International Journal of Systematic and Evolutionary Microbiology (2003), **53**, 1689–1695 **DOI** 10.1099/ijs.0.02762-0

Taxonomic Note

Classification and nomenclature of *Agrobacterium* and *Rhizobium* – a reply to Farrand *et al.* (2003)

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Farrand *et al*. [*Int J Syst Evol Microbiol* **53** (2003), 1681–1687] have presented a critique of the proposal of Young *et al*. [*Int J Syst Evol Microbiol* **51** (2001), 89–103] to revise the nomenclature and classification of *Rhizobium*. They argued that Young *et al*. (2001) are mistaken in their reclassification of all *Agrobacterium* species within *Rhizobium*, and that the resulting nomenclatural revision is 'unnecessary and unwarranted'. These objections arise because the authors appear not to understand the role of formal nomenclature, and fail to distinguish between formal and special-purpose nomenclatures (*Bacteriological Code*, 1990 Revision). The arguments set out by Farrand *et al*. (2003) can be addressed in terms of (1) the taxonomic status of the genera *Agrobacterium* and *Rhizobium*; (2) the status of species and biovars and their nomenclature; and (3) the role of transmissible genomic elements in classification and nomenclature. Finally, an attempt is made to unravel the confusion underpinning their discussion with a consideration of the relationship between formal and special-purpose nomenclatures.

Agrobacterium and *Rhizobium* as genera

Commentaries on recent approaches to bacterial classifications suggest that they can validly be based on different premises that lead to different nomenclatures (Young, 2001a). *Phenetic* classification is based on the overall similarities and differences between bacteria. *Phylogenetic* classification is based on the inferred ancestral relationships of bacteria. These classifications have been critically discussed by Young *et al.* (1992). *Polyphasic* classification is based on a consensus of phenotypic data (gathered by all available methods) that is consistent with phylogenetic classification established using 16S rRNA or 23S rRNA sequence data (Vandamme *et al.*, 1996). The status of *Agrobacterium* and *Rhizobium* can be discussed in terms of these different approaches to classification.

The extent to which horizontal gene transfer occurs and hence gives rise to misleading classifications is unclear at present (Broughton, 2003; Young, 2001b). Clearly, if this phenomenon were widespread, i.e. there is extensive chromosomal heterogeneity between strains of individual taxa, then both phylogenetic and phenetic classifications will be corrupted and attempts to derive natural classifications will be in vain. While chromosomal variation by horizontal gene transfer may occur extensively in some genera, expression of transferred genes is not necessarily a universal phenomenon. For instance, plant-pathogencity genes, most of which appear to be on chromosomes, are only expressed in about 20 of the 1146 known bacterial genera.

Agrobacterium has long been recognized as closely related to *Rhizobium*, and amalgamation of these two genera has often been suggested (Graham, 1964; Heberlein *et al.*, 1967; De Ley, 1968; White, 1972; Graham, 1976; Kerr, 1992). These essentially phenotypic comparisons of *Agrobacterium* spp. and *Rhizobium* spp. have subsequently been supported by phylogenetic inferences based on 16S rDNA sequence analyses which showed that the genera could not be distinguished as separate monophyletic clades (Amarger *et al.*, 1997; Chen *et al.*, 1997; de Lajudie *et al.*, 1994, 1998a, b; Jarvis *et al.*, 1997; Nour *et al.*, 1994; Rome *et al.*, 1996; Sawada *et al.*, 1993; Tan *et al.*, 1997; Wang *et al.*, 1998; Willems & Collins, 1993). Phenotypic data of de Lajudie *et al.* (1994) and a study of fatty acid profiles by Tighe *et al.* (2000) further confirmed the integrity of individual agrobacterial and rhizobial species, but in both studies the species of the two genera were intermingled. Because there was no basis for considering them as separate genera, either in phenotypic terms or in terms of inferred phylogenies, Young *et al.* (2001b) proposed that all species be amalgamated in a single genus, *Rhizobium*, comprising pathogenic, symbiotic nitrogen-fixing, and unspecialized soil populations.

Farrand *et al.* (2003) are correct when they note how well each species of *Agrobacterium* and *Rhizobium* is supported in phenotypic terms, *as an individual species*; however, species discrimination should not be confused with the ability to differentiate *Agrobacterium* and *Rhizobium* as separate genera. Farrand *et al.* (2003) claim that the phenotypic data of Holmes & Roberts (1981), de Lajudie *et al.* (1994) and Young *et al.* (2001b) all give support to this differentiation. Unfortunately, Farrand *et al.* (2003) do not indicate the specific data to which they refer and careful review of publications they cite does not reverse the earlier conclusions of Young *et al.* (2001b). Reference by Farrand *et al.* (2003) to the report of Allen & Allen (1950) is to studies made at a time when bacterial systematics was relatively unsophisticated and which study compared only three or four of the rhizobial species recognized today with *Agrobacterium radiobacter* alone. None of the differentiating tests described by Allen & Allen (1950) are acknowledged in the literature today. Farrand *et al.* (2003) indicate that they regard *Rhizobium galegae*, *Rhizobium huautlense*, *Agrobacterium rubi* and *Agrobacterium vitis* as being atypical either in phenotypic or phylogenetic terms. There appears to be little or no evidence for this special notice.

Farrand *et al.* (2003) considered that comparative 16S rDNA sequence analyses support the allocation of *Agrobacterium* and *Rhizobium* species to two clades comprising:

Clade 1. 'biovar 1 agrobacteria' and *Agrobacterium rubi*, together with *Rhizobium galegae*, *Rhizobium huautlense* and *Allorhizobium undicola*;

Clade 2. 'biovar 2 agrobacteria', together with *Rhizobium leguminosarum*, *Rhizobium etli* and *Rhizobium tropici*.

A comprehensive record of the members of these clades is (Squartini *et al.*, 2002; Tan *et al.*, 2001; Wei *et al.*, 2002; Young *et al.*, 2001b):

Clade 1. *Agrobacterium tumefaciens* (the type species of *Agrobacterium*) (='biovar 1 agrobacteria'), *Agrobacterium rubi*, *Agrobacterium vitis*, *Rhizobium galegae*, *Rhizobium huautlense* and *Allorhizobium undicola*.

Clade 2. *Agrobacterium rhizogenes* (='biovar 2 agrobacteria'), *Rhizobium leguminosarum* (the type species of *Rhizobium*), *Rhizobium etli*, *Rhizobium gallicum*, *Rhizobium giardinii*, *Rhizobium indigoferae*, *Rhizobium hainanense*, *Rhizobium mongolense*, *Rhizobium sullae*, *Rhizobium tropici* and *Rhizobium yanglingense*.

Formal division into genera would see an emended genus *Agrobacterium* (=Clade 1) and an emended genus *Rhizobium* (=Clade 2). Notwithstanding their interpretation of the *Agrobacterium–Rhizobium* clade structure, Farrand *et al.* (2003) do not pursue the nomenclature implied by this phylogenetic classification. The analysis of other genes may influence the interpretation of the 16S rDNA sequences alone but it seems improbable that additional sequence data will establish separate clades for *Rhizobium* and *Agrobacterium* as presently described. As noted in a discussion of *Allorhizobium* and of *Delftia* (Young, 2001b), differentiation of taxa based on perceived gaps between clades is completely arbitrary if clades are considered without reference to phenotypic data. The ambivalence of Farrand *et al.* (2003) to interpretations of 16S rDNA sequence analysis is emphasized by their wish to minimize the implications of the intermingling of *Agrobacterium* and *Rhizobium* sequences, while claiming that the sequence data give support for separation of the two genera.

Farrand *et al.* (2003) claim that members of *Agrobacterium* and members of *Rhizobium* differ in various aspects of chromosomal structure as reported by Goodner *et al.* (2001) and Wood *et al.* (2001). However, in the studies cited, only one *Agrobacterium* strain was examined. Farrand *et al.* (2003) also claim that the data of Jumas-Bilak *et al.* (1998), in which the genomic structures of *Agrobacterium*, *Rhizobium* and *Sinorhizobium* are compared, support the differentiation of *Agrobacterium* from *Rhizobium*. A careful reading of Jumas-Bilak *et al.* (1998) suggests that the differences merely reflect a high level of diversity among related strains, also by their data on *Rhizobium leguminosarum*, *Rhizobium fredii* and *Sinorhizobium* (as *Rhizobium*) *meliloti*.

Farrand *et al.* (2003) also suggested that rhizobium-specific intergenic mosaic elements (RIMEs) described by Østerås *et al.* (1995) provide evidence of differences between *Agrobacterium* and *Rhizobium*. Østerås *et al.* (1995) reported the presence of RIMEs for *Sinorhizobium* (as *Rhizobium*) *meliloti*, *Rhizobium leguminosarum* and unassigned '*Rhizobium* spp.', as well as for '*Agrobacterium rhizogenes*', but not for '*Agrobacterium tumefaciens*'. However, because they present data from only a few strains, Østerås *et al.* (1995) do not make a contribution to the discussion of generic differences between *Agrobacterium* and *Rhizobium*.

Species and biovars

When considering the nomenclature of groups within *Agrobacterium*, Farrand *et al.* (2003) expressed their preference for that originally used by Keane *et al.* (1970) and elaborated later by Kerr & Panagopoulos (1977), in which groups were differentiated within the genus as biovars 1, 2 and 3. These studies showed that phenotypic differences were not congruent with pathogenic populations; differentiation within *Agrobacterium* was made at the infrasubspecific level as biovars, while species names were retained for pathogenic populations. When this nomenclature was proposed, plant-pathogenic ability and expression were believed to represent a substantial component of the bacterial phenotype which, although still to be discovered, would ultimately give support to species names. Such a nomenclature no longer conforms to our understanding of species in *Agrobacterium.* It is clear that the biovars are so well supported in phenotypic and genomic terms that they can be considered to be species, whereas pathogenic characters alone do not justify species differentiation. The elevation of biovars 1, 2 and 3 to species rank has been widely endorsed (Holmes & Roberts, 1981; Kersters & De Ley, 1984; Holmes, 1988; Ophel & Kerr, 1990; Sawada *et al.*, 1993; Bouzar, 1994; Bouzar & Jones, 2001).

Various proposals have been made to provide a species nomenclature for *Agrobacterium* (Holmes & Roberts, 1981; Bradbury, 1986; Holmes, 1988; Ophel & Kerr, 1990) in which biovars 1, 2 and 3 are replaced by species *Agrobacterium tumefaciens*, *Agrobacterium rhizogenes* and *Agrobacterium vitis*, respectively. Kersters & De Ley (1984) supported the revision of species nomenclature but did not follow Holmes & Roberts (1981). The application of these phenotypic species epithets is now supported for pathologists working with *Agrobacterium* (Moore *et al.*, 2001). Biovar nomenclature for *Agrobacterium* was continued almost exclusively in the literature of *Rhizobium* taxonomy until about 1994, after which species nomenclature was increasingly adopted (Nour *et al.*, 1994; Rome *et al.*, 1996; Amarger *et al.*, 1997; Chen *et al.*, 1997; Tan *et al.*, 1997).

Farrand *et al.* (2003) do not give detailed consideration to the application of the epithets *tumefaciens*, *rhizogenes* and *vitis* to species described on the basis of stable characters but not including pathogenicity. This may arise from their failure to distinguish between species, as taxonomic groups, and species epithets, as names. According to Farrand *et al.* (2003), names should be 'meaningful' and 'should be designed for easy recognition and recollection'; in other words, names should be descriptive and informative. There has been a strong tradition of proposing names for bacteria that are descriptive of a significant character of the taxon. However, such descriptive epithets may refer to one or a few characters not necessarily present in all members of the taxon, especially if subsequent revisions result in dissociation between a taxon's description and the descriptive element referred to by the name. For instance, when first proposed *Agrobacterium* and *Rhizobium* were considered to refer to 'field bacterium' and to 'root bacterium' respectively. Species names can best be considered as proper nouns, sometimes originally having had descriptive meanings, but now no more than nomenclatural labels. This misunderstanding has been canvassed in detail (Sneath, 1984; Young, 2000b).

Transmissible genomic elements in classification and nomenclature

The discussion of 'species designations' by Farrand *et al.* (2003) is difficult to follow because they do not distinguish between species as taxonomic groups and species epithets as names. Young *et al.* (2001b) contend that tumorigenic and rhizogenic populations cannot be circumscribed as species in formal nomenclature because the different pathogenic characters are borne on transmissible Ti or Ri plasmids. Acquisition, exchange or loss of one of these plasmids by a bacterial strain would thus lead to a change in its species identity (Kersters & De Ley, 1984). For this reason, the epithets *tumefaciens* and *rhizogenes*, if restricted to use for populations defined by their pathology, could not refer to stable taxa. Farrand *et al.* (2003) are right to note that the laboratory experiments in which Sym plasmids from nitrogen-fixing species are transferred to plant-pathogenic species, and those in which Ti and Ri plasmids from plant-pathogenic species are transferred to nitrogen-fixing species, are almost certainly not indicative of promiscuity between these two groups of bacteria; root isolations of such complementary recombinations have never been reported from field studies. However, these experiments do indicate how closely related are nitrogen-fixing and plant-pathogenic species. Part of the solution to the nomenclatural issue is to accept an informal terminology for tumorigenic and rhizogenic strains as proposed by Holmes & Roberts (1981).

Formal and special-purpose nomenclatures

Most illuminating of the approach to formal nomenclature by Farrand *et al.* (2003) is their view, in discussing the status of the epithets *tumefaciens* and *rhizogenes*, that 'retention of one but not the other cannot be excused on the basis of established rules for assigning species names'. In other words, the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992) (the Code) should be ignored if it impedes choices of names preferred by the authors. When applying names to taxa, the Code requires that rules of priority be adhered to, and that in revisions of taxa, names are allocated according to the priority of type (name-bearing) strains (Young, 2000b). Within these constraints bacteriologists are free to use any valid nomenclature (Young, 2001a; Young *et al.*, 2001a). Over the years, the Code has been refined and become accepted as the basis for bacterial nomenclature. The creation of the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) (the Approved Lists) resulted in nomenclature unencumbered by the ~26 000 uninformative species names that had been generated in the past. Nowhere do Farrand *et al.* (2003) cite or refer to the Code (except incidentally where they refer to 'established rules for assigning species names') or to the Approved Lists. If the attitude of Farrand *et al.* (2003) to the Code were widely accepted, it is hard to see how a return to unregulated and chaotic bacterial nomenclature could be avoided.

Since the development of the Approved Lists, some authors have assumed that the most recent reclassification and formal nomenclatural revision of genera or species is invariably to be preferred. However, there have been many revisions, some of which, though sound, are incomplete. Other reclassifications, sometimes leading to comprehensive nomenclatural revisions, may be unsound. Particular nomenclatural proposals are not always the most suitable for some applications, and therefore it is for bacteriologists to choose classifications and nomenclature that best serve their purposes (Young, 2001a; Young *et al.*, 2001a). This point is made explicitly by the Committee on Taxonomy of Plant Pathogenic Bacteria of the International Society for Plant Pathology (Young *et al.*, 1996) and in a recent report by the International Committee on Systematics of Prokaryotes Subcommittee on the taxonomy of *Agrobacterium* and *Rhizobium* (Lindström & Martínez-Romero, 2002), referring to alternative formal nomenclatures of these two genera, and by Broughton (2003).

Farrand *et al.* (2003) draw their particular conclusions because they do not distinguish formal nomenclature (governed by the Code) from special-purpose nomenclatures that serve the interests of particular research groups. Special-purpose nomenclatures, as infrasubspecific subdivisions, are discussed in Appendix 10 of the Code. Infrasubspecific names are for convenience, generally making no claim to expressing natural relationships, but having the benefit of nomenclatural stability because of their general acceptance. Nothing impedes Farrand *et al.* (2003) or their supporters from using a special-purpose nomenclature referring to *Agrobacterium* biovars, but not covered by the Code, if they have understanding referees and journal editors.

Conclusions

Symbiotic nitrogen-fixing and plant-pathogenic bacteria are likely to represent only a part of the overall diversity in the genera of the *Rhizobiaceae*. The present record of characterized species is strongly biased in favour of organisms of anthropocentric interest. There is little basis at present for believing that symbiotic nitrogen-fixing strains are the predominant populations in species originally identified by their nitrogen-fixing ability. As the catalogue of bacterial diversity is expanded, names based on specific descriptive characters such as nitrogen fixation can be expected only to become more confusing (Young, 2003).

According to Farrand *et al.* (2003), Young *et al.* (2001b) fail to make a compelling case for the amalgamation of *Agrobacterium* and *Rhizobium*. Alternatively it might be asked whether, in their review of 50 years of systematics data, Farrand *et al.* (2003) have made any case for continuing to consider these as separate genera. There is nothing conceptually radical in amalgamating pathogenic, symbiotic nitrogen-fixing, and non-pathogenic species in a generic circumscription of *Rhizobium* comparable to those of *Burkholderia* (Moulin *et al.*, 2001), *Ensifer* (Young, 2003), *Methylobacterium* (Sy *et al.*, 2001) and *Ralstonia* (Chen *et al.*, 2001).

Farrand *et al.* (2003) claim that 'there exist sets of like traits among members of the genus *Rhizobium* and other sets of like traits among members of the genus *Agrobacterium*, and the two groups do not share these sets in common'. Where they have attempted to illustrate the differences between the two sets, it seems that they have misread the literature, or have extrapolated studies of individual strains to represent whole genera.

Farrand *et al.* (2003) justify their argument for separation of *Agrobacterium* and *Rhizobium* because, although they 'are related but comprise a large group of diverse bacteria', there is 'insufficient reason to place all of these different species in a single genus' when 'there is such diversity among these groups'. Whether or not this is so, Farrand *et al.* (2003) provide scant, if any, support for their claim that the agrobacteria and rhizobia are 'a paradoxically diverse group of related members'. Certainly they provide no evidence that the claimed diversity can be resolved by maintaining the division between these two genera. The claims of Farrand *et al.* (2003) would be better supported if they provided unique phenotypic and genotypic circumscriptions of *Agrobacterium* and *Rhizobium* such as are canvassed for genera of the *Proteobacteria* (Murray *et al.*, 1990) and according to the criteria outlined by Graham *et al.* (1991). [The authors of Young *et al.* (2001b) were also authors of the *Agrobacterium* and *Rhizobium* chapters in the forthcoming second edition of *Bergey's Manual of Systematic Bacteriology*. The chapters were prepared before publication of Young *et al.* (2001b), but were retained at the request of the editors, and hence refer to the genera separately as *Rhizobium* (Kuykendall *et al.*, 2003) and *Agrobacterium* (Young *et al.*, 2003).]

Taxonomic studies that give rise to formal nomenclature necessarily involves an ongoing dynamic interaction between classification, nomenclature and identification (Young *et al.*, 1992). Classification is the ordering of bacteria into natural groups. Formal nomenclature entails naming the groups as taxa according to the Code. Identification requires the application of methods derived from studies of classification to allocate bacterial isolates either to previously described taxa or to new groupings that may, after further study, stand as novel taxa. Over time, the refinement of classification and attendant changes in nomenclature have served the bacteriological community very well as they express, with increasing accuracy and reliability, phenotypic or inferred phylogenetic bacterial relationships. The revision of *Phytomonas*, a genus in which almost all plant-pathogenic species used to be included, and the more recent revision of *Pseudomonas*, are examples. A point not often emphasized is that, to support the dynamic by which taxonomy proceeds, revisions of classification must provide methods of identification as well as formally accepted names, and identified strains must be deposited in publicly accessible culture collections for long-term safe storage as resources for future comparison.

Most bacteriologists assume that refinements in classification and nomenclature will support their fields of research by more clearly demonstrating similarities and differences in bacterial populations. The last 30 years of taxonomic research has illuminated relationships that reflect models of evolution of natural bacterial groups. Sometimes, the reasons for revisions are not immediately obvious to practitioners, although they are rational and justified, and they become assimilated with time. Elsewhere (Young, 2000a) the need for caution in nomenclatural revisions has been urged, but there can be no argument for stasis in approaches to classification and its resulting nomenclature.

Revisions of *Agrobacterium* and *Rhizobium* may cause uncertainty, especially among those with long experience of past nomenclature. Regardless of the implied 'meaning' of the names, such names are usually quickly assimilated as nomenclatural labels. However, there are institutions, as in the public health community, where the need for nomenclatural stability is dominant, and whose diagnostic routines allow no room for confusion. Farrand *et al.* (2003) touch on this point incidentally when they note that the nomenclature of *Escherichia*, *Shigella* and *Salmonella* is in need of substantial revision. However, the likely confusion that nomenclatural changes would cause in medical diagnostic and public health laboratories has led, understandably, to a conservative approach to nomenclatural revisions of the *Enterobacteriaceae*.

Concluding their communication, Farrand *et al.* (2003) list 82 colleagues who have indicated their support for the 'taxonomic validity of the genus *Agrobacterium*'. The paramount interest of most of these workers appears to be in a nomenclature that maintains continued stability and applicability. The convenience of special-purpose nomenclatures set against perceived difficulties in application of descriptive names of species produced by formal revisions of *Agrobacterium* seems to have been the reason for failure to adopt these revisions previously. As noted above, new formal nomenclatural proposals do not disqualify the use of previously published nomenclature and there is no insuperable impediment to using nomenclature that is not covered by the Code where there is a consensus on its use. Of course, the discussion on these nomenclatural matters is not settled here. Younger, fresh-minded scientists will consider the issues and make their own decisions. It will, however, be interesting to see whether the matter is not settled decisively one way or the other within this decade.

Acknowledgements

The New Zealand Foundation for Research, Science and Technology funded this paper under contract C09X0201. We thank S. R. Pennycook, P. K. Buchanan, Landcare Research, Auckland, and D. R. W. Watson (retired), Plant Diseases Division, DSIR, Auckland, for critically reading the manuscript and for their helpful suggestions.

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