LETTER TO THE EDITOR

Taxonomic ambiguities: a case history

Rhodospirillum centenum is a remarkable non-sulfur purple photosynthetic bacterium isolated in our laboratory in 1987. The first published description of the organism (Favinger *et al*., 1989) detailed an assortment of characterisics that placed it in the genus *Rhodospirillum* (morphology, *in vivo* absorbancy spectrum of photopigments, general physiology, nutritional requirements). We named the organism *Rhodospirillum centenum* in recognition of the fact that it was discovered on the 100th anniversary of the isolation of the first pure culture of an anoxyphototroph, *Rhodospirillum rubrum*. The latter became an important experimental organism whose study revealed many of the basic aspects of anoxygenic photosynthesis, including the discovery of photophosphorylation. Soon after isolation of the original type strain of *Rhodospirillum centenum*, it was deposited in the American Type Culture Collection as ATCC 43720 (non-phototactic variant); a phototactic variant was later deposited as ATCC 51521.

Unusual properties of *Rhodospirillum centenum*

Our original publication (Favinger *et al*., 1989) and subsequent papers (Gest, 1995; Jiang *et al*., 1997; Nickens *et al*., 1996; Ragatz *et al*., 1994, 1995; Yildiz *et al*., 1991a, b, 1992) described the following unusual characteristics of *Rhodospirillum centenum*: (a) Under conditions favourable for synthesis of poly-β-hydroxybutyrate, vegetative cells become converted to thick-walled cysts, which may be 'survival forms' suggestive of a life cycle; (b) in contrast to virtually all other non-sulfur purple bacteria, the production of photosynthetic pigments by *Rhodospirillum centenum* is not appreciably repressed by molecular oxygen; (c) some cells contain complex refractile structures identical in appearance to the 'R bodies' observed in a few species of non-photosynthetic bacteria, notably endosymbionts of paramecia. 'R bodies' are believed to be protein products of a defective phage; (d) on agar, *Rhodospirillum centenum* cells differentiate from swim cells, which bear a single polar flagellum, into swarm cells that are hyperflagellated with polar and lateral flagella. Colonies of swarm cells display authentic phototactic behaviour, a property never before observed in anoxyphototrophs. (e) *Rhodospirillum centenum* cells contain a phytochrome-like protein that may be ancestral to cyanobacterial and plant phytochromes (see Jiang *et al*., 1999).

Proposal of a taxonomic disarrangement

Five years after our original publication, Kawasaki *et al*. (1992) published a paper with a title that indicated they discovered a previously unknown bacterium that they named *Rhodocista centenaria*. In fact, the latter was *Rhodospirillum centenum*. Kawasaki *et al*. deposited our original type strain (ATCC 43720) in the Japanese Institute of Applied Microbiology culture collection using their proposed new genus/species names and a different catalogue number. Naturally, we considered this to be a violation of acceptable practices. Curiously, the alternative designation (*Rhodocista centenaria*) of *Rhodospirillum centenum* was eagerly adopted by several taxonomists despite inconsistencies with regulations of the International Code of Nomenclature of Bacteria (1990 Revision) (Lapage *et al*., 1992). Since most microbiologists are not familiar with the quasi-legalistic regulations of the Code, the case history of *Rhodospirillum centenum* is instructive of a more general problem that has developed recently, namely, proposed renaming of numerous bacterial species that have been prominent in experimental research during the past 50 years.

'Effective publication', validation and priority of a name

Rule 24b of the 1990 Code revision states that 'Where two names, previously published in other journals [*that is*, *other than the International Journal of Systematic Bacteriology* (*IJSB*)], are validated by announcement on the same Validation List in IJSB, priority is established by the sequence number on the list.' The designation *Rhodospirillum centenum* was 'effectively published' in our original 1989 paper. *Rhodospirillum centenum* and the name *Rhodocista centenaria* both appear in the same Validation List in IJSB, namely, List no. 48 [*Int J Syst Bacteriol* **44** (1994), 182–183]. The list assigns a 'priority number' determined by the 'date the documentation and request for validation are received'. Inexplicably, Validation List no. 48 gave both names the same (inscrutable) priority number, namely '2'. In our opinion, this was inconsistent with the aims of developing a fair, appropriate and useful system for nomenclature of bacteria.

Some basic principles of nomenclature

Principle 1 of the 1990 Code states that: 'The essential points in nomenclature are as follows. (1) Aim at stability of names; (2) avoid or reject the use of names

which may cause error or confusion; and (3) avoid the useless creation of names.' Moreover, Rule 55 of the Code specifies 'A legitimate name or epithet may not be replaced merely because of the following: (1) it is inappropriate; (2) it is disagreeable; (3) another name is preferable; (4) another name is better known; (5) it no longer describes the organism; (6) it has been cited incorrectly; an incorrect citation can be rectified by a later author.' To this, we add that the reasons for creation of a new genus must be compelling and well documented in order to conform to Principles 1, 4, 6 and 9 of the 1990 Code.

The Chairman of the Judicial Commission of the International Committee on Systematic Bacteriology informed us privately that in using the species name *centenum* we did not follow Rule 52(2) of the Code, which states that 'ordinal numbers cannot be used as specific epithets'. We disagree with his contention because *centenum* is not an ordinal number. The Latin word *centenum* can be either a cardinal or multiplicative number, which is permitted by the Code.

Several other reasons for rejecting the name *Rhodocista*

Even though we discovered the capacity of *Rhodospirllum centenum* to form cysts, we dismissed the notion of applying this to propose a new genus designation for several reasons. Use of the name *Rhodocista* immediately invites confusion with the genus *Rhodocystis* (which does not form cysts) proposed by Molisch in 1907 (Molisch, 1907). The name *Rhodocystis* is still listed in *Bergey's Manual of Systematic Bacteriology*, but has been changed several times (see Gest & Favinger, 1998). Furthermore, *Rhodospirillum centenum* forms cysts only under special nutritional conditions. If a property of this kind were emphasized in devising alternative names of various bacterial genera, the result would be chaos. Should the genus name *Rhodomicrobium* be changed to *Rhodoexosporus* simply because this bacterium has the capacity to produce exospores? It is inevitable that new discoveries of significant characteristics will be made after an organism is named. Obviously, frequent renaming would be in basic conflict with the aim of developing a stable and useful nomenclature.

The title of the paper by Kawasaki *et al*. (1992) was '*Rhodocista centenaria* gen. nov., sp. nov., a cyst-forming anoxygenic photosynthetic bacterium and its phylogenetic position in the Proteobacteria alpha group.' Among other lapses, the taxonomy committee that approved validation of the name proposed by Kawasaki *et al*. (1992) seems to have overlooked some basic nomenclatural rules. Most importantly, Kawaski *et al*. (1992) did not discover a 'new' organism. They might have proposed their alternative name as 'comb. nov.' [for 'new combination' of terms (*combinatio nova*)], but this also would be incorrect since neither of their terms was used previously. In any event, they might have used the term 'nom. nov.' (*nomen novum*), but this requires reference to the original name as the basonym, which was not done. Such details are grist for the mill of taxonomists.

16S rRNA as the major criterion for nomenclature changes

Kawasaki *et al*. (1992) proposed changing the name of *Rhodospirillum centenum* largely on the basis of 16S rRNA sequence data. In the 16S rRNA tree they presented, however, the relatively low confidence levels do not exclude *Rhodospirillum centenum* and *Rhodospirillum rubrum* from the same clade. Moreover, as far as we know, there is no 'magic' percentage sequence similarity that justifies creation of a new genus. In this instance, creation of a new monotypic genus (*Rhodocista*) based on a trivial difference in 16S rRNA sequence does not contribute to a better understanding of the organism and can only lead to confusion in the literature. It should also be noted that in a phylogenetic tree generated by Kawasaki *et al*. (1993), *Rhodospirillum centenum* is in fact most closely related to *Rhodospirillum rubrum*, the type species of the genus. Using their logic, *Rhodospirillum* would also become a monotypic genus. Moreover, magnetotactic non-photosynthetic bacteria would have to be irrationally included in a genus with certain *Rhodospirillum* species.

Our criticism of using 16S rRNA sequences as the primary basis for changing names of bacteria is supported by new information on 'lateral genomics'. Thus, Doolittle (1999) has commented on how lateral (horizontal) gene transfer may have affected bacterial evolution: 'New genes from far away should impart new tempo and new modes in prokaryotic evolution. Laterally transferred genes, because they can confer radically new and complex phenotypes, might often result in adaptive radiations and the formation of new subpopulations (bacterial clades or even 'phyla')-perhaps in fact more often than can mutation and selection operating on already resident genes. Lawrence & Ochman (1998) could be right when they suggest that, unlike eukaryotic speciation, bacterial speciation might be ''driven by a high rate of horizontal transfer, which introduces novel genes, confers beneficial phenotypic capabilities, and permits the rapid exploitation of competitive environments.'''

The difficulties involved in defining bacterial genera and species are still with us (see Stackebrandt & Goebel, 1994) and have been added to by numerous recent proposals to change the names of various bacteria using 16S rRNA sequences as the primary basis for assigning taxonomic and phylogenetic relationships. Murray & Schleifer (1994) have pointed out that the International Code of Nomenclature of Bacteria 'is not able to provide sensible regulation of nomenclature for new taxa defined by very limited data, such as a nucleotide sequence for a small portion of the genome' (see also Murray, 1998, for a very useful summary of current problems and procedures in connection with taxonomy and nomenclature of bac-

teria). Despite the popularity of the notion that 16S rRNA sequences can suffice to define phylogenetic relationships with accuracy, the case has yet to be proven. In fact, this hypothesis has recently been weakened by new information indicating widespread occurrence of lateral gene transfer among bacterial species and genera. It seems likely that rRNA sequences will prove to be very useful eventually for identifying certain kinds of taxonomic relationships, but we believe it is doubtful that they will provide an unambiguous evolutionary phylogeny of bacteria.

'Does 16S rRNA provide all the answers?'

This question was posed by Gupta (2000), who concluded that 'the answers to some of the central issues in prokaryotic phylogenies will likely emerge from consideration of other molecular sequences and by alternate approaches'. It has become obvious that elucidation of the complex course of microbial evolution will require a number of alternative approaches, including one noted by Murray (2000): '… there are enough problems in unraveling evolution, establishing phylogenetic order, and in the interpretations required for making trees to make it truly important to be precise about the alternatives based on proteins'. A similar thought is embodied in remarks of Ernst Mayr (1998): 'Evolution is an affair of phenotypes. It is phenotypes, not genes, that are the objects (targets) of selection. This is now generally accepted by evolutionists after 50 years of controversy. Indeed, the significance of a molecular change is usually best indicated by its phenotypic consequences.'

How do new names enter the scientific literature?

Despite the International Code of Nomenclature of Bacteria and Validation Lists in the IJSB, new names appear in the literature as a result of passive acceptance by editors. A relevant comment by R. G. E. Murray (1998): 'There is no such thing as "official sanction" by any body; since the science and understanding is continually evolving, it would seem undesirable. Names are now validated to the extent that the requirements of the Code are met and assessment that the science is good. Even then, *what lasts is determined by general acceptance* [*emphasis added*], which is eventually summarized in compendia such as *Bergey's Manual* and *The Prokaryotes* in the authoritative secondary literature … we can only hope that the current validation process can be even more thorough and effective than it is now without imposing any further delay.'

We trust that the microbiological community will use common sense and continue to employ the designation *Rhodospirillum centenum* for an exceptional bacterium whose study can be expected to enrich our understanding of anoxygenic bacterial photosynthesis and photosensory behaviour of cells.

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