Other

Classification of Procaryotic Organisms and the Concept of Bacterial Speciation

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Taxonomy is the science of classification of organisms. Bacterial taxonomy consists of three separate, but interrelated areas: classification, nomenclature, and identification. Classification is the arrangement of organisms into groups (taxa) on the basis of similarities or relationships. Nomenclature is the assignment of names to the taxonomic groups according to international rules (*International Code of Nomenclature of Bacteria*). Thus classification has been used to organize the bewildering and seemingly chaotic array of individual bacteria into an orderly framework. Classification and adequate description of bacteria require knowledge of their morphologic, biochemical, physiological, and genetic characteristics. The basic and most important taxonomic group in bacterial systematics is the species. The concept of a bacterial species is less definitive than for higher organisms.

Classification nomenclature and identification

Taxonomy is the science of classification of organisms. Bacterial taxonomy consists of three separate, but interrelated areas: classification, nomenclature, and identification. Classification is the arrangement of organisms into groups (taxa) on the basis of similarities or relationships. Nomenclature is the assignment of names to the taxonomic groups according to international rules (*International Code of Nomenclature of Bacteria* [Sneath, 1992]). Identification is the practical use of a classification scheme to determine the identity of an isolate as a member of an established taxon or as a member of a previously unidentified species.

Some 4000 bacterial species thus far described (and the tens of thousands of postulated species that remain to be described) exhibit great diversity. In any endeavor aimed at an understanding of large numbers of entities it is practical, if not essential, to arrange, or classify, the objects into groups based upon their similarities. Thus classification has been used to organize the bewildering and seemingly chaotic array of individual bacteria into an orderly framework. Classification need not be scientific. Mandel said that "like cigars, … a good classification is one which satisfies" (Mandel, 1969). Cowan observed that classification is purpose oriented; thus, a successful classification is not necessarily a good one, and a good classification is not necessarily successful (Cowan, 1971, 1974).

Classification and adequate description of bacteria require knowledge of their morphologic, biochemical, physiological, and genetic characteristics. As a science, taxonomy is dynamic and subject to change on the basis of available data. New findings often necessitate changes in taxonomy, frequently resulting in changes in the existing classification, in nomenclature, in criteria for identification, and in the recognition of new species. The process of classification may be applied to existing, named taxa, or to newly described

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TABLE 1. Taxonomic ranks

Formal rank	Example
Domain	*Bacteria*
Phylum	*Proteobacteria*
Class	*Alphaproteobacteria*
Order	*Legionellales*
Family	*Legionellaceae*
Genus	*Legionella*
Species	*Legionella pneumophila*
Subspecies	*Legionella pneumophila* subsp. subsp. *pneumophila*

organisms. If the taxa have already been described, named, and classified, new characteristics may be added or existing characteristics may be reinterpreted to revise existing classification, update it, or formulate a new one. If the organism is new, i.e., cannot be identified as an existing taxon, it is named and described according to the rules of nomenclature and placed in an appropriate position in an existing classification, i.e., a new species in either an existing or a new genus.

Taxonomic ranks

Several levels or ranks are used in bacterial classification. The highest rank is called a Domain. All procaryotic organisms (i.e., bacteria) are placed within two Domains, *Archaea* and *Bacteria*. Phylum, class, order, family, genus, species, and subspecies are successively smaller, non-overlapping subsets of the Domain. The names of these subsets from class to subspecies are given formal recognition (have "standing in nomenclature"). An example is given in Table 1. At present, neither the kingdom nor division are used for *Bacteria*. In addition to these formal, hierarchical taxonomic categories, informal or vernacular groups that are defined by common descriptive names are often used; the names of such groups have no official standing in nomenclature. Examples of such groups are: the procaryotes, the spirochetes, dissimilatory sulfate- and sulfur-reducing bacteria, the methane-oxidizing bacteria, methanogens, etc.

Species The basic and most important taxonomic group in bacterial systematics is the species. The concept of a bacterial species is less definitive than for higher organisms. This difference should not seem surprising, because bacteria, being procaryotic organisms, differ markedly from higher organisms. Sexuality, for example, is not used in bacterial species definitions because relatively few bacteria undergo conjugation. Likewise, morphologic features alone are usually of little classificatory significance because the relative morphologic simplicity of most procaryotic organisms does not provide much useful taxonomic information. Consequently, morphologic features are relegated to a less important role in bacterial taxonomy in comparison with the taxonomy of higher organisms.

The term "species" as applied to bacteria has been defined as a distinct group of strains that have certain distinguishing features and that generally bear a close resemblance to one another in the more essential features of organization. (A strain is made up of the descendants of a single isolation in pure culture, and usually is made up of a succession of cultures ultimately derived from an initial single colony). Each species differs considerably and can be distinguished from all other species.

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. (See the chapter on Nomenclature for more detailed information about nomenclatural types). 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. Any strain can be designated as the type strain, although, for new species, the first strain isolated is usually designated. The type strain need not be a typical strain.

The species definition given above is one that was loosely followed until the mid-1960s. Unfortunately, it is extremely subjective because one cannot accurately determine "a close resemblance", "essential features", or how many "distinguishing features" are sufficient to create a species. Species were often defined solely on the basis of relatively few phenotypic or morphologic characteristics, pathogenicity, and source of isolation. The choice of the characteristics used to define a species and the weight assigned to these characteristics frequently reflected the interests and prejudices of the investigators who described the species. These practices probably led Cowan to state that "taxonomy … is the most subjective branch of any biological science, and in many ways is more of an art than a science" (Cowan, 1965).

Edwards and Ewing (1962, 1986) were pioneers in establishing phenotypic principles for characterization, classification and identification of bacteria. They based classification and identification on the overall morphologic and biochemical pattern of a species, realizing that a single characteristic (e.g., pathogenicity, host range, or biochemical reaction) regardless of its importance was not a sufficient basis for speciation or identification. They employed a large

number of biochemical tests, used a large and diverse strain sample, and expressed results as percentages. They also realized that atypical strains, when adequately studied, are often perfectly typical members of a given biogroup (biovar) within an existing species, or typical members of a new species.

Numerical taxonomic methods further improved the validity of phenotypic identification by further increasing the number of tests used, usually to 100–200, and by calculating coefficients of similarity between strains and species (Sneath and Sokal, 1973). Although there is no similarity value that defines a taxospecies (species determined by numerical taxonomy), 80% similarity is commonly seen among strains in a given taxospecies. Despite the additional tests and added sensitivity of numerical taxonomy, even a battery of 300 tests would assess only between 5–20% of the genetic potential of bacteria.

It has long been recognized that the most accurate basis for classification is phylogenetic. Kluyver and van Niel (1936) stated that "many systems of classification are almost entirely the outcome of purely practical considerations … (and) are often ultimately impractical …" They recognized that "taxonomic boundaries imposed by the intuition of investigators will always be somewhat arbitrary—especially at the ultimate systematic unit, the species. One must create as many species as there are organisms that differ in sufficiently fundamental characters" and they realized that "the only truly scientific foundation of classification is in appreciating the available facts from a phylogenetic view". The data necessary to develop a natural (phylogenetic) species definition became available when DNA hybridization was utilized to determine relatedness among bacteria.

DNA hybridization is based upon the ability of native (double-stranded) DNA to reversibly dissociate or be denatured into its two complementary single strands. Dissociation is accomplished at high temperature. Denatured DNA will remain as single strands when it is quickly cooled to room temperature after denaturation. If it is then placed at a temperature between 25 and 30°C below its denaturation point, the complementary strains will reassociate to again form a double-stranded molecule that is extremely similar, if not identical, to native DNA (Marmur and Doty, 1961). Denatured DNA from a given bacterium can be incubated with denatured DNA (or RNA) from other bacteria and will form heteroduplexes with any complementary sequences present in the heterologous strand–DNA hybridization. This is the method used to determine DNA relatedness among bacteria.

Perfectly complementary sequences are not necessary for hybridization; the degree of complementary required for heteroduplex formation can be governed experimentally by changing the incubation temperature or the salt concentration. Increasing the incubation temperature and/or lowering the salt concentration in the incubation mixture increases the stringency of heteroduplex formation (fewer unpaired bases are tolerated), whereas decreasing the temperature and/or increasing the salt concentration decreases the stringency of heteroduplex formation. The percentage of unpaired bases within a heteroduplex is an indication of the degree of divergence present. One can approximate the amount of unpaired bases by comparing the thermal stability of the heteroduplex to the thermal stability of a homologous duplex. This is done by stepwise increases in temperature and measuring strand separation. The thermal stability is calculated as the temperature at which 50% of strand separation has occurred and is represented by the term "$T_{m(e)}$".

The ΔT_m values of heteroduplexes range from 0 (perfect pairing) to ~20°C, with each degree of instability indicative of approximately 1% divergence (unpaired bases). As DNA relatedness between two strains decreases, divergence usually increases.

A number of different DNA–DNA and DNA–RNA hybridization methods have been used to determine relatedness among bacteria (Johnson, 1985). Two of these, free solution reassociation with separation of single- and double-stranded DNA on hydroxyapatite (Brenner et al., 1982) and the S-1 endonuclease method (Crosa et al., 1973) are currently the most widely used for this purpose. These methods have been shown to be comparable (Grimont et al., 1980). An in-depth discussion of DNA hybridization methods has been presented by Grimont et al. (1980) and by Johnson (1985).

Experience with thousands of strains from several hundred well-established and new species led taxonomists to formulate a phylogenetic definition of a species (genomospecies) as "strains with approximately 70% or greater DNA–DNA relatedness and with 5°C or less ΔT_m. Both values must be considered" (Wayne et al., 1987). They further recommended that a genomospecies not be named if it cannot be differentiated from other genomospecies on the basis of some phenotypic property. DNA relatedness provides a single species definition that can be applied equally to all organisms and is not subject to phenotypic variation, mutations, or variations in metabolic or other plasmids. The major advantage of DNA relatedness is that it measures overall relatedness, and therefore the effects of atypical biochemical reactions, mutations, and plasmids are minimal since they affect only a very small percentage of the total DNA.

TABLE 2. Classification of atypical strains that could be *E. coli*

Relatedness of biogroup to typical *E. coli*	Characteristic
80% or more	Urea positive and KCN positive
	Mannitol negative
	Inositol positive
	Adonitol positive
	H_2S positive or H_2S positive and yellow pigmented
	H_2S positive and citrate positive
	Citrate positive
	Phenylalanine deaminase positive
	Lysine and ornithine decarboxylase and arginine dihydrolase negative
	Indol negative
	Methyl red negative
	Methyl red negative and mannitol negative
	Urea positive and mannitol negative
	Anaerogenic, nonmotile, and lactose negative
60% or less	Yellow pigment, cellobiose positive, and KCN positive = *Escherichia hermannii*
	Urea positive, KCN positive, citrate positive, cellobiose positive = *Citrobacter amalonaticus*

Once genomospecies have been established, it is simple to determine which variable biochemical reactions are species specific, and therefore to have an identification scheme that is compatible with the genetic concept of species. The technique is also extremely useful in determining the biochemical boundaries of a species, as exemplified for *Escherichia coli* in Table 2. The use of DNA relatedness and a variety of phenotypic characteristics in classifying bacteria has been called polyphasic taxonomy (Colwell, 1970), and seems to be the best approach to a valid description of species. DNA relatedness studies have now been carried out on more than 10,000 strains representing some 2000 species and hundreds of genera, with, to our knowledge, no instance where other data invalidated the genomospecies definition.

Stackebrandt and Goebel (1994) reviewed new species descriptions published in the *International Journal of Systematic Bacteriology*. In 1987, 60% of species descriptions included DNA relatedness studies, 10% were described on the basis of serologic tests, and 30% did not use these approaches. In 1993, 75% of species descriptions included DNA relatedness data, 8% used serology, and 3% used neither method. In the remaining 14%, 16S rRNA sequence analysis was the sole basis for speciation. As 16S rRNA sequence data have accumulated, the utility of this extremely powerful method for phylogenetic placement of bacteria has become evident (Woese, 1987; Ludwig et al., 1998b). The number of taxonomists using 16S rRNA sequencing is or soon will be greater than the number using DNA hybridization (Stackebrandt and Goebel, 1994), and many of them were creating species solely or largely on the basis of 16S rRNA sequence analysis. It soon became evident, however, that 16S rRNA sequence analysis was frequently not sensitive enough to differentiate between closely related species (Fox et al., 1992; Stackebrandt and Goebel, 1994). Stackebrandt and Goebel (1994) concluded that the genetic definition of 70% relatedness with 5% or less divergence within related sequences continues to be the best means of creating species. They concluded that 16S rRNA sequence similarity of less than 97% between strains indicates that they represent different species, but at 97% or higher 16S rRNA sequence similarity, DNA relatedness must be used to determine whether strains belong to different species.

The validity and utility of the DNA relatedness based genetic definition of a species has been questioned (Maynard Smith, 1995; Vandamme et al., 1996a; Istock et al., 1996). These criticisms fall into several categories: (a) DNA relatedness (and any other current means of speciation) does not sufficiently sample bacterial diversity by employing large numbers of wild isolates from many different habitats; (b) it employs an arbitrary cutoff for a species whereas evolution is a continuum; (c) the DNA-relatedness based definition does not achieve standardization of species; (d) bacterial species are not real entities—named species are useful but not meaningful from an evolutionary standpoint; (e) DNA relatedness results are not comparable due to different methods; (f) DNA relatedness tests are too difficult and/or tedious to perform. In view of these perceived problems, it has been recommended that the best solution to the species problem in the absence of a "gold standard", which has not been provided by DNA relatedness, is a pragmatic polyphasic (consensus) taxonomy that integrates all available data.

Each of these criticisms has some merit; however each can be addressed, and none, in our opinion, represent fatal flaws nor significantly negate the usefulness of the DNA-relatedness based definition of a species. Large numbers of diverse strains (50–100) have been tested for DNA relatedness in a number of species including *E. coli*, *Legionella pneumophila*, *Enterobacter agglomerans*, *Klebsiella oxytoca*, *Yersinia enterocolitica*. In no case did the sample size or the diversity of sources and/or phenotypic characteristics change the results. For many other species only one or a few strains were tested—usually because that was the total number of strains available.

It is true that the 70% relatedness and 5% divergence values chosen to represent strains of a given species are arbitrary, and that there is a "gray area" around 70% for some species. Nonetheless, these values were chosen on the basis of results obtained from multiple strains, usually 10 or more, of some 600 species studied in a number of different reference laboratories. There are few, if any cases, in which the species defined in this manner have been shown to be incorrect.

The DNA relatedness approach has standardized the means of defining species by providing a single, universally applicable criterion. Since it has been successful, one must believe that it generates species that are compatible with the needs and beliefs of most bacteriologists. There are two areas in which genomospecies have actually or potentially caused problems. One of these is where two or more genomospecies cannot be separated phenotypically. In this case it has been recommended that these genomospecies not be formally named (Wayne et al., 1987). Alternatively, especially if a name already exists for one of the genomospecies, the others can be designated as subspecies. In this way there is no confusion at the species level and, one can, if one wishes, distinguish between the genomospecies using a genetic technique. The other "problem" is with nomenspecies that were split or lumped, usually on the basis of pathogenicity or phytopathogenic host range. These include species in the genera *Bordetella*, *Mycobacterium*, *Brucella*, *Shigella*, *Klebsiella*, *Neisseria*, *Yersinia*, *Vibrio*, *Clostridium*, and *Erwinia*. In some of these cases (*Klebsiella*, *Erwinia*) the classification has been changed and is now accepted. In the others, changes have not yet been proposed or, as in the case of *Yersinia pestis* and *Yersinia pseudotuberculosis*, which are the same genomospecies, the change was rejected by the Judicial Commission because of possible danger to public health if there was confusion regarding *Y. pestis*, the plague bacillus.

If one agrees that a true species definition is not possible, the genomospecies definition is still useful in providing a single, universally applicable basis for designating species.

To criticize DNA relatedness because results obtained using different methods may not be totally comparable seems somewhat unjustified. When compared, the most frequently used methods have given similar results. Obviously, one should be careful in comparing data from various laboratories, especially when different methods are used. However, this is at least equally true for sequence data and phenotypic tests.

It is true that large amounts of DNA are required for the DNA relatedness protocols now used for taxonomic purposes, and that it is necessary to use radioactive isotopes. As for the difficulty involved and the limitations in strains that can be assayed (it is not uncommon to do 40–80 DNA relatedness comparisons daily), surely these are not credible reasons to stop using the method. Efforts can and should be made to automate the system, to miniaturize it, and to substitute nonradioactive compounds for the radioactive isotopes. With these improvements, the method will be available for use in virtually any laboratory. Even without them, one can argue that DNA hybridization is more affordable and practical than a consensus classification system in which several hundred tests must be done on each strain.

It is noteworthy that bacterial species can be compared to higher organisms on a molecular basis using mol% G + C range, DNA–DNA or DNA–rRNA relatedness, and similarity of 16S vs. 18S rDNA sequences (Staley, 1997, 1999). Thus, *E. coli* can be compared with its primate hosts based on the results of DNA–DNA hybridization. When this is done, it is apparent that the bacterial species is much broader than that of its hosts. For example, humans and our closest relative, the chimpanzee (*Pan troglodytes*), show 98.4% relatedness by this technique (Sibley and Ahlquist, 1987; Sibley et al., 1990). Indeed, even lemurs, which exhibit 78% DNA relatedness with humans, would be included in the same species as humans if the definition of a bacterial species was used. Furthermore, none of the primates would be considered to be threatened species using the bacterial definition. Likewise, the range of mol% G + C and the range of small subunit ribosomal RNA within *E. coli* strains shows a similar result, namely, that the bacterial species is much broader than that of animals (Staley, 1999).

One consequence of the broad bacterial species definition is that very few species have been described, fewer than 5000, compared with over a million animals. This has led some biologists to erroneously conclude that bacteria comprise only a minor part of the biological diversity on Earth (Mayr, 1998). In addition, with such a broad definition, not a single free-living bacterial species can be considered to be threatened with extinction (Staley, 1997). Therefore, biologists should realize, as mentioned earlier in this section, that the bacterial species is not at all equivalent to that of plants and animals.

In summary, the genetic definition of a species, if not perfect, appears to be both reliable and stable. DNA relatedness studies have already resolved many instances of confusion concerning which strains belong to a given species, as well as for resolving taxonomic problems at the species level. It has not been replaced as the current reference standard. It should remain the standard, at least until another approach has been compared to it and shown to be comparable or superior.

TABLE 3. Infrasubspecific designations

Preferred name	Synonym	Applied to strains having:
Biovar	Biotype	Special biochemical or physiologic properties
Serovar	Serotype	Distinctive antigenic properties
Pathovar	Pathotype	Pathogenic properties for certain hosts
Phagovar	Phage type	Ability to be lysed by certain bacteriophages
Morphovar	Morphotype	Special morphologic features

Subspecies A species may be divided into two or more subspecies based on consistent phenotypic variations or on genetically determined clusters of strains within the species. There is evidence that the subspecies concept is phylogenetically valid on the basis of frequency distribution of ΔT_m values. There are presently essentially no guidelines for the establishment of subspecies, which, although frequently useful, are usually designated at the pleasure of the investigator. Subspecies is the lowest taxonomic rank that is covered by the rules of nomenclature and has official standing in nomenclature.

Infrasubspecific Ranks Ranks below subspecies, such as biovars, serovars, phagovars, and pathovars, are often used to indicate groups of strains that can be distinguished by some special character, such as antigenic makeup, reactions to bacteriophage, etc. Such ranks have no official standing in nomenclature, but often have great practical usefulness. A list of some common infrasubspecific categories is given in Table 3.

Genus All species are assigned to a genus, which can be functionally defined as one or more species with the same general phenotypic characteristics, and which cluster together on the basis of 16S rRNA sequence. In this regard, bacteriologists conform to the binomial system of nomenclature of Linnaeus in which the organism is designated by its combined genus and species names. There is not, and perhaps never will be, a satisfactory definition of a genus, despite the fact that most new genera are designated substantially on the basis of 16S rRNA sequence analysis. In almost all cases, genera can be differentiated phenotypically, although a considerable degree of flexibility in genus descriptions is often needed. Considerable subjectivity continues to be involved in designating genera, and considerable reclassification, both lumping and splitting, is still occurring at the genus level. Indeed, what is perceived to be a single genus by one systematist may be perceived as multiple genera by another.

Higher Taxa Classificatory relationships at the familial and higher levels are even less certain than those at the genus level, and descriptions of these taxa are usually much more general, if they exist at all. Families are composed of one or more genera that share phenotypic characteristics and that should be consistent from a phylogenetic standpoint (16S rRNA sequence clustering) as well as from a phenotypic basis.

Major developments in bacterial classification

A century elapsed between Antony van Leeuwenhoek's discovery of bacteria and Müller's initial acknowledgement of bacteria in a classification scheme (Müller, 1786). Another century passed before techniques and procedures had advanced sufficiently to permit a fairly inclusive and meaningful classification of these organisms. For a comprehensive review of the early development of bacterial classification, readers should consult the introductory sections of the first, second, and third editions of *Bergey's Manual of Determinative Bacteriology*. A less detailed treatment of early classifications can be found in the sixth edition of the *Manual*, in which post-1923 developments were emphasized.

Two primary difficulties beset early bacterial classification systems. First, they relied heavily upon morphologic criteria. For example, cell shape was often considered to be an extremely important feature. Thus, the cocci were often classified together in one group (family or order). In contrast, contemporary schemes rely much more strongly on 16S rRNA sequence similarities and physiological characteristics. For example, the fermentative cocci are now separated from the photosynthetic cocci, which are separated from the methanogenic cocci, which are in turn separated from the nitrifying cocci, and so forth; with the 16S rRNA sequences of each group generally clustered together. Secondly, the pure culture technique which revolutionized bacteriology was not developed until the latter half of the 19th century. In addition to dispelling the concept of "polymorphism", this technical development of Robert Koch's laboratory had great impact on the development of modern procedures in bacterial systematics. Pure cultures are analogous to herbarium specimens in botany. However, pure cultures are much more useful because they can be (a) maintained in a viable state, (b) subcultured, (c) subjected indefinitely to experimental tests, and (d) shipped from one laboratory to another. A natural outgrowth of the pure culture technique was the establishment of type strains of species which are deposited in repositories referred to as "culture collections" (a more accurate term would be "strain collections"). These type

strains can be obtained from culture collections and used as reference strains to duplicate and extend the observations of others, and for direct comparison with new isolates.

Before the development of computer-assisted numerical taxonomy and subsequent taxonomic methods based on molecular biology, the traditional method of classifying bacteria was to characterize them as thoroughly as possible and then to arrange them according to the intuitive judgment of the systematist. Although the subjective aspects of this method resulted in classifications that were often drastically revised by other systematists who were likely to make different intuitive judgments, many of the arrangements have survived to the present day, even under scrutiny by modern methods. One explanation for this is that the systematists usually knew their organisms thoroughly, and their intuitive judgments were based on a wealth of information. Their data, while not computer processed, were at least processed by an active mind to give fairly accurate impressions of the relationships existing between organisms. Moreover, some of the characteristics that were given great weight in classification were, in fact, highly correlated with many characteristics. This principle of correlation of characteristics appears to have started with Winslow and Winslow (1908), who noted that parasitic cocci tended to grow poorly on ordinary nutrient media, were strongly Gram-positive, and formed acid from sugars, in contrast to saprophytic cocci which grew abundantly on ordinary media, were generally only weakly Gram-positive and formed no acid. This division of the cocci studied by the Winslows (equivalent to the present genus *Micrococcus* (the saprophytes) and the genera *Staphylococcus* and *Streptococcus* (the parasites) has held up reasonably well even to the present day.

Other classifications have not been so fortunate. A classic example of one which has not is that of the genus "*Paracolobactrum*". This genus was proposed in 1944 and is described in the Seventh Edition of *Bergey's Manual* in 1957. It was created to contain certain lactose-negative members of the family *Enterobacteriaceae*. Because of the importance of a lactose-negative reaction in identification of enteric pathogens (i.e., *Salmonella* and *Shigella*), the reaction was mistakenly given great taxonomic weight in classification as well. However, for the organisms placed in "*Paracolobactrum*", the lactose reaction was not highly correlated with other characteristics. In fact, the organisms were merely lactose-negative variants of other lactose-positive species; for example "*Paracolobactrum coliform*" resembled *E. coli* in every way except in being lactose-negative. Absurd arrangements such as this eventually led to the development of more objective methods of classification, i.e., numerical taxonomy, in order to avoid giving great weight to any single characteristic.

Phylogenetic Classifications

We have already discussed the impact of DNA relatedness at the species level. Unfortunately, this method is of marginal value at the genus level and of no value above the genus level because the extent of divergence of total bacterial genomes is too great to allow accurate assessment of relatedness above the species level. At the genus level and above, phylogenetic classifications, especially as based on 16S rRNA sequence analysis, have revolutionized bacterial taxonomy (see Overview: A Phylogenetic Backbone and Taxonomic Framework for Procaryotic Systematics by Ludwig and Klenk).

Official Classifications

A significant number of bacteriologists have the impression that there is an "official classification" and that the classification presented in *Bergey's Manual* represents this "official classification". It is important to correct that misimpression. There is no "official classification" of bacteria. (This is in contrast to bacterial nomenclature, where each taxon has one [and usually only one] valid name, according to internationally agreed-upon rules, and judicial decisions are rendered in instances of controversy about the validity of a name.) The closest approximation to an "official classification" of bacteria would be one that is widely accepted by the community of microbiologists. A classification that is of little use to bacteriologists, regardless of how fine a scheme or who devised it, will soon be ignored or significantly modified. The editors of *Bergey's Manual* and the authors of each chapter make substantial efforts to provide a classification that is as accurate and up-to-date as possible, however it is not and cannot be "official".

It also seems worthwhile to emphasize something that has often been said before, viz. bacterial classifications are devised for microbiologists, not for the entities being classified. Bacteria show little interest in the matter of their classification. For the systematist, this is sometimes a very sobering thought!

Note added in proof

Recently a committee of bacterial taxonomists met to re-evaluate the bacterial species definition (Stackebrandt et al., 2002b). The committee recognized that, since the report by Wayne et al. (1987), several new methods have been developed that greatly aid in bacterial taxonomy, including 16S rDNA sequence analyses, restriction enzyme typing methods, multilocus sequencing, whole genome

sequence analyses, Fourier-Transformed Infrared Spectroscopy and pyrolysis-mass spectrometry. Special methods noted by the committee that show great promise for taxonomists include sequencing of housekeeping genes, DNA profiling and the application of DNA arrays. Microbiologists were encouraged to develop new methods that would allow data to be compared to DNA–DNA reassociation, which the committee concluded should remain the standard for species circumscription for *Bacteria* and *Archaea*. Other recommendations were made to base the species description on more than a single strain, to follow guidelines established by the subcommittees of ICSP (International Committee on Systematics of Prokaryotes) for minimal characterization of a species, and to recognize the importance of phenotypic properties for species identification. Also, because electronic databases are an immensely important aid for the international community of bacterial systematists, the committee recommended the development of standards for electronic exchange of taxonomic information.

Acknowledgments

This chapter is dedicated to the memory of John L. Johnson, a consummate scientist, trusted colleague and friend, whose search for truth was uncompromising and unhindered by personal ego.

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