too small to see, their classification presents special difficulties. van Leeuwenhoek's invention of the microscope not only rendered bacteria visible (9) but also permitted sorting them into morphological groups (cocci, spirals, and short and elongated rods [8]). In 1884 Christian Gram devised a procedure that separated bacteria into two major staining-reaction groups (47). A second era began when biochemical and physiological characters were used to identify and classify cultures (36). A third revolution followed Sanger, Gilbert, and Maxam's development of methods for sequencing DNA in the 1970s (2, 23). Sequence variation in genes that encode essential functions is obviously restricted to those base changes that do not affect viability. It is assumed that any changes that have occurred must have been acquired slowly and possibly also at a constant rate. Obviously, transcription and translation are central to all organisms, and for this reason ribosomal genes have found particular favor.

In other words, technological advances have driven each of the three (the morphological, the physiological, and the sequence) epochs of bacterial taxonomy. As with all new methods, they have to be finely tuned before they are of widespread utility, and as the paper by van Berkum et al. in this issue (57) shows, attempts to use sequence data to classify bacteria need reexamination.

Symbiotic, nitrogen-fixing bacteria interact with legumes in a readily identifiable manner (producing root nodules). Partly for this reason, they have been classified and studied since the dawn of bacteriology. *Bacillus radiocola* was probably the first name used, but when Nobbe et al. (32, 33) found that bacteria isolated from *Pisum sativum* nodules were unable to nodulate plants belonging to the legume tribes *Genisteae* and *Hedysareae,* a simple solution presented itself—to name the bacterium after the host plant (19). Later, many taxonomic proposals were made (for examples, see reference 16), but all strongly emphasized the host from which the *Rhizobium* was isolated (28, 51, 60).

There are many problems with this approach, including the fact that about 18,000 species of legumes as well as countless rhizobia exist. Also, the "host range" of both bacteria and plants varies from pairs that are more or less faithful to one another to combinations in which almost all traces of specificity have vanished (4, 38). As examples, a number of genera within the *Phaseoleae* (e.g., *Phaseolus* and *Vigna*) form nodules with about half of all rhizobia presented to them (27, 31) and some individual rhizobia (e.g., the broad host range *Rhizobium* species NGR234) are able to nodulate about 50% of all legumes (41). A group such as the "cowpea" miscellany (by definition, members of this group nodulate cowpea [*Vigna unguiculata*] in addition to the host from which they were isolated) eventually contained rhizobia isolated from the majority of all nodulated legumes (34).

LA MODE—THE 16S rRNA GENE

As similar problems existed with other groups (e.g., *Pseudomonas* [37]), taxonomists desperately sought new methods to classify bacteria. Characters such as DNA base ratios, amino acid sequences of proteins, DNA-DNA as well as DNA-RNA hybridizations, the constituents of ribosomes and of cell walls, etc., have all been used, often with surprising consequences. Reviewing this work in 1981, Trüper and Krämer (53) asked, "Which systematic basis will prevail; morphology, physiology or chemical composition of cellular components?" and then replied, "There is no answer yet to the question and there may never be a final answer." Nevertheless, sequencing conserved genes (or parts of genes) is a simple way to provide insights that elude morphological and physiological methods. In themselves, improvements in sequencing technologies would have accelerated the use of sequence data in bacterial taxonomy, but a further development, that of the PCR, greatly simplified the task. Carefully designed oligonucleotide primers allowed amplification and sequencing of only the variable portion of a target gene that could be as short as 200 bp. A single sequencing gel could thus provide taxonomic information on many accessions. Furthermore, these same techniques could be applied to nonpurified DNA or even to "environmental samples." An explosion of papers purporting new taxonomic relationships resulted. Some of them were greeted with enthusiasm, while others seeded confusion.

Using sequence variation of the 16S rRNA gene (or any

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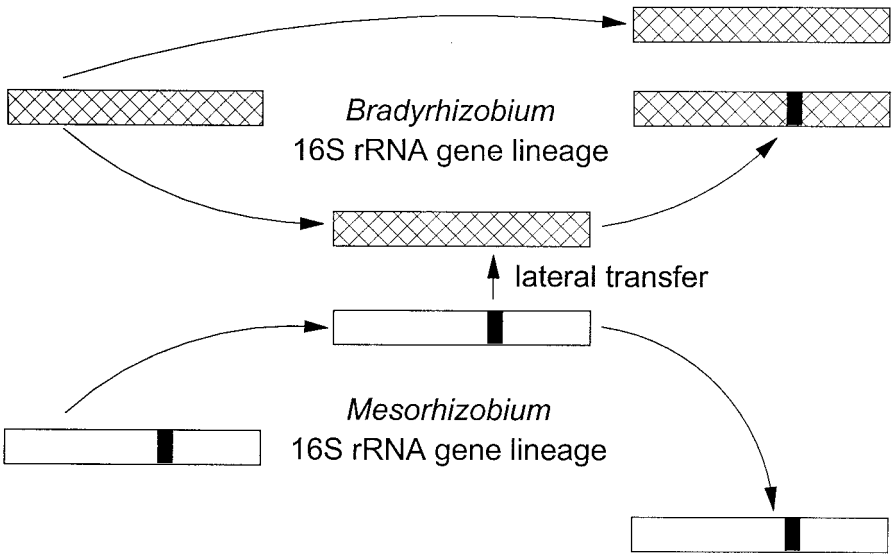

FIG. 1. Model showing how recombination between short segments of the 16S rRNA genes of *B. elkani* and species of *Mesorhizobium* may have occurred, resulting in the lateral transfer of the 16S rRNA gene from *Mesorhizobium* to *Bradyrhizobium*. See the text for further details.

other gene for that matter) for taxonomic purposes presupposes that evolution of the genome progresses at a constant rate and that genes are inherited in a strictly hierarchical manner—in other words, that genes are passed from generation to generation and are not shared between existing cells via horizontal or lateral transfer. Suspicions that this might not always be the case arose from the findings that many taxa, including *Clostridium* (42), *Escherichia coli* (seven alleles) (7), *Haloarcula* (5% difference between the two expressed copies [1]), and *Rhodobacter* (12), contain multiple and often-divergent 16S rRNA genes. The most damning example is that of *Thermobispora bispora*, however, which contains two similar copies of the 16S rRNA gene (as well as three copies of the 23S rRNA gene) that differ from each other by 6.4% at the nucleotide level (59). As these copies of the 16S rRNA gene are on the same chromosome within the same cell, their sequence divergence suggests that the rather arbitrary 5% mismatch that had previously been used to place bacteria into separate genera is untenable.

INCONSISTENCIES IN 16S rRNA, ITS, AND 23S rRNA SEQUENCES

Reexamination of this problem by van Berkum et al. (57) as it applies to the *Rhizobiaceae* is timely not only because of these problems but also because Young et al. (62) claim that the close relatedness of 16S rRNA sequences of *Agrobacterium* and *Rhizobium* species (<7% mismatch) warrants regrouping the agrobacteria and rhizobia into a single genus, *Rhizobium*. What van Berkum et al. did was to sequence the 16S rRNA and the 23S rRNA genes as well as the internally transcribed space (ITS) region that is located between the conserved portions at the 3′ end of the 16S rRNA gene and the 5′ end of the 23S rRNA gene of a number of α-*Proteobacteria* (*Agrobacterium, Rhizobium*, and related genera). Standard computational analyses were then performed on these sequence data to construct phylogenetic relationships among the bacteria. Their results show that the ITS region and the 23S rRNA gene provide phylogenetic signals which are different from those derived from the 16S rRNA gene. In other words, the three sets of data produced three morphologically distinct phylogenetic trees that are impossible to combine into a single tree. In part, this is due to multiple copies of the 16S rRNA gene referred to above (which copy is representative of the species?), but the major contribution of van Berkum et al. concerns the discovery that allelic variation within the *rrn* locus is due to gene conversion. Their data show that a small portion of the 16S rRNA gene of *Bradyrhizobium elkanii* originated from *Mesorhizobium* by lateral transfer (Fig. 1). If this is true, it negates the principle that rRNA genes are inherited only by vertical descent (see above). And if mother-to-daughter transfer is not the only mechanism by which rRNA genes are inherited, further use of 16S rRNA sequence data to construct phylogenetic trees is no longer justified.

GENE CONVERSION

Lateral transfer of genes is known to produce extremely dynamic genomes in which substantial amounts of DNA are introduced into and deleted from bacterial chromosomes (35). To test whether gene conversion is at least partly responsible for the discordant phylogenies within the *Rhizobiaceae*, van Berkum et al. searched among specific alleles of the 16S rRNA genes that may have a history of recombination. Potential recombination events between short segments of the 16S rRNA genes of *B. elkani* and species of *Mesorhizobium*, as well as between *Sinorhizobium* and *Mesorhizobium*, were identified (see Fig. 5 in reference 57). This suggests that divergent genera of the α-*Proteobacteria* are not as genetically isolated as previously claimed (17).

For gene conversion to occur, bacteria must exchange genetic information among themselves. Do they? Laboratory experiments have clearly shown that *Agrobacterium tumefaciens* carrying symbiotic (Sym) plasmids of various *Rhizobium* spe-

TABLE 1. Proposed changes in the nomenclature of some genera and species of the *Rhizobiaceae* based primarily on the DNA sequence of the 16S rRNA gene[a]

Old name	Proposed or new name	Special features	Reference(s)	Suggested name
A. tumefaciens (bv. 1) (includes *Agrobacterium rubi*)	*Rhizobium radiobacter*	Provokes galls; circular and linear chromosomes; no RIMEs[b]	14 vs 62	*Agrobacterium tumefaciens* (tumor forming, regardless of biovar)
Agrobacterium rhizogenes (bv. 2)	*Rhizobium rhizogenes*	Provokes hairy roots	14 vs 62	*A. rhizogenes* (root forming, regardless of biovar)
A. rubi	*Rhizobium rubi*	Provokes galls	14 vs 62	*A. rubi*
Agrobacterium vitis	*Rhizobium vitis*	Provokes galls	14 vs 62	*A. vitis* (bv. 3)
Allorhizobium undicola	*Rhizobium undicola*	Nodulates *Neptunia natans*	11 and 62 vs 14	*R. undicola*
Mesorhizobium loti MAFF303099	*Mesorhizobium huakuii* bv. loti	Nodulates *Lotus corniculatus*; completely sequenced	54	*M. huakuii*
Rhizobium	*Sinorhizobium*	Nodulates many legumes; two circular chromosomes; many RIMEs	10 vs 14 and 55–57	*Rhizobium* (or *Sinorhizobium*[c]); *R. fredii*, *R. meliloti*, *R. saheli*, *R. terangae*, etc.

[a] As the scientific basis for these name changes has been questioned by the findings of others (listed under references), I propose that the former names be used until a detailed revision of the family is made.
[b] RIMEs, *Rhizobium*-specific intergenic mosaic elements.
[c] Although a consensus is now forming that changing the name from *Rhizobium* to *Sinorhizobium* is not warranted (14, 55–57), recently many authors adopted the convention of referring to some of these bacteria as *Sinorhizobium.* It goes against the spirit of this commentary to dictate that *Sinorhizobium* should be abandoned at this time. Furthermore, Euzéby (http://www.bacterio.cict.fr) said ". . .it is possible for two or more validly published names to remain in use."

cies produce atypical, Fix$^-$ nodules (3, 6, 20, 21, 24, 52, 61), although *A. tumefaciens* containing a *Rhizobium etli* plasmid forms nitrogen-fixing nodules (29). Ti plasmids of *A. tumefaciens* are self-conjugal elements (13). Nevertheless, despite proper virulence gene induction and T-strand formation, transconjugants of *Rhizobium meliloti* harboring Ti plasmids of *A. tumefaciens* do not produce tumors on plants (58), suggesting that genetic barriers between the two organisms exist. Here the point is not that *Agrobacterium* harboring *Rhizobium* plasmids produces effective, nitrogen-fixing nodules on legumes (or that *Rhizobium* transconjugants containing Ti plasmids provoke crown galls) but that the plasmids are maintained in the heterologous backgrounds, and this is plainly the case.

Thus, the next question is: does horizontal transfer of genetic information occur under natural conditions, e.g., in the rhizosphere? Two different Sym plasmids of *Rhizobium leguminosarum* readily complemented a nonattaching, nonnodulating mutant of *R. meliloti* in the rhizosphere of *Medicago sativa* (5). Although certain plasmid-chromosome combinations are favored, natural populations of *R. leguminosarum* also display extensive transfer of symbiotic plasmids in the field (18, 26, 43, 45). Moreover, structural rearrangements among the plasmids of the transconjugants also occur (18), using well-documented mechanisms (15, 30, 44). Undoubtedly, the most striking evidence of horizontal transfer concerns the "symbiosis islands" of *Mesorhizobium loti*. Genetically diverse "mesorhizobia" were isolated from nodules of *Lotus corniculatus* growing in fields that were devoid of indigenous *Lotus* rhizobia, but which had been inoculated with a single *M. loti* isolate (48). All contained a 502-kb chromosomally integrated element that transfers to nonsymbiotic mesorhizobia, converting them to *Lotus* symbionts. This symbiotic island integrates into a phenylalanine tRNA gene on the chromosome of the host, in a process mediated by a P4-type integrase encoded at one end of the element (48–50).

NAMES OF THE ROSES

There is little doubt that soil bacteria are not unchangeable, static organisms. On the contrary, plasmids and well-defined parts of chromosomes are freely exchanged among bacteria, especially when they congregate at the root surface (the rhizoplane) or within the nodule (40). Furthermore, a small (53-kb) plasmid of *Bacillus megaterium* harbors a functional rRNA operon that is probably transferable to other bacteria (25). Since bacterial genomes are much more fluid than previously thought, there is little reason to doubt that acquisition of foreign DNA, followed by recombination into the parental genome, is an important driving force in evolution. That essential genes are targets for conversion may come as a surprise, but as Flores et al. (15) have shown, repeated sequences are "hot spots" for genomic rearrangements. As complete DNA sequences of other *Rhizobiaceae* become available (at the time of writing, only those of *A. tumefaciens*, *Bradyrhizobium japonicum*, *M. loti*, and *R. meliloti* have been published), more concatameric 16S rRNA genes will undoubtedly be found. In their paper, van Berkum et al. (57) suggest that rather than being the dominant character used in bacterial taxonomy, the DNA sequence of the 16S rRNA gene should be only one of many used. If this principle is to be applied, it means, however, that some of the recent name changes based on analysis of the 16S rRNA gene need to be rethought (Table 1). Several groups have made cogent arguments against the adoption of the new names (14, 52–54). The report by Farrand et al. (14) also contains a list of over 100 bacteriologists who are opposed to the proposal of Young et al. (62).

There are really only two reasons for giving names to living objects—to pinpoint them so that others will understand which one is being talked about and, if possible, to group them so that their interrelationships are obvious. Essentially, these are the differences between taxonomy (which could be achieved by a sort of "bacterial bar code") and phylogeny, which is the evolutionary history of a species or other taxonomic group. Superficially, many flowers look like roses but their scent sets them apart. So too with the *Rhizobiaceae—Agrobacterium* makes crown galls, *Rhizobium* makes nodules. One is a pathogen, the other is a symbiont. Whether or not these traits are reflected in the 16S rRNA sequence is of lesser importance in giving names, since we have an obligation to ensure (i) that the

name reflects an easily discernible reality (e.g., a rose, a gall, or a nodule), (ii) that the name is not a source of error, (iii) that the name is not equivocal, (iv) that the name is maintained for as long as possible, and (v) that the name is commonly accepted.

van Berkum et al. (57) have done the scientific community a large service by pointing out that names based solely on 16S rRNA sequence data satisfy few of these criteria. Or, as Postgate (39) wrote, ". . .new rRNA phylogeny is the phylogeny of rRNA genes, not of their hosts. . . ." A moratorium or at least a cooling-down period on renaming the *Rhizobiaceae* (and probably other groupings) is thus called for. It would be sensible to wait until further data are available on a variety of conserved genes (23S rRNA, the ITSs, *glnA*, *nodA*, *recA*, etc.). Some of this will be provided by current whole-genome sequencing projects, but more could be gathered by using current techniques (54, 57). When data are available, and after a suitable period of reflection, perhaps it would be appropriate if the editor of the *Journal of Bacteriology* or the editor of the *International Journal of Systematic and Evolutionary Bacteriology* commissioned an "outsider" to revise the genera *Agrobacterium* and *Rhizobium*, etc., which would be published in their respective journals.

One final point concerns precedent. Many think that if there are compelling morphological and behavioral reasons for reclassifying competitors as *Rattus erectus*, rules of precedent require that if this is published, the name *R. erectus* would have to be used in place of *Homo sapiens sapiens* in the scientific literature. This is not the case. Extracts from J. P. Euzéby's *List of Bacterial Names with Standing in Nomenclature* (http://www.bacterio.cict.fr) (updated 28 January 2003) include the following:

(i) "There is no official classification of bacteria, but the names given to bacteria are regulated."

(ii) ". . . the name of a taxon is validly published, and therefore has standing in nomenclature, if one of the following criteria is met: 1) the name is cited in the *Approved Lists of Bacterial Names.* 2) The name is published in papers in the *International Journal of Systematic and Evolutionary Microbiology* (and its predecessor). 3) The name is validated by announcement in a Validation List."

(iii) But in a nota bene he adds, "1) The names in this list are 'valid' only in the sense of being validly published as a result of conformity with the Rules of Nomenclature. **The names which are to be used are those which are correct in the opinion of the bacteriologist (especially a combinatio nova or a nomen novum), and a particular name does not have to be adopted** . . . .." This was confirmed by the International Committee on Systematics of Prokaryotes (22), who said, "Consequently, the committee suggest that it is up to the individual experts and/or authors to choose . . . which name they want to use."

The "take-home message" is thus clear. Use the names that you think best describe the organism in light of the five taxonomic rules mentioned above. In time, rhizobial taxonomy will stabilize and form a consensus that we can all live with, and van Berkum et al. will be thanked for helping with that.

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