THE PRINCIPLES OF MICROBIAL CLASSIFICATION

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Introduction: The Philosophy of Classification

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In the biological sciences, classification is a compromise between the dynamics of evolution and the stasis of the present; with microbes there is less apparent stasis, for with rapid multiplication there are greater opportunities for observing evolution in action. All classifications are subjective, and, like religious and political opinions, have a large element of aesthetic unreason about them. A classification consists of two elements: the objects to be classified, and the subject who feels the urge to classify them. There cannot be a classification without a classifier or taxonomist, and no two taxonomists will approach objects in the same way. Thus a given set of objects may be classified in different ways by different taxonomists; even when the purpose is identical, the similarities may not be numerous. We cannot expect any particular classification to be permanent: Turrill (1952) has developed the idea of alpha to omega taxonomy, alpha classifications being the best that can be done with the information available; with increasing knowledge gaps are filled and errors corrected, and the classifications proceed along the Greek alphabet. Omega classifications are the ideals that never will and cannot be reached while evolution continues.

Why do we make classifications? If our intention is to pigeon-hole objects, all we need do is to give each a distinctive name or number, and arrange them in alphabetical or numerical order. But this is merely cataloguing, it is not grouping objects together, which is the essence of classification. Classifications may have a limited and specific purpose, as the water bacteriologists have classified coliform bacteria, or may be more generalized. General schemes, e.g. of Kingdoms, may be purely arbitrary in character and utilitarian in purpose, and may differ little from the dichotomous schemes used for identifications; on the other hand, they may attempt to be 'natural' and to show the relations of one group to another. The maker of a natural classification assumed that the creation of the world had been orderly and, by correlating the characters of his objects, he hoped to reconstruct the plan of the Creator. In a modern biological sense, a natural system is described as phylogenetic, and is supposed to reveal evolutionary trends, but we should remember that without an adequate fossil background much of our taxonomy is a mixture of speculation and wishful thinking.

Since classifications are largely subjective, their creation is an art, and the 'best' classifications are those made by artists with the keenest appreciation of what is both useful and intellectually satisfying. The classifier sorts objects so that the likes are brought together. If we knew everything about our objects we should be in a better position to make the sorting; in fact we must base our sorting on the known part. We can define a microbial classification as the sorting of micro-organisms in such a way that those with similar characters (or attributes) are brought together into units and those with dissimilar characters are separated and put into other units. The number of shared characters determines the homogeneity of the unit; with many characters shared the units will be small; with only a few characters in common, the association will be loose and the units large. The difference in unit size constitutes the basis for the ranks of hierarchical classifications.

Should we start at the top with big units and subdivide them, or should we work from the bottom, building small into larger units? Starting at the top we have, as primary units, algae, protozoa, fungi, bacteria, yeasts and viruses, and we divided them into subunits and sub-subunits. On the other hand, starting from the bottom, we can take the individual cell, the micro-organism, the colony, or the isolate, as the primary unit. The individual micro-organism may have one or more cells; theoretically such an organized whole makes the ideal unit, but as it is technically difficult to isolate and has a relatively short life it is too ephemeral. A colony forms a population which has more substance and permanence, but may not be entirely homogeneous. In general an isolate is derived from a colony after technical procedures to ensure that it is 'pure', by which we mean that it is not composed of a mass of individuals showing widely divergent characters. It is convenient to make the isolate the smallest unit; similar isolates are collected together into the next larger group, the species.

Species concept. When biologists discuss classification they always come up against the species concept; for larger plants and animals the species is easier to define: Julian Huxley (1940) says that species are natural units, which (*a*) have a geographical distribution-area; (*b*) are self-perpetuating; (*c*) are morphologically distinguishable from other related groups; and (*d*) normally do not interbreed with related groups. Microbial species cannot be defined in this way; few microbes have a particular geographical distribution-area, though their economic effects may be localized, as in fungi of the order Uredinales in which host specialization plays an important part in the species concept (Ainsworth, 1954). Among algae, fungi and protozoa, species may be distinguished on morphological grounds, distinctions often emphasized by complicated life cycles. In bacteria gross morphology barely distinguishes higher ranks; cytology is useful at the generic level; at the species level the bacteriologist relies more on physiological than on morphological differences. Apart from certain protozoa and heterothallic fungi, most micro-organisms reproduce asexually so that interfertility is not a species character. In spite of this, satisfactory classifications can be made using other criteria, as in *Aspergillus* in which colony form and colour are combined with morphology. In microbiology we introduce characters not utilized by botanists and zoologists, such as nutritional requirements, metabolic and catabolic products, antigenic structure and pathogenicity; their use will be developed by other speakers.

The species concept is old, and originally was applied to any group of objects or ideas which had certain stated characteristics in common. We owe its use in the modern biological sense to the seventeenth-century English naturalist, John Ray. It was firmly established when Linnaeus grouped species into larger units, genera, which he also regarded as 'natural' units. We should remember that at this time naturalists believed that the species was preordained and the result of a divine creation; it was, therefore, unchanging. Linnaeus believed that there were as many species as there had been different forms created at the beginning of the world. Thus to the concept of a natural system was grafted an element of the supernatural (Heslop-Harrison, 1953).

In the seventeenth century John Locke wrote that 'the animal and vegetable kingdoms are so nearly joined, that,...[when] we come to the lowest and most inorganical parts of matter, we shall find everywhere that the several species are linked together, and differ but in almost insensible degrees'. This is still true more than 250 years later. How, then, at the level of micro-organisms can we define a species? Topley & Wilson (1929) summed up the situation aptly in the sentence: 'The terms "genus" and "species", as applied to bacteria, seem to us to defy definition, except as designations for two convenient groupings, of which the genus is the larger including group, and the species the smaller included group.'

A few examples will give a better idea of microbial species. Some protozoan species, such as *Entamoeba histolytica* and *E. coli*, are distinguished by essential differences associated with both the nucleus and the cytoplasm. Species of the *Aspergillus niger* series are distinguished by the dimensions of the primary sterigmata and of conidia, those of *Saccharomyces* by fermentation reactions. Among bacteria some species differ only in antigenic make-up, as in the salmonella group; in the genus *Clostridium* species are distinguished by the shape and position of the endospore, and by the toxicity of metabolic products. These examples show that the word species has different values in the different disciplines, and even in one it is used to describe different concepts in different genera. What is called a species of *Salmonella* would be a serotype of a streptococcal species, and the whole genus *Salmonella* is no more heterogeneous than the species *Escherichia coli*. Another example of the uncertain nature of a bacterial species can be found in the organism originally named *Salmonella arizona*, which was removed from the genus *Salmonella* because it fermented lactose. Other serologically related, but not identical, strains were found and an Arizona group or genus was formed: but not all these strains ferment lactose so that if we now apply the criterion upon which *Arizona* was separated from *Salmonella* the non-fermenters could legitimately be classified in the genus *Salmonella*, thus completing a circular argument.

The problem before us is to decide whether the species concept is tenable in microbiology, and if it is not, what we are to substitute for it. In the study of flowering plants, the older, or 'classical taxonomy' based on species is being supplemented by an 'experimental taxonomy' with new and diverse basic units, in which a general classification of the plant kingdom is not attempted (Heslop-Harrison, 1953). Compared with classical taxonomy, the experimental variety is essentially dynamic. Short generation times give microbiologists better chances to see evolution at work, and it is clear that the concept of static species must be abandoned in favour of something more elastic.

The nomenclatural type concept. In biological nomenclature the conception of nomenclatural types has developed, each type serving as a fixed point about which other isolates of similar characters can be grouped. These points are called nomenclatural types (Ainsworth & Cowan, 1954). Their value in classification depends on the validity of the taxonomic group (taxon) that each represents; if this is well founded, the type—of whatever rank—will, if it can be maintained without mutational change, be useful to the taxonomist. Does this type concept help us to classify microbes continually undergoing evolutionary changes? The types represent organisms at the time of their description and can be likened to genotypes; in the laboratory or under natural conditions the organisms may undergo changes, the characters of the changed organisms, or phenotypes, being so different from the original that they might be classified in different groups (or species).

Distinguishing characters. The choice of good distinguishing characters is the art of taxonomy, and we should consider what qualities these 'good' characters possess. To the taxonomist, a good character is readily observable, and is not easily altered by changes in environment. For example, the typhoid bacillus has certain characters that are reliable because, in the natural state, variation from them is not observed: these characters are all negative and indicate lack of appropriate enzymes: they are the anaerogenic fermentation of sugars, the inability to ferment lactose, and the inability to produce indole from tryptophan. *In vitro* the last two characters can be changed by growth under special conditions, but the first is a character that, so far, has not been altered by the most devilish tricks of the students of bacterial variation. It is, therefore, a particularly good character, but so are the others, because in the wild state variation has not been found. In general, positive characters are regarded as more useful than negative ones, but most of them can be lost. Thus we may suppose that *Bacillus coli mutabile* is *Escherichia coli* with a partial loss of lactose-fermentation, and paracolons of the Ballerup-Bethesda series may be *E. coli* which have completely lost the enzyme lactase. The value of a positive character to the taxonomist depends on its regularity, thus the capacity to produce urease is a good character of *Proteus vulgaris* and a poor one for the Providence group, in which it is only sometimes present on first isolation and is quickly lost on subculture. Even a labile character may have value when the lability is regular, as the lactose- and sucrose-fermenting variants of *Shigella sonnei* (Cook, Knox & Tomlinson, 1951).

In different groups of organisms there is emphasis on different characters; morphology plays a greater part in the classification of algae and fungi than of bacteria, yeasts and viruses; serology is a bacteriologist's tool and is little used by workers in other disciplines. Microbiologists have great opportunities for borrowing techniques from each other: for example, serology can be applied to cultivatable protozoa or to fungi, but lack of familiarity with these techniques often obscures the obviousness of their application. The result is that in the characterization of micro-organisms there is a great unevenness of description, or great variation in quality of description. We need more fundamental knowledge, such as essential nutritional requirements, to supplement empirical observations. Other speakers will deal with the different criteria used in classification: all I stress is the inequality in value of different criteria in different groups of organisms. One of the newest approaches to the make-up of living organisms has been made by the geneticists, who have found in rapidly multiplying micro-organisms a happy hunting ground. Their evolutionary experimentation is showing how unrealistic is our species concept, for they find that certain transformations can be made almost as readily as a successful subculture.

Whither taxonomy? Where, taxonomically, will this lead to? Many workers look upon micro-organisms as bags of enzymes or protein molecules, and it is difficult to see how a system based on family relations can logically be applied to them. If we abandon our species concept, what alternative is offered? Mycologists have 'series' with an intermediate and undefined place between genus and species (see Baldacci, Spalla & Grein, 1954). Some serologists subdivide a group of bacteria by antigenic structure (group in this case corresponding roughly to a genus); in some groups (e.g. Salmonella) they give each serotype a distinctive name so that the scheme has the superficial appearance of a Linnaean system, but in others (e.g. Klebsiella) second names are dispensed with, and species lose their identity. This system satisfies those experts who use it, but its inconsistencies do not attract the less specialised taxonomist.

In virology, the infective agents have been classified by the diseases they produce in susceptible hosts, and attempts are now being made to classify the viruses themselves. This cannot be done adequately until more is known of their nature and their properties, and it may be necessary to classify one group as living entities and another as complex proteins.

It is my purpose to sow doubt in your minds, and to leave you with a number of unanswered questions: (1) Does the hierarchical structure of a Linnaean system satisfy the requirements of microbiologists? (2) Can we accept the species concept, and all that this implies, or must we view our organisms as a huge spectrum composed of gradually merging forms? (3) How can we improve the descriptions which characterise our organisms so that the relations of one group to another can be more clearly seen? (4) Does the nomenclatural type play a useful part in building up a taxonomic scheme? (This, apart from its value in relation to nomenclature.) (5) Finally, are we wasting our time in trying to classify microbes?

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DISCUSSION

By J. W. Howie, *University of Glasgow*

It seems to me that bacteriologists would do well to learn from the modest attitude of virologists, most of whom have resolved that they do not yet know enough about viruses to classify them in a manner justifying the identification of species and the use of Linnaean binomials. We are still without enough knowledge about a great many bacteria to know which of their characters are sufficiently stable and important to define genera and species. Our premature use of specific binomials has been repeatedly made ridiculous by swiftly changing ideas about classification. The resulting instability of nomenclature may be very stimulating to ourselves, but it is a constant source of irritation to those who are not greatly interested in systems of bacterial classification, but whose daily work is much concerned with bacteria. In medical work, for example, the typhoid bacillus has changed its name rather frequently with new ideas on classification and nomenclature. Within the past 20 years it has been *Bacillus typhosus*, *Eberthella typhosa*, *Bacterium typhosum* and *Salmonella typhi*. For the purposes of medical reporting it is best referred to by its common name: the typhoid bacillus. To assign the organisms to the genus *Salmonella* may be perfectly sound on grounds of immunology, but very confusing to a clinician to whom enteric fever caused by the typhoid and the paratyphoid bacilli means one thing and food-poisoning caused by one of the other salmonellas means quite a different clinical condition with an entirely different epidemiology. This is not a plea to abandon attempts at systematic classification but a suggestion that we should be modest enough about our tentative efforts to be content to use common, well-established names to designate familiar organisms to those who cannot reasonably be expected to be regular readers of the *International Bulletin of Bacteriological Nomenclature and Taxonomy*. Within the family of bacteriologists there is need for a great deal of work on bacterial systematics and for debate and discussion about valid criteria for classification; but until agreement on this subject rests on a much wider and sounder basis than at present, our constant although desirable changes of outlook should not be reflected in repeated alterations of the names that we use to describe bacteria to those whose interest is not primarily in the bacteria themselves, but in the important effects which they produce.

At present, our classification rests upon a largely subjective assessment of various attributes: morphological, cultural, biochemical, nutritional, immunological and ecological. Our knowledge of these attributes for any particular group of bacteria often depends more upon the special interests, aptitudes and facilities of those who have worked with the group than upon which of the attributes are useful and valid for classification. Our classifications often rest, therefore, upon partial knowledge acquired for purposes which have nothing to do with taxonomy. We should recognize these limitations by keeping our nomenclature on a basis of common names when we address our remarks to those who are not bacteriologists.

Dr Cowan has asked five questions, and I offer the following answers.

Question 1. Is the heirarchical system suited to bacterial classification?

Answer. Not in our present state of knowledge.

Question 2. Is the species concept of value in bacterial classification?

Answer. Not yet at any rate. Bacteria form something much more like a continuous spectrum than definable species.

Question 3. How can we improve our descriptions of bacteria?

Answer. By much work and sound thinking.

Question 4. Has the nomenclatural type a place in classification?

Answer. Yes—a very useful place if it remains stable and if it is what its label says it is.

Question 5. Are we wasting our time in attempting to classify bacteria?

Answer. Certainly not. We are making a very poor job of it only because we know too little. The attempt, however, is well worth while. Bacteria exist, so we must attempt to classify them. In any case, the exercise keeps Dr Cowan alive and happy.

By G. S. WILSON, *Public Health Laboratory Service, London*

It is difficult to draw up principles of classification of bacteria on any but arbitrary lines. The conception of species as held by Linnaeus was based on the belief in a fixed natural order in the world of living things. Experience, however, has shown that the demarcation of species is often far from clear, and that in many instances gradations can be traced between them. During the past 20 years or so the intensive study of bacterial variation has revealed in some groups, particularly the Enterobacteriaceae, so many intermediate types, not only between what were previously regarded as valid species, but even between different genera, that the task of defining species seems to be almost insuperable. To abandon the concept of species would be unfortunate, partly because it introduced order into the systematic study of bacteria, and partly because of the convenience it affords to descriptive bacteriology. In these circumstances it seems that the only practicable method of classifying and naming micro-organisms is to establish a series of nodal points along the continuous chain of variants, and to regard the organisms at these nodes, and for some distance on either side of them, as constituting species. The criteria used for selecting these nodes, and for determining the distance on either side of these nodes within which variants of the species might be included, will clearly need considerable discussion; but if this task were entrusted to a small group of bacteriologists of wide experience whose hands were not tied too rigidly by the present code of nomenclature, it should not prove impossible.