Implications of alternative classifications and horizontal gene transfer for bacterial taxonomy

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Following the publication of the Approved Lists, there has been a tendency to regard all subsequent revisions of classification as providing improved nomenclature, to be accepted without question. This takes no account of the fact that such revisions may be based on one of three alternative concepts, phenetic, phylogenetic or polyphasic classification, sometimes leading to different, valid, but incompatible nomenclature, or that some investigations are based only on subsets of relevant taxa and on limited data, leading to incomplete and sometimes confusing revisions of nomenclature. The polyphasic approach to classification has widespread support, although there appears to be a tendency to allow comparative sequence analyses of 16S rDNA to determine classification contrary to the indications of other data. In some cases, classification is based solely on 16S rDNA data. Examples are considered. Consideration is given to the criteria by which taxa are circumscribed, particularly at the level of genus and species. It is suggested that there is a need for reconciliation of the criteria by which taxa at these levels are circumscribed. Recent studies demonstrating the widespread occurrence of horizontal gene transfer suggest that there is a need for caution in monophyletic interpretations, especially when these are based on the analysis of single sequences.

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Introduction

Although Staley & Krieg (1984) wrote that ‘bacterial classifications are devised for microbiologists, not for the entities being classified’, bacteriologists do not necessarily agree about the aims of classification. The several systems of bacterial classification that are in vogue at present are incompatible, sometimes leading to different classifications and to different schemes of bacterial nomenclature. Unless the principles underlying different classifications are understood, these different applications of names cause bewilderment. The thrust of this article is to show that no one system of classification is supreme, each offering a different perspective on evolutionary processes and serving different purposes in systematics. One problem that particularly besets discussions on bacterial taxonomy is that some terms derived elsewhere and often in the distant past are poorly defined or are used in different ways by different writers. For example, see the history of the usage of ‘polyphasic’ described below. Ambiguity and lack of precision of usage are real causes of confusion. In attempting to maintain simplicity without confusion, terms are referred to Cowan (1978) and Lincoln et al. (1998) unless further explanation is felt necessary. Terms used here are not inconsistent with Tindall (1997).

In 1980, the Approved Lists (Skerman et al., 1980) recorded 1792 validly published species names in 290 genera (6–2 species per genus) (Euzéby, 1997). One expectation of modern systematics has been that the increasing numbers of methods and sophisticated tools for analysis would form congruent circumscriptions of taxa; that, in future, genera, species and, perhaps, higher taxa would be more precisely and clearly circumscribed and that methods would be available for allocation of isolates to those groups (Murray et al., 1990; Young et al., 1992; Goodfellow & O’Donnell, 1993; Vandamme et al., 1996; Goodfellow et al., 1997). Of the new species and genera proposed since 1980, many are the result of revisions of existing...
taxa, with a trend towards subdivision of previously published genera. This is indicated by the fact that, by January 2001, an estimated 4518 species in 998 genera (4-5 species per genus) had been validly described (Euzéby, 1997).

Approaches to classification

In the period of development of the Approved Lists, bacterial systematics was guided almost entirely by phenetic concepts of classification. After 1980, with the flourishing of molecular methods, particularly the analysis of DNA by hybridization and sequencing, classifications based on the inference of phylogenetic relationships became popular. More recently, attention has turned to polyphasic taxonomy as a preferable basis for classification (Goodfellow & O'Donnell, 1993; Vandamme et al., 1996).

Phenetic classification. The goal of phenetic classification is to create clustered groups of strains, established as a hierarchy of species and genera on the basis of their overall similarities (Cowan, 1978; Goodfellow & O'Donnell, 1993; Lincoln et al., 1998). Members of a species share high levels of similarity and are more similar to species in their own genus than to species of other genera. At every taxonomic level, taxa share common characters and can be circumscribed by a description that distinguishes their members from others at the same level. Phenotypic data include single-character data and multiple-character data. Single-character biochemical and physiological reactions (staining reactions, cell and flagellar morphology, pigment production and biochemical and nutritional characters) describe a small component of the total bacterial phenotype. Early phenetic classifications relied on data provided by single-character tests. The approach at that time, even involving numerical methods of analysis (Sokal & Sneath, 1963; Sneath, 1985), produced overly simple and not very accurate classifications because expression was given only to a relatively small proportion of the total bacterial phenotype. Even so, at the species level, many taxa based on these methods have been supported by more comprehensive studies (see below). It is at the generic level and above that single-character tests are found to be inadequate when more extensive data are included. Multiple-character tests [also called polyphasic tests by Young et al. (1992) and including chemotaxonomic tests], such as comparisons of cell wall composition, fatty acid and protein profiling, isoprenoid quinone and DNA composition and DNA and RNA sequences when considered as part of the bacterial phenotype, have the advantage that large components of a phenotype could be compared directly. Some multiple-character tests can also be analysed and interpreted by quantitative, numerical methods (Sneath, 1985; Kersters, 1985). For multiple-character methods, particularly those involving the capture of data by gel scanning, problems of standardization still limit their portability. In spite of this limitation, such methods are a step towards the phenetic ideal of comparing total bacterial phenotypes and have played an important role in circumscribing specific bacterial groups. The strength of nomenclature based on phenetic classification is that it allows users to make predictions about the characters of related taxa (Heslop-Harrison, 1962; Goodfellow & O'Donnell, 1993). Phenetic classification can be considered to indicate evolutionary relationships in the sense of showing the end product of evolution (Cowan, 1978). However, it does not have specific evolutionary implications, accepting the possibility that there may be taxa whose phylogenetic relationships can no longer accurately be ascertained, and takes no account of the effects of parallel or convergent evolution upon taxonomic interpretations.

Phylogenetic classification. An alternative goal of systematics, which has been widely adopted in recent years, is the creation of a phylogenetic classification of bacteria based on ancestral relationships (Woese, 1987; Swofford et al., 1996). Surprisingly, the term phylogeny is rarely defined precisely. A central outcome of phylogenetic classification is that taxa be monophyletic. By this, it is meant that all members of any taxon under consideration shall share the same common ancestor. A further requirement is that taxa sharing more recent common ancestry in time or in surrogates of time (Sneath, 1988) are considered to be more closely related to one another than they are to other taxa (Lincoln et al., 1998). Without this requirement, phylogenetic classifications of different lineages could not be calibrated; inferred phylogenies that placed the origins of (say) the Archaea before the Cambrian or in the Quaternary would have equal validity. Microbes have left no detailed fossil record, and phylogenetic inferences depend almost entirely on the indirect evidence provided by the analysis of sequence data that are considered to express rates of genetic change with time; for micro-organisms, it is assumed that changes in molecular sequences can be used to infer reliable historical relationships. Estimates of time are usually given as expressed rates of nucleotide base mutation. This assumption, together with the ready ability, by using PCR, to amplify conserved 16S rDNA or 23S rDNA found universally in bacteria and the ability to sequence this DNA, has seen an explosion of phylogenetic studies. The comparative analysis of rDNA sequences has seemed to provide a key to the systematization of relationships at the generic level and higher taxonomic levels (Stackebrandt & Woese, 1984; Woese, 1987). It was this phylogenetic philosophy and these molecular methods that were responsible for many recent revisions. However, although rDNA is ubiquitous, this is not, of itself, the basis for asserting that such analyses give accurate phylogenies, particularly at lower taxonomic levels (Sneath, 1989). A disconcerting aspect of phylogenetic inference is that reliance cannot be placed on any particular sequence comparison, because
analyses give differing results depending on the algorithm chosen and particularly on the selection of included sequences. In addition, phylogenetic inferences based on 16S rDNA are supported by only a small number of other gene sequences (Ludwig, 1999; Ludwig & Schleifer, 1999). Notwithstanding these issues, there remains widespread confidence in 16S rDNA as a reference for phylogenetic inference (Vandamme et al., 1996; Ludwig & Schleifer, 1999).

Increasingly, however, it is recognized that analyses based on this single sequence should be supported by other sequence data. Certainly, phylogenetic classifications have led to radical proposals for the reclassification of taxa and these have been supported by phenotypic data. For instance, the confirmation, by molecular methods, of the blue-green algae as photosynthetic bacteria and their transfer from the Cyanophyceae to the Cyanobacteria is perhaps the best example. This has led to the assumption that, where phenotypic support has not been obtained for phylogenetic classifications based on sequence analyses, future phenotypic studies will provide justifications. However, unless evolutionary change occurs at similar rates in different phylogenetic branches, concordance of phylogenetic and phenetic taxonomic groups cannot always be expected (Young et al., 1992).

Phenotypic similarity does not necessarily reflect phylogenetic relatedness. Where congruence between phylogenetic relatedness and phenotypic similarity is not established, it follows that robust phenotypic descriptions will not be derived for taxa established by phylogenetic inference. In practice, establishing genera and species on the basis of genomic data for phylogenetic classification has weakened the emphasis on phenotypic descriptions for taxa (Young et al., 1992; Young, 2000).

**Polyphasic classification.** Colwell (1970) introduced the term *polyphasic taxonomy* to refer to classifications based on a consensus of all available methods: single-character tests, as well as multiple-character tests, including phenotypic and genomic data. There appears to be no difference in principle between *polyphasic taxonomy* (Colwell, 1970) and *phenetic classification* as defined by Cowan (1978) or used subsequently (Young et al., 1992; Goodfellow & O’Donnell, 1993; Goodfellow et al., 1997). By contrast, Vandamme et al. (1996), reinterpretting Colwell (1970), who made no reference to phylogenetic interpretations, proposed *polyphasic taxonomy* for systematics processes that generated classifications based on a consensus of data gathered by all available methods ‘that would be consistent with phylogenetic classification’. In their view, phylogenies were to be established using 16S rRNA or 23S rRNA sequence data. Nevertheless, their account is open to a number of interpretations, of which two are discussed here. In the first, they may derive taxonomic relationships, the distribution of genera and of species in genera, on the basis of the analysis of sequence data as phylogenetic inference, and only take account of phenotypic data that are consistent with such analyses (interpretation 1). In the second, they may interpret polyphasic taxonomy as meaning that, at the higher levels of phylum, division, class, order and family, taxa are ordered in phylogenetic terms (based on sequence analysis) but at lower levels, at species and perhaps genus levels, taxa are established as phenetic groups based on a consensus of phenotypic data (interpretation 2). If they take this latter view, then they appear to have the support of Murray et al. (1990) and Mayr (1998) in their description of *Darwinian taxonomy* and of Colwell (1970) for species and genera in her original interpretation of polyphasic taxonomy. Both Murray et al. (1990) and Mayr (1998) emphasized the importance of integrating phenotypic data as support for taxa, with the proviso that integration was consistent with monophyletic classifications; that groups of taxa were descended from a single ancestral taxon. This interpretation makes the tacit assumption that phenotypically similar organisms will be closely related in phylogenetic terms, which assumes of necessity that evolutionary rates of character changes are similar. Although this assumption is not necessarily supported by observation, it may be a sensible approach to bacterial classification if it is understood that accurate phylogenetic inferences may be impossible to achieve in consistent and reliable ways and that such syntheses will usually be unable to take account of all available information. The implications of these alternatives can best be understood by consideration of examples where the process has specifically been applied, as will be discussed later.

**Species and genus concepts.** The basic biological unit for asexually reproducing organisms such as bacteria is the clonal genomic group. Clonal groups collectively form distinguishable taxa that are based on shared phenotypic characters. The circumscriptions of these taxa, like the higher taxa of sexually reproducing organisms, are arbitrary. Nevertheless, evolutionary diversification is assumed, and does appear to a greater or lesser degree, to have resulted in organisms expressed as an array of taxa in a series of phenotypic hierarchies. It is on the understanding that such arrays exist that modern taxonomy, including that of micro-organisms, is conducted. Modern taxonomy entails the systematic cataloguing of taxa to reflect their phenotypic relationships, underpinned by their phylogenetic (monophyletic) relationships (Murray et al., 1990; Mayr, 1998). The catalogue is of the phenotypic diversity. As greater attention was turned to genomic and multiple-character methods, so Wayne et al. (1987) attempted to give guidance to the practices associated with the proposal of new species. They suggested a quantitative definition of the bacterial species as the population whose strains share more than 70% DNA–DNA hybridization and have a $T_m$ of less than 5 °C. This definition has come to form the standard for species circumscriptions in the past decade. Wayne et al. (1987) did not choose this reassocation value for arbitrary reasons. They did so...
because this reassociation value seemed to discriminate species already well established on phenotypic assessments. Wayne et al. (1987) also urged, almost incidentally, that hybridization data be supported by phenotypic data; like Murray et al. (1990), they anticipated that phenotypic data would support the genomic framework, that there would be a congruence of systematics using different methods and that this would give expression to phylogenetic relationships. Significant difficulties for DNA–DNA reassociation studies are the high level of experimental error (Sneath, 1989) and the intractability of the method, coupled with the need to include increasing numbers of species in hybridization experiments as they are allocated to genera (Young et al., 1992). The implications of and problems with this species standard have been discussed (Young et al., 1992; Palys et al., 1997). These problems are a serious hindrance to the naming of new species.

Bacterial taxa above species are also formally recognized when they can be represented by unique phenotypic circumscriptions. Although there are no specific requirements for the descriptions of genera, there is a requirement that Minimal Standards for genera provide phenotypic circumscriptions. For example, for the genus Rhizobium (Graham et al., 1991) and the genus Staphylococcus (Freney et al., 1999), comprehensive accounts of the principles and procedures for generic circumscriptions are presented. At higher taxonomic levels, phenotypic circumscriptions become increasingly general. Thus, for Divisions, the sole specific phenotypic criterion is cell wall structure, as expressed by the Gram reaction (Gibbons & Murray, 1978). Only for the class Proteobacteria is there as yet no phenotypic circumscription (Stackebrandt et al., 1988). However, Murray et al. (1990) made it clear that there is an expectation that, at the generic level at least, taxa should be supported with phenotypic descriptions. Their note on Taxonomic Consequences is worth quoting in full. ‘The first step in the identification of bacteria is the assignment of organisms to genera. Therefore, the greatest clarity in circumscription and utility in the choice of characteristics must be accorded to the level of genera. It is completely impracticable to define genera solely on the basis of phylogenetic data. Genera need to be characterized by using phenotypic properties, even if the choice of phenotypic markers might change given the development of better tests. A degree of flexibility is necessary in the definition of genera. In cases in which there is disparity between phylogenetic and phenotypic data, priority should be provisionally given to the latter. In such instances, further detailed comparative studies of the phenotype should be encouraged to resolve the apparent disparity so that classification reflects phylogenetic relationships.’

Considering their respective emphases of a species definition based on phylogeny and a generic definition based on phenotype, it is hard to read the papers of Wayne et al. (1987) and Murray et al. (1990) in conjunction (written by a largely common authorship with an interval of 3 years) without concluding that there is a need for reconciliation into a single rationale of the principles that guide species and generic circumscriptions.

**Polyphasic classification in practice.** In the examples below, applications are given of polyphasic taxonomy in proposals published in the *International Journal of Systematic Bacteriology*. These are considered because of their influence as precedents for the interpretation of this methodology.

(i) **Delftia** is the validly published, monospecific name for the genus comprising *Delftia acidovorans* (Wen et al., 1999). By comparative analysis of 16S rDNA sequence, the genus is represented by a single sequence as an outlier of the genus Comamonas. There is no generic circumscription for the new genus. In proposing this new genus, Wen et al. (1999) offered no evidence or rationale for distinguishing *Delftia* as a genus separate from *Comamonas* except for suggested ‘deeply rooted’ branches between *Comamonas* species. Because the genus is defined by sequence analysis alone as a monospecific taxon separate from other individual *Comamonas* species, acceptance of *Delftia* as a validly published genus name means that there is no rational basis for choosing between the following alternatives: (1) *Delftia acidovorans* and the remaining *Comamonas* species, (2) further renaming of all *Comamonas* species in novel, monospecific genera or (3) renaming all present members of *Comamonas*, except for the type species, in *Delftia*. These alternative proposals may seem bizarre, but they are no less rational than the original proposal of *Delftia*. Without independent support for each genus as a distinguishable taxon, all three alternatives have equal claim.

(ii) **Allorhizobium** is a monospecific genus, validly published by de Lajudie et al. (1998b), comprising the nitrogen-fixing species *Allorhizobium undicola*. Comparative analysis of 16S rDNA sequence data show the organism as an outlying branch of the Agrobacterium–Rhizobium cluster of species. Its nearest neighbour is *Agrobacterium vitis*. Considering the description of *Allorhizobium undicola*, it is well supported as a species distinct from *Agrobacterium vitis* (and from all other members of Agrobacterium–Rhizobium) by DNA–DNA reassociation data, SDS-PAGE of proteins, PCR-RFLP of the internal transcribed sequence (ITS) region between 16S rDNA and 23S rDNA and nutritional data. Inspection of SDS-PAGE, ITS and nutritional data give no support for a closer relationship between *Agrobacterium vitis* and *Allorhizobium undicola* than to other species in Agrobacterium, Rhizobium, Sinorhizobium or Mesorhizobium. The generic relationships proposed between these species are therefore based entirely on the comparative 16S rDNA sequence data; on a perceived low percentage similarity (95.5%) and the lack of bootstrap support for the branch. However, reliance cannot be placed on any particular sequence comparison,
because analyses give differing results depending on the algorithm chosen and particularly on the selection of included sequences. This is shown by comparison of inferred phylogenies in recent reports (Amarger et al., 1997; Chen et al., 1997; de Lajudie et al., 1994, 1998a, b; Nour et al., 1994; Rome et al., 1996; Tan et al., 1997; van Berkum et al., 1998; Wang et al., 1998; Willems & Collins, 1993). The reasons given by de Lajudie et al. (1998b) for proposing the new genus were the unsettled state of Agrobacterium nomenclature and the evidence of heterogeneity (in phylogenetic terms) in Rhizobium, which implied to them the need for the creation of a new genus separate from Agrobacterium and Rhizobium. Allorhizobium undicola shares a common generic circumscription with Agrobacterium vitis and with all other members of Agrobacterium and Rhizobium. Acceptance of Allorhizobium undicola requires revision of the outlying group of Rhizobium species, and of Agrobacterium vitis, according to one of several possibilities: (1) proposal of the genus Allorhizobium to include Allorhizobium undicola, Agrobacterium vitis, Rhizobium galegae and Rhizobium huaultense; (2) amalgamation of Allorhizobium undicola and Agrobacterium vitis in Allorhizobium and the creation of a new genus to recognize Rhizobium galegae and Rhizobium huaultense; or (3) proposal of a new genus for Agrobacterium vitis as a new monospecific sister genus with Allorhizobium (and the consequent creation of a new genus to recognize Rhizobium galegae and Rhizobium huaultense). Indeed, these nomenclatural iterations can be extended logically across Agrobacterium, Rhizobium and Sinorhizobium. In the absence of distinct generic circumscriptions for each genus, there is no rational way to choose between these alternatives.

(iii) Pantoea was proposed by Gavini et al. (1989) as a genus to distinguish strains previously included in Erwinia as Erwinia herbicola (syn. Enterobacter agglomerans). The plant-pathogenic species Erwinia ananas and Erwinia stewartii were subsequently included in the genus by Mergaert et al. (1993). Although all species were well defined, neither when the genus was first proposed, nor subsequently, has a circumscription of Pantoea been published that distinguishes it from Erwinia. Recently comparative 16S rDNA sequence data were reported that showed that Pantoea species formed a cluster contiguous with Erwinia species (Kwon et al., 1997; Hauben et al., 1998). Percentage similarity values for sequence data within and between individual species for the two genera were usually 95–98%. Bootstrap support for the clusters was not provided.

(iv) Hauben et al. (1998) proposed a revision of the genus Erwinia. They transferred Erwinia cacticida, Erwinia carotovora, Erwinia chrysanthemi and Erwinia cyripedii to an emended genus Pectobacterium and proposed a new genus, Brenneria, for Erwinia ali, Erwinia nigrifluens, Erwinia paradisiaca, Erwinia quercina, Erwinia rubrifaciens and Erwinia salicis. Percentage similarity values for sequence data within and between individual species of the Brenneria–Erwinia–Pantoea–Pectobacterium cluster were usually 95–98%. Included in the phylogenetic dendrogram were sequences located between the Erwinia–Pantoea cluster and the Pectobacterium cluster, representing the genera Citrobacter, Enterobacter, Escherichia, Klebsiella, Salmonella and Shigella. Sequences representing Ewingella, Hafnia, Rahnella and Yersinia were between Brenneria and the other genera. Hauben et al. (1998) only provided percentage similarity data for Erwinia, Pantoea, Pectobacterium and Brenneria, but it is axiomatic that the interleaved genera must have equivalent values (95–98%). Bootstrap support for the clusters was not provided. No individual circumscriptions for the new genera were proposed. The animal-pathogenic genera Salmonella, Escherichia/Shigella, Enterobacter, Klebsiella and Yersinia and the other distinct genera, Citrobacter, Ewingella, Hafnia and Rahnella, can be discriminated on the basis of distinct phenotypic characters. Although a polyphasic framework is considered to be an ideal, the classification proposed is based on the phylogenetic analysis of data (Hauben et al., 1998) using the neighbour-joining algorithm alone. Few phylogeneticists would now accept this method as the sole arbiter of bacterial classification. More recently, Spröer et al. (1999), in a study of a similar selection of sequences from the Enterobacteriaceae, reported Brenneria and Pectobacterium as a single cluster, indicating the fragility of analyses at this taxonomic level. There is no phenotypic basis for the differentiation of the plant-pathogenic genera Brenneria or Pectobacterium from Erwinia. Young et al. (1992) noted the need for a comprehensive revision of the Enterobacteriaceae, which has not yet been accomplished.

(v) In a revision of Rhizobium, the new genus Sinorhizobium was proposed for Sinorhizobium fredii and Sinorhizobium xinjiangense by Chen et al. (1998) on the basis of nutritional and biochemical tests. The validity of the genus was questioned by Jarvis et al. (1992) on the basis of partial 16S rDNA analysis and on the interpretation of numerical data. This genus has since been examined in greater detail and an emended circumscription of the genus has been produced (de Lajudie et al., 1994), together with the proposal of additional species. However, this circumscription does not delineate a taxon distinct from Agrobacterium or Rhizobium and the data reported for different methods (PAGE of total proteins and carbon-source utilization tests) did not support the genus as a coherent taxon; that is, the protein data showed Sinorhizobium fredii (the type species) as an outlier to the other species, but carbon-source utilization data showed Sinorhizobium species intermingled with Azorhizobium, Bradyrhizobium and Rhizobium. Similar incoherence of the genus was demonstrated by the data of de Lajudie et al. (1998b). Fatty acid data (Jarvis et al., 1996) indicate a single cluster of species, representing Agrobacterium, Rhizobium and Sinorhizobium. With the exception of a
quantitative difference in a single fatty acid (Tighe et al., 2000), support for Sinorhizobium as distinct from Rhizobium is on the basis of comparative 16S rDNA sequence data alone (de Lajudie et al., 1994, 1998b).

Most of these examples are specifically cited as involving the application of polyphasic taxonomy. The genera described are based on inferred phylogenetic (monophyletic) clusters without circumscriptions; it is only at the species level that taxa are supported by phenotypic data. In these cases, it is the first interpretation of Vandamme et al. (1996) that has been adopted.

The genera described in the examples above can therefore be regarded as evolutionary clusters rather than as adequately proposed taxa. Proposed monophyletic clusters cannot, of themselves, be considered as taxa, because they do not have circumscriptions; we do not know, except arbitrarily, where such a taxon begins and ends (Mayr, 1998). These examples highlight an increasing tension developing between nomenclatural proposals based on inferred phylogenetic proposals, usually based on comparative analyses of 16S rDNA data alone, and nomenclature based on variations of polyphasic classification (Young et al., 1992; Goodfellow & O'Donnell, 1993; Goodfellow et al., 1997) or Darwinian interpretations (Mayr, 1998).

**Horizontal gene transfer**

A basic assumption of phylogenetic inference is that bacteria taxa are monophyletic and that there is not sufficient exchange of genetic material between unrelated taxa by recombination or transduction events significantly to obscure phylogenetic interpretations. However, if it is a widespread phenomenon, genetic recombination between unrelated bacteria could confound orthodox taxonomic interpretations. Transfer of genes between unrelated bacteria was observed as long ago as 1928 (Veal et al., 1992). Antibiotic and heavy-metal resistance and the transfer of nitrogen-fixing genes have long been recognized as commonly occurring events. The mechanisms by which exchange occurs in nature, for example direct exchange of genes between bacteria or exchange mediated by infectious viruses, have been the subject of intensive study. In general, gene transfer has been considered to be confined to the exchange of small, adaptive, functional genes (Goodfellow et al., 1997). However, increasing numbers of reports suggest that genetic interchange may be more common than has been appreciated (Cohan, 1994, 1996; Jaenecke et al., 1996; Lorenz & Wackernagel, 1994). Recently, detailed evidence of sequence similarities, implying gene homologies, in unrelated bacteria has raised questions as to the relative significance in bacterial evolution of mutation lines as compared with horizontal gene transfer. For example, homologous hrp genes, implicated in the pathogenicity and resistance mechanisms that regulate the interactions between bacterial pathogens and their plant and animal hosts (Gabriel, 1999; Gough et al., 1992), are found in separate subclasses of the Proteobacteria. These observations are explained more readily by horizontal gene transfer than by lineal gene inheritance. Sequence discontinuities in Escherichia coli also suggest gene transfer (Milkman & Bridges, 1993) and Haubold & Rainey (1996), studying Pseudomonas, concluded that there was frequent large-scale genetic transfer between strains. This is supported further by analysis of the complete sequence of the Escherichia coli genome (Lawrence & Ochman, 1998), from which it was inferred that ~18% of the genome resulted from the stable integration of transferred genes. The presence of unexpected sequences in particular bacterial species (Lan & Reeves, 1996; Sagerström et al., 1997) is strong support for exchange. An integrase gene system with the specific function of facilitating gene exchange has been reported to be widespread in bacteria (Hall & Collis, 1995; Mazel et al., 1998), together with evidence for the presence of introduced functional genes. Perhaps most striking, the transfer of parts of 16S rRNA sequences has been suggested between genomic species in Aeromonas (Sneath, 1993); reports that the highly conserved state of 16S rRNA makes it a prime candidate as a vector for gene transfer (Strätz et al., 1996) and evidence for its recombination (Wang et al., 1997; Yap et al., 1999) are especially thought-provoking. The exchange of house-keeping genes or of extended chromosomal sequences containing large numbers of genes that are central to bacterial metabolic activity has not been documented extensively, although there are tantalizing indications of this possibility (Syvanen, 1994). Genetic exchange representing more than 5% of the bacterial chromosome and involving most of the genes associated with symbiosis in nitrogen-fixing bacteria has been demonstrated in nature (Sullivan & Ronson, 1998) and in the pathogenicity islands reported for species in the Enterobacteriaceae. Recently, Dubnau (1999) reviewed the specific mechanisms of DNA uptake by Gram-positive and Gram-negative bacteria and concluded that their most likely function was to promote genetic and hence phenotypic diversity in order to maintain the evolutionary fitness of populations.

**Evolution and classification**

There is nothing in evolutionary theory to contradict the possibility of exchange of any component of the bacterial genome. In fact, theory and observation both support the promiscuous exchange of any and all genes (though not between any and all bacterial taxa, and not leading inevitably to expression), providing a potential for adaptability to novel environments by the recipient bacteria. If horizontal gene transfer is a significant factor in bacterial genetics, bacterial genera and species might better be viewed as groups of organisms that share a common core of chromosomal structures (whose stability is maintained by environmental selection) while individually having the potential to receive genes for many possible metabolic processes from unrelated bacteria. If this is the case,
the current view of bacterial evolution and diversity, and the assumptions supporting bacterial classifications based on monophyly, may well need re-evaluation (Syvanen, 1994). As yet, there is insufficient evidence from which to determine the significance for bacterial systematics of horizontal gene transfer, although it is regarded by molecular ecologists as a natural explanation of commonly observed, systematics-related phenomena involving gene variation. Its consequences for taxonomy can no longer be ignored.

A major consequence of modern systematic analyses has been to expose the two contrasting poles of our assumptions concerning bacterial evolution. Phylogenetic systematics and classification assume the monophyletic origins of present day taxa and our ability to trace their ancestry, in the way that is supposed to be the case for higher organisms. For this to be possible, evolutionary change is assumed to occur through the natural selection of mutations occurring in a relatively stable genome, and environmental forces function primarily to induce change.

According to this view, stability is a function of genome structure and evolutionary change is the result of environmental pressures. An alternative view is that the bacterial genome is highly mutable, both because mutation rates are high and because horizontal gene transfer is a common activity involving all sites of the genome. According to this view, natural selection plays a primary role both in maintaining the stability of observed taxa, sometimes as unrecognized polyphyletic entities, and in producing adaptational changes that lead to the evolution of new taxa. [This concept is not new; it was proposed by Baas Becking (1934) – ‘Everything is everywhere, but the environment selects’ – who attributed the idea to Beijerinck (1913). While these authors were surely referring to species diversity, the application to the diversity of genes follows from their assumptions.] For phenetic, polyphasic and phylogenetic classifications, it is assumed that the phenotype and genotype are relatively stable in the face of environmental selection pressures. If variability is high, the natural hierarchies expressed in different classifications will prove to be not as coherent as has been assumed in the current development of nomenclature. Systematic interpretations based on assumptions of genotype and phenotype stability may have to give way to those predicated on assumptions of greater plasticity.

Epilogue

Schleifer & Stackebrandt (1983) noted that ‘modern systematists should not be content to work with two types of classification: an artificial one for practical purposes – and a natural one with no practical application’. This is an artificial dichotomy. Phenetic, phylogenetic and polyphasic classifications are all natural in the sense that they reflect evolutionary trends according to different criteria. One solution to the problem might be to insist that, for the purposes of formal nomenclature, one system of classification should form the basis of interpretation. The polyphasic (Colwell, 1970; Young et al., 1992; Goodfellow et al., 1997), Darwinian (Mayr, 1998) approach might be adopted. If it was, there would be a need for clear definition and rigorous scrutiny of proposals to ensure that they were consistent with this classification in future. The requirement that one system of classification be the basis of formal nomenclature is not supported by the International Code of Nomenclature of Bacteria (Lapage et al., 1992), which is neutral in this regard; nevertheless, it appears to find support with some practitioners of bacterial systematics. An alternative view is to accept all taxonomic approaches and to recognize that there can be more than one legitimate classification, each with its implied nomenclature. In this case, the application of classification may need to be viewed more flexibly and account taken of nomenclature based on these different assumptions. For applied bacteriologists, who depend on nomenclature generated by systematics specialists, a proper caution, even scepticism, in accepting new proposals in nomenclature at face value as definitive will not be out of place. Systematists may need to give more consideration to the practical impact of nomenclatural revisions based on partial information and to avoid the dogmatism of asserting that any classification, phenetic, phylogenetic or polyphasic, is paramount, or that they offer more than interim answers to questions of relationship.

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