

Bacterial classification and taxonomy: a 'primer' for the new millennium

Howard Gest

The notion that a single molecular marker, 16S rRNA sequence, can serve to decipher the evolutionary phylogeny of bacteria has apparently led to significant effects on the minds of many investigators. These effects include: (a) a general amnesia about the long history of thought and debate on the classification/taxonomy of bacteria, (b) some confusion about the distinction between actual bacteria and what can be called 'virtual computer bacteroids', (c) a mental void on the long, rich and important history of research into microbial nutrition, which led to the elucidation of many basic principles of biology and cell biochemistry, (d) belief in new myths such as the recently trumpeted 'discovery' of a great diversity of free-living, 'uncultivable' microbes as indicated by molecular biological probes, and (e) implantation of the misconception that the classification of bacteria should be largely, if not entirely, based on evolutionary phylogeny.

The immediate inspiration for this article was a sentence used by Sydney Brenner (1997) in an imaginary letter sent to a mythical nephew, namely "I can't see the wood for the phylogenetic trees". To help see the 'wood', I have selected items from the extensive research literature that illustrate the problems encountered in the historical development of bacterial classification and taxonomy schemes. This 'primer' is offered as an elementary guide, as we enter the next century, to the somewhat chaotic status of this important aspect of microbiology.

● **The situation in 1946 as viewed by C.B. van Niel**
In 1946, the great microbiologist C.B. van Niel published a thoughtful essay on 'The classification and natural relationships of bacteria' in which he reviewed the history of earlier work aimed, in part, at developing a stable and generally accepted nomenclature that would eliminate duplication or multiplication of names for the same organism. He emphasized that even if we knew the phylogenetic relations among bacteria, a classification based on such relations would not necessarily be the best or most efficient for determinative purposes.

● **Changing names of bacteria**
Although determinative keys are very important in practical matters (for example in medical microbiology, public health microbiology and plant pathology), this tends to be forgotten by those probing evolutionary relations using molecular markers. The latter press for revised taxonomic schemes and this inevitably leads to proposals for changing names of bacteria.

Particularly egregious instances of name changing have afflicted the anoxygenic photosynthetic bacteria group. Example – The purple photosynthetic bacterium *Rhodocystis gelatinosa* isolated in 1907 was renamed *Rhodopseudomonas gelatinosa* in 1944, redesignated *Rhodocyclus gelatinosus* in 1984 and in 1991 the name *Rubrivivax gelatinosus* was proposed. The bacterium does

indeed hydrolyse gelatin, but these reincarnations do not reflect appreciable advances in an understanding of its basic features or evolutionary relationships. This example indicates how confusion is introduced into the research literature, text books and computer information retrieval. It is already happening.

● Genus and species

How are bacterial genera and species defined? An authority on this matter is *Bergey's Manual of Systematic Bacteriology*. The definitions are, in fact, a bit fuzzy, especially that of genus. From the *Manual*:

Genus: "The bacterial genus is usually a well-defined group that is clearly separated from other genera, and the thorough descriptions of genera in the 1984 edition of *Bergey's Manual* exemplify the depth to which this taxonomic group is usually known. However, there is so far no general agreement on the definition of a genus in bacterial taxonomy, and considerable subjectivity is involved at the genus level. Indeed, what is perceived to be a genus by one person may be perceived as being merely a species by another systematist."

Species: "A bacterial species may be regarded as a collection of strains that share many features in common and differ considerably from other strains... One strain of a species is designated as the type strain; this strain serves as the name-bearer strain of the species and is the permanent example of the species, i.e. the reference specimen for the name. The type strain has great importance for classification at the species level, because a species consists of the type strain and all other strains that are considered to be sufficiently similar to it as to warrant inclusion with it in the species..."

● Numerical taxonomy

In 1963, Sokal and Sneath described an application of the Adamsonian approach to taxonomy in which as many diagnostic characteristics as possible are used and given equal weight. The degree of relationship between organisms was considered to be a function of the number of similar characteristics and is expressed as a 'similarity coefficient.' The utility of numerical taxonomy has been demonstrated in a number of studies. Sneath reviewed the 30-year history of numerical taxonomy in 1995 and concluded that "Numerical taxonomy in the broad sense is the greatest advance in systematics since Darwin or perhaps Linnaeus. It has stimulated several new areas of growth, including numerical phylogenetics, molecular taxonomy, morphometrics and numerical identification." Even though numerical taxonomy does not assume phylogenetic relationships, it is obvious that close correspondence of a large number of phenotypic characteristics has something to say about genetic connections.

● Cowan's comments on taxonomy (1970)

"A hitherto undetected similarity exists between Lewis Carroll's *Alice and taxonomists, and bacterial taxonomists in particular... taxonomy can – and does – drive taxonomists to a topsy-turvy Wonderland...*" [Author's note: this paper was



based on a seminar *Alice in Taxonomyland* at the University of Maryland, 5 May 1969.]... “It is surprising how many so-called microbiologists look upon the schemes published in *Bergey’s Manual (1923–1957)* as if they were not only useful general classifications of bacteria, but ones that have received universal approval, both on earth and in heaven. I am assured by my colleagues that approval of *Bergey’s Manual* is not universal, even on earth; I am not yet able to judge its reception in heaven...”

Cowan pointed out that elaborate rules have been stipulated in codes of nomenclature in the attempt to regulate the formation and use of names, “but these codes would delight the hearts of lawyers because they are too detailed and try to cater for all eventualities. In the event, they are confusing and self-contradicting...”

“The Bacteriological Code [i.e., *International Code of Nomenclature of Bacteria*] should be simplified by deleting the Rules and Recommendations. It should consist of Principles, and discretion should be given to bacteriologists to apply them intelligently.” The magnitude of the problem that Cowan discussed is indicated by List no. 22 published in the *International Journal of Systematic Bacteriology* (36, 1986), which contains 44 new names, new combinations, synonyms or revived names.

● Example of a rule from the Code

“Rule 56b. A conserved name (*nomen conservandum*) is a name which must be used instead of all earlier synonyms and homonyms. Note 1. A conserved name (*nomen conservandum*) is conserved against all other names for the taxon, whether these are cited in the corresponding list of rejected names or not, so long as the taxon concerned is not united with another taxon bearing a legitimate name. In the event of union or reunion with another taxon, the earlier of the two competing names is adopted in accordance with Rules 23a,b.”

The intent of this rule, to stabilize nomenclature, is excellent and is quoted here only to illustrate the quasi-legal (sometimes confusing) language of the Code.

● Sneath’s 1989 assessment of the utility of molecular sequence markers in classification and taxonomy

“A major difficulty with currently available molecular sequences is the fact that they are only samples of relatively small size. Sampling error will therefore continue to dominate the picture...”

One area that is now being explored is the use of molecular ‘signatures’, – small sub-sequences that are characteristic of various bacterial groupings. Their value will depend considerably on how constant these signatures are within the newly defined bacterial taxa. At present there has been no detailed analysis of this, though some within-taxon variation is evident...

Another more pragmatic question is how far we can safely revise bacterial classifications on present sequence data. In many of the earlier papers on rRNA, strain numbers were seldom given, so we do not know which were type strains. Most workers examined only a single strain of a taxon such as a genus, yet the analyses were displayed as representative of the entire taxon – sometimes when the

validity of adjacent taxa was being questioned. Many genera are heterogeneous from the molecular viewpoint and this would imply that all their species must be studied before deciding on the new disposition of the genus. This still leaves unanswered the question of how homogeneous the species are.”

● More on molecular data

In 1994, Murray and Schleifer pointed out that the international nomenclature code “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. The constructors of the original code (1957) and the Judicial Commission considering the 1976 and 1990 revisions did not foresee or act upon the possibilities for molecular description and typification of prokaryotes that were not yet cultivable. As a result, formal names are being proposed for uncultivated prokaryotes whose uniqueness is defined only by very limited characteristics, such as differences in a molecular sequence...”

A novel sequence isolated from nature merely indicates that there may be a unique organism in the environment. However, one has to take into consideration that sometimes sequencing and/or amplification errors simulate a novel sequence, suggesting some intraspecies diversity, or that formations of chimeric sequences may be possible. Moreover, the possibility that the sequence was retrieved from cell-free (naked) DNA cannot be excluded, and it may have originated from an organism not originally growing in the examined habitat...”

In fact, it was recently demonstrated in an ‘environmental ecology’ study, that RNA sequence data supposedly indicating the presence of certain bacteria in natural samples were due to contaminating 16S rDNA introduced in the exquisitely sensitive polymerase chain reaction.

● Concluding remarks

The assumption that our current knowledge is sufficient to warrant new, presumably ‘final’, names for many genera and species is obviously questionable and arrogant, and evokes Cowan’s admonition: “It is often easier to create a new genus or species than to do the comparative work necessary to put an organism into its rightful place in an existing genus or species. The temptation to designate a new genus or species should be resisted.” Obviously, as knowledge advanced, numerous name changes were proposed and many were reasonable and useful, for example the change from *Streptococcus lactis* to *Lactococcus lactis*. During the past two decades, however, the notion that a single molecular marker can accurately reveal evolutionary phylogeny has driven a deluge of premature name changes. Since recent research suggests that microbial evolution was far more complex than commonly supposed, and probably involved extensive lateral gene transfer, conservatism in nomenclature changes seems well advised.

Carl Linnaeus originated the systematic classification of plants and animals. In 1737, before microbiology became a science, he said “What difficulty has been caused to botanists from the revival of the sciences down to the present day by the invention of new names is known to everyone who has handled the subject.” Alas,

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In 1949, while still a graduate student, Professor Gest made the unexpected discovery that anoxygenic photosynthetic bacteria fix molecular nitrogen. He and his colleagues have isolated and characterized a number of new species of purple and green anoxyphototrophs, including the remarkable bacterium *Rhodospirillum centenum*. The latter was isolated in 1987 and given the species name in commemoration of the fact that it was discovered 100 years after the first isolation of a photosynthetic bacterium in pure culture (*Rhodospirillum rubrum*). When grown on agar, *R. centenum* cells produce numerous lateral flagella and the swarming colonies exhibit phototaxis (the first known example in such organisms). In conversation, Professor Gest refers to over-zealous bacterial taxonomists who would like to change the name of *R. centenum* as 'taxonomic pirates'.



microbiology today is faced with this old problem. Santayana's 1905 observation is timely: "Those who cannot remember the past are condemned to repeat it."

There is every reason to believe that as current genome sequencing projects mature and expand, we will be in a much better position to evaluate organismal relationships and deal with classification/taxonomy schemes more effectively. In the meantime, readers are referred to a very useful summary by R.G.E. Murray (1998) of current problems and procedures in dealing with taxonomy and nomenclature of bacteria.

Attention is also directed to a thoughtful review by Palleroni (1997) that discusses the limitations in using only molecular data for analysis of prokaryote systematics. On the brink of the new millenium, the significance of 16S rRNA as a major taxonomic criterion is receding. At the same time, recognition of lateral (horizontal) gene transfer as an important element in prokaryote evolution is gaining momentum (Lake *et al.*, 1999).

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