Bacterial Taxonomy: a Critique

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INTRODUCTION

With the recent advances in molecular biology has come the entrance of the chemist and the geneticist into the field of bacterial taxonomy. Within the last few years, the so-called numerical or neo-Adansonian taxonomy has also created considerable interest. That our conventional taxonomy has many shortcomings is obvious, and the purpose of this paper is to review the history of some areas of conventional taxonomy, to state my personal views for improvements, and to evaluate critically some publications on numerical taxonomy. If this paper is to have an impact for improvement, it seems to me best to be specific, even though such specificity is bound to cause some antagonism.

CULTURE IDENTIFICATION AND LABELS

Chemists in general and, unfortunately, many bacteriologists tend to regard a culture of bacteria as if it were a chemical and to accept the label at its face value much as they would the label on a bottle from a reputable manufacturer. The literature shows numerous examples of this attitude, with papers in which cultures were used without any mention of tests for either purity or identity. Even though the paper may have no direct bearing on taxonomy, the scientific import of the paper is bound to be enhanced by proper identification of the cultures used. The morphology of the bacteria appears to have been most neglected, with a culture of a flagellated organism simply stated as being motile. This neglect of morphology is found even in taxonomic studies, and many original descriptions, with proposals for new taxa, have been made without flagellar description and proper illustrations. Such taxa are often unidentifiable. A number of papers have appeared during the last few years on the deoxyribonucleic acid base composition of bacteria. Since such papers have a direct bearing on taxonomy, it would seem doubly important that the cultures used be properly identified. Unfortunately, we frequently find only labels, and antiquated labels at that, such as Bacterium coli, Vibrio percolans, V. cuneatus, Proteus hydrophilla, Flavobacterium piscicida, etc.

In the past several years, the situation has improved somewhat with the increased use of cultures from public culture collections. Even such cultures carry no absolute guarantee of purity or even identity. Contrary to what could be expected, not all cultures received for deposit by the American Type Culture Collection (ATCC) have been properly identified, or at least do not conform to descriptions in the literature or as recorded in Bergey's Manual. During the past year, I have checked the flagellation of all motile cultures received by the ATCC. Aside from one or two cultures which were obviously mislabeled, perhaps contaminated somewhere along the line, the following are some recent examples of flagellar types not ordinarily included in the specific genera or species: Vibrio marinus ATCC 15382, with lophotrichous flagella; Cell-vibrio polythrophicum ATCC 14774, with lophotrichous flagella; Pseudomonas schuylkillesiensis ATCC 15916, with lophotrichous flagella; P. piscicida ATCC 15802, with lophotrichous flagella. Bacteriologists who make no distinction between polar monotrichous and lophotrichous flagellation may find nothing improper in the above labels. P. piscicida, however, has been carefully described and illustrated and it is difficult for me to accept a lophotrichous organism in this species. Xanthomonas citri ATCC 15923, with peritrichous flagella, is certainly inappropriate, as is also Pseudomonas oxalaticus ATCC 11883, 11884, 11451, and 11452, with peritrichous flagella. Vibrio percolans ATCC 15922, with peritrichous flagella of normal wavelength, is not acceptable; neither is Achromobacter cholino-phagum ATCC 15918, with polar monotrichous flagellation. The following were so obviously mislabeled that we may suspect a contaminant to have replaced the original culture: Moraxella

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liquefaciens ATCC 15099, with lophotrichous flagella; Bordetella bronchiseptica ATCC 15101, with polar monotrichous flagellation and somatic rosette formation, no apparent stalks, but otherwise a typical Caulobacter sp.; Caulobacter variabilis ATCC 15255, with peritrichous flagella; C. fusciformis ATCC 15257, with peritrichous flagella. If public culture collections are to serve as reliable sources of type cultures or of cultures with known characteristics, it appears to be necessary for the personnel of the collections to characterize and perhaps identify all cultures received for deposit. Much effort would be spared and much confusion prevented if the donors would properly identify the cultures and submit to the collections photomicrographs of pertinent morphological characteristics.

**NEW TAXA**

As our knowledge of bacteria increases, the original and the older descriptions of genera and species may need alteration and clarification. In many instances, the original description is not adequate to establish definitively, in contemporary terms, the identity of the organism. The alternatives are either to discard such taxa or to modify the original descriptions sufficiently to be definitive. By long-established convention, each genus has a type species which was either specified by the author of the genus or by a later author. Few of our present genera and their type species originated in this simple manner. The history of most genera and their type species is very involved, with emendations of great variety, as illustrated by numerous examples in Bergey's Manual. To introduce more order and to stabilize our taxonomy, so-called type culture collections were established. From the taxonomic standpoint, the idea was and is to preserve in a viable and stable condition the original strain to which a specific name was given. Where the original strain is lost, which is the case with most of those described 50 or more years ago, a nontype strain would be chosen and preserved. Every bacteriologist who proposes a new species should feel obligated to deposit a culture of the type strain of the species in the appropriate culture collection. The editors of journals which publish the proposals should require that such deposits be made.

Although living types are taxonomically important, they do not replace or obviate a careful description and adequate illustrations by means of photomicrographs or electron micrographs. A live culture may be lost or contaminated, or it may mutate; a description of physiological characteristics may become inadequate by contemporary standards, but good illustrations are timeless. However, it cannot be denied that bacterial taxonomy in the future may come to rely much more than at present on cytological and chemical features. If all published species of bacteria had been properly illustrated, much of the confusion in bacterial taxonomy would not have occurred. It is in the matter of illustrations that American bacteriologists have failed so miserably. Except for a period in Germany, from Loeffler's discovery of flagella staining in 1889 to about the world war in 1914, bacteriologists in other countries have not done much better. American bacteriologists have been very prolific in proposing new species and genera, but with little or no regard for detailed morphology. Even at present, one often finds in descriptions of proposed new species, under the heading of morphology, only a statement regarding the soma and whether or not the culture was motile. Textbooks often state that flagella staining is difficult, tricky, and rarely successful. The result has been that many new species have been given an incorrect morphological description. This is one of the major reasons for the taxonomic confusion and the many changes in the seven editions of Bergey's Manual. The Editors of Bergey's Manual cannot be blamed for most of this confusion. The blame rests on the authors of the species and genera and on the editorial boards of journals which accept the papers for publication.

The major journal in the United States for the publication of papers on bacterial taxonomy has been the Journal of Bacteriology. The Journal of Bacteriology was founded in 1918. From 1918 until 1960, it published proposals for names of some 84 new motile species of bacteria or emendations of previously described motile species. Of these 84 species, only 5 were properly illustrated. Of the other 79 species, 51 were described as peritrichous or polar flagellate and 28 were described simply as motile. Several of the species originally described as polar flagellate were subsequently found to be peritrichous flagellate, and vice versa.

A study of the various editions of Bergey's Manual shows the difficulties and confusion caused by inadequate and careless descriptions. The flagellation of the vinegar bacterium, Acetobacter, is described in the consecutive editions as: all nontomote; all nontomote; motility variable; motility variable; motile with polar flagella or nontomote; single polar flagellum or nontomote; and (in the 7th edition) polar flagella or nontomote. It is almost unbelievable that the morphology of an important group of bacteria as widely distributed and well known as the vinegar bacteria should first be described as nontomote and later as polar monotrichous flagellate.
Thiobacillus spp. are described in the first five editions as either simply motile or as peritrichous flagellate; not until the 6th edition are they correctly described as polar monotrichous. Azobacter spp. are polar flagellate in the first five editions, and first become peritrichous in the 6th edition. Chromobacterium is described in all seven editions as having some species polar flagellate and others peritrichous flagellate, when in reality the various species show characteristically a mixed polar-peritrichous flagellation. Listeria was polar monotrichous flagellate up to the 6th edition, when it became peritrichous.

Proteus hydrophila was described as peritrichous in the 3rd, 4th, and 5th editions, before being removed from the genus Proteus and correctly described as primarily polar monotrichous flagellate. The minor taxonomic significance attached to flagellar arrangement by American bacteriologists is illustrated in a paper by Kulp and Borden (8) from which I quote: "'Red-leg' disease, an epizootic of aquarium frogs, is a form of septicemia that is caused by an organism which is a member of, or so closely related to, the Proteus group as to merit the name Proteus hydrophilus." This statement was made in spite of further statements by the authors that the organism is a polar monotrichous flagellate (fairly well illustrated by a photomicrograph), does not swarm on agar, and does not decompose urea.

Spirillum virginianum was originally described as polar monotrichous flagellate, and was so described in the 5th, 6th, and 7th editions. Cultures with this label in culture collections are lophotrichous flagellate. Species with the label in the genus Corynebacterium were transferred to the appendix of the 6th edition, because they supposedly were polar flagellate. In the 7th edition, they are described as having a single polar or a single lateral flagellum. These bacteria are peritrichous.

Many more examples could be given of false descriptions, but the above examples suffice to illustrate the point that faulty descriptions of bacterial morphology are numerous and would not have been published if proper illustrations had been presented.

The time and effort expended in arriving at the proper classification of an incompletely described species is nicely illustrated by Pseudomonas stutzeri. The following account is taken from the paper by van Niel and Allen (24). In 1895, Burri and Stutzer described two cultures which they labeled Bacillus denitrificans I and Bacillus denitrificans II. The morphology was not illustrated. Lehmann and Neumann (1896) included both organisms in their "Handbuch" under the new names of Bacterium denitrificans and Bacterium stutzeri, respectively. In Migula's System der Bakterien (1900), the two organisms are renamed Pseudomonas stutzeri and Bacillus nitrogenes, respectively. Migula, for some reason now unknown, must have been led to believe Bacillus denitrificans I to be polar flagellate and Bacillus denitrificans II to be peritrichous flagellate. Chester (1901) restored the specific epithet stutzeri to Bacillus denitrificans II, following Lehmann and Neumann, and placed it in his genus Bacillus, implying peritrichous flagellation. Orla-Jensen (1909) also regarded this latter organism as peritrichous flagellate. In the various editions of Bergey's Manual, the organism is placed in the genus Achromobacter because of its supposed peritrichous flagellation. Bacillus denitrificans II Burri and Stutzer (1895) has thus been named Bacterium stutzeri Lehmann and Neumann; Bacillus nitrogenes Migula; Bacillus stutzeri Chester; Achromobacter sewerini Bergey et al.; Achromobacter stutzeri (Lehmann and Neumann) Bergey et al.; Achromobacter pestifer (Frankland and Frankland) Bergey et al.; Pseudomonas stutzeri (Lehmann and Neumann) van Niel and Allen.

Van Niel and Allen feel quite certain that the organism originally named Bacillus denitrificans II is the same as the polar monotrichous organism which they named Pseudomonas stutzeri. The major reason it has been regarded as peritrichous flagellate is the classification by Migula in his genus Bacillus, implying peritrichous flagellation. Van Niel and Allen feel that Migula did not personally study the organism, guessed at the flagellation, and guessed wrong. I quote: "Certain it is that some of Migula's Bacillus species had been arbitrarily assigned to the genus. This is clear from the description of Bacillus punctatus of which it was stated that the type of flagellation is unknown. Another instance is furnished by the inclusion of Henneberg's two species of mollicute acid bacteria as Bacillus oxydans and Bacillus acetigenus; like all other Acetobacter species these are now known to be polarly flagellated (Vaughn, 1942; Frateur, 1950) [italics mine]. In consequence of these patent cases of arbitrariness, it would seem reasonable to infer that also with respect to Bacillus nitrogenes Migula simply guessed at the flagellation and guessed wrong."

Whether or not Migula was arbitrary is certainly not substantiated by the examples given, but rather the opposite. The vinegar bacteria of Henneberg may have been peritrichous flagellate as stated by Migula, but certainly not polar monotrichous flagellate as implied by van Niel and Allen. The paper by van Niel and Allen illustrates the confusion which may result if we
rely on verbal descriptions and poor or questionable illustrations. The major difficulty is with poorly flagellate peritrichous types. Any peritrichous flagellate culture may show some individuals with a single polar flagellum. If the flagellation is poor, or the preparation is poor, the technician by chance may find an organism with a single polar flagellum and, finding no other flagellated individuals, conclude that the culture is polar monotrichous flagellate. I assume this to be the case with Acetobacter spp. How species of the polar multirichous Acetomonas, which are well flagellated, could have been found to be polar monotrichous is more difficult to explain, and again emphasizes the importance of adequate illustrations. Another source of error with poorly flagellated cultures is the distilled water used in washing the bacteria. Unless freshly distilled, the water may contain an appreciable number of flagellated bacteria. The majority of these are polar monotrichous, but all flagellar types may be found. With very poorly flagellated cultures, it is advisable to wash and suspend the bacteria in bacteria-free distilled water.

Although I have not made a detailed survey of new taxa proposed in journals other than the Journal of Bacteriology, the latter has not been unique in its disregard for flagellar characteristics and proper illustrations. As a rather extreme example, I submit the paper by Stevenson (23) in the Journal of General Microbiology entitled: "A Note on the Genus Aeromonas." The author studied three cultures: Aeromonas maritima, personally isolated from diseased locusts; Pseudomonas noctuarum (Serratia marcescens) strain 006, from the Laboratory of Insect Pathology, Prague, Czechoslovakia; and Serratia marcescens, from the London School of Tropical Medicine. I quote: "The flagellation of Aeromonas and Serratia strains was apparently polar, though in one or two preparations observed under the light microscope, there were organisms which may have possessed peritrichate flagellation. The only strain examined for flagellation under the electron microscope was Aeromonas maritima strain SL 10a; in this case all the flagellated organisms were monotrichate. The flagellation of P. noctuarum strain 006 was peritrichate." No illustrations are presented. In the Summary we read: "Previous workers have shown that the flagellation of certain bacteria is variable, becoming lateral or polar according to age and conditions of culture. In view of these findings, and because of the morphological and biochemical similarities of the organisms, Lysenko (1958) has equated Pseudomonas noctuarum with Serratia marcescens. It is now suggested that Pseudomonas noctuarum would be better placed in the genus Aeromonas, and that it is similar to an organism provisionally named A. maritima. It is further suggested that all members of the genus Aeromonas could be regarded as non-chromogenic species of the genus Serratia." It is hard to believe that the editorial board of a leading British journal of microbiology would accept this paper as a contribution to bacterial taxonomy. Such indifference to bacterial morphology can only lead to endless confusion. Does the London School of Tropical Medicine actually have cultures of Serratia marcescens with polar flagellation? With proper attention to flagellation, much of the devious history of Pseudomonas noctuarum (White) Lysenko could have been prevented: Bacillus noctuarum White → Proteus noctuarum (White) Bergey et al. → Escherichia noctuarii Bergey et al. → Pseudomonas noctuarum (White) Weiser and Lysenko → Aeromonas noctuarum (White) Stevenson → Serratia noctuarum (White) Lysenko. The present Aeromonas maritima was originally classified as a paracolon bacillus and later placed in the genus Aeromonas. If I interpret correctly, the author suggests that it be renamed Serratia maritima. I am still in doubt as to the flagellation of this organism.

The relatively infant Canadian Journal of Microbiology does not seem to have done much better. Let me give just one recent example: Shieh (19) proposed the new species Achromobacter cholinophagum. Illustrations are not presented, and the organism is simply described as a gram-negative motile rod. The culture deposited with ATCC and numbered 15918 is polar monotrichous flagellate. A definitive characteristic of this type of organism is the arrangement of the flagella, which the author does not mention at all. Are we to assume that both the author and the editors of the Canadian Journal of Microbiology are indifferent to flagellar morphology and that they do not subscribe to the differentiation of Pseudomonas and Achromobacter on the basis of flagellar arrangement? If the physiological characteristics are interpreted correctly, the organism is a species of Pseudomonas. For lack of proper illustrations, we add yet another mislabeled organism to a list which is overly and inexcusably long.

**Conventional Taxonomy**

What may be called conventional taxonomy selects certain characteristics as pre-eminent over others, and these determine the various taxa. It is the choice of these characteristics, which I shall term definitive, that has led to the major changes, and some controversy, in bacterial taxonomy. With the majority of bacteriologists, the genus and the species are the only two taxa with which
they have much familiarity, since these give the name to the organism. In bacterial taxonomy, the genus has become the pre-eminent taxonomic group. Each genus should have just a few definitive characteristics which are not shared in toto by any other genus. A single definitive characteristic, either morphological or physiological, may be sufficient justification for a genus but should be avoided as much as possible. Genera such as Flavobacterium and Xanthomonas, which may differ only in flagellar arrangement, appear justified. Others, such as Methanomonas, Mycoplana, Agar bacterium, Alginomonas, Alginobacter, Photobacterium, Cellulomonas, and Cellvibrio, are probably not justified. For a detailed and excellent discussion of the taxonomy of Methanomonas (Pseudomonas) methanica, see the paper by Dworkin and Foster (5). Definitive characteristics which are highly variable or which require very unusual techniques should be avoided, such as pathogenicity for plants and animals. Without knowledge of the source and pathogenicity, cultures of Erwinia and Agrobacterium may be difficult to identify. Pathogenicity certainly is an acceptable definitive characteristic, but is more appropriate on the species or perhaps still lower level. The concept of a type species for each genus may be of some value, but it does little to define the parameter of the genus.

It would be naive to expect universal agreement among bacteriologists on the definitive characteristics for all genera, not to mention species. Specialists invariably have a tendency to want to create more genera and species than a nonspecialist would deem appropriate. As an example we may use the genus Haemophilus, out of which was created Bordetella. This may seem neither logical nor practical to a nonspecialist, since the distinction between the two is hardly more than antigenic constitution, which in other groups seldom serves for separation of species. If we had to deal only with Bordetella pertussis, we would have two definitive characteristics which could justify generic rank, namely, pathogenicity and antigenic constitution. But there is also Bacillus bronchisepticus Ferry, alias Alcaligenes bronchisepticus, Brucella bronchiseptica, Haemophilus bronchisepticus, and now Bordetella bronchiseptica. Aside from being nonfermentative gram-negative rods, Bordetella bronchiseptica and Bordetella pertussis have little in common except some antigens. To justify the classification, it is stated in the 7th edition of Bergey's Manual that Bordetella bronchiseptica is morphologically similar to Bordetella pertussis. If this is so, then so are Escherichia spp., Salmonella spp., Achromobacter spp., and Alcaligenes spp., etc. similar morphologically to Bordetella pertussis. The first edition of Bergey's Manual seems to me to be the most logical in naming the organism Alcaligenes bronchisepticus.

Another example of the "splitting" tendency of the specialist is found in the Enterobacteriaceae. The definitive characteristics of this family are clear-cut: gram-negative rods; peritrichous flagellation, if any; and fermentative metabolism of carbohydrates. The creation of genera which are based almost entirely on physiological characteristics can easily get out of hand, as it seems to have in this family. Characteristics which in other groups of bacteria usually serve to differentiate species are here used to differentiate genera. A nonspecialist, already confused by the taxonomy of this family, may find the latest (?) addition, Edwardsiella, hard to justify. If the same physiological criteria were used for the creation of genera in the family Enterobacteriaceae, we would soon have dozens of new genera in this family.

There are many examples of genera which are difficult to differentiate with certainty. The only distinguishing characteristic between Alcaligenes and Achromobacter is a variable degree of oxidative metabolism of carbohydrates by the latter and not by the former. Agrobacterium and Achromobacter are difficult, if not impossible, to differentiate in the laboratory without knowing the phytopathogenicity. A peritrichous type of Rhizobium could easily be confused with either of the above genera. Species of Corynebacterium and Brevibacterium may be impossible to differentiate. It might be well to remove the soil and plant types from the genus Corynebacterium and place them in a separate genus, perhaps Brevibacterium.

Aside from such definitive characteristics as gross morphology, Gram reaction, and spore formation, the most valuable definitive characteristics of bacteria are the flagellar morphology and the nature of the carbohydrate metabolism. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates was first effectively established by Hugh and Leifson (7) and appears to have been almost universally recognized. The pseudomonas-achromobacter groups are thus clearly differentiated from the aeromonas-enteric groups and the staphylococci from the micrococi. The taxonomic significance of flagellar morphology, however, is not as universally recognized. The difficulty here may be one of technique. Many bacteriologists cannot stain flagella properly and find the electron microscope too laborious or not available. It is probably a universal failing in all of us to minimize the importance of what we cannot do properly. After the publication by Loeffler of a staining
technique in 1889, flagellar morphology quickly became a dominant definitive taxonomic characteristic. The Loeffler technique is very tricky, but by meticulous care and patience some excellent preparations were obtained, as evidenced, for example, in Migula’s System der Bakterien. Since the time of Loeffler, many flagella staining techniques have been published, some of which are just as tricky as Loeffler’s. At the present time, however, the technique is so well standardized that only the most inept technicians have any difficulties. In spite of this, it is rare to see really good photomicrographs in the bacteriological journals. Most proposed new species of motile bacteria have been published without flagellar illustrations. This is a great disservice to bacterial taxonomy, and the journals are as much to blame as the authors.

In several recent publications, many of which deal with numerical taxonomy, we find a tendency to belittle the significance of flagellar morphology, and we also find statements which are contrary to the facts. In the Annual Review of Microbiology, De Ley (4) writes: “Furthermore, Aeromonas, Chromobacterium and many marine bacteria are peritrichous when young, and polar when old.” This is a completely false interpretation of published work. Species of these genera and many marine bacteria frequently show mixed flagellation, particularly in young cultures and on agar, but the polar flagella are present throughout the growth cycle.

In an article in the Journal of General Microbiology, Lysenko (13) states: “The predominant type of flagellation was 1–2 flagella per cell. The importance of flagellation has been discussed by many workers and most recently by Rhodes (1959) and it is generally agreed that the type of flagellation is not an important feature in pseudomonas taxonomy.” In this same publication, Lysenko proposes a number of his cultures as neotypes or lectotypes without illustrations, and the following description of the flagellation (words in parentheses are mine): P. aeruginosa, one or two polar flagella (typically monotrichous); P. pseudomallei, one or two polar flagella (typically polar multirichious); P. fluorescens, polar flagella (typically polar multirichious). P. putida, P. geniculate, and P. synxantha are described as having polar flagella without any details as to how many. P. chlororaphis, P. aureofaciens, and P. ovalis are described as having tufts of polar flagella. If flagellation is not an important feature in pseudomonas taxonomy, why make any statement at all regarding flagellation except that it is polar? Anyone who has studied a number of strains of P. aeruginosa knows that two flagella on the same pole are quite rare; to describe the type culture as having one or two polar flagella is very misleading.

Lautrop and Jessen (9) counted the flagella on 6,025 cells of 122 strains of P. aeruginosa, and found 97% with one flagellum, 3% with two flagella, 0.05% with three flagella, and none with four flagella. A similar count of the flagella on 4,990 cells of 63 strains of two biotypes of P. fluorescens showed 31% with two flagella, 13% with three flagella, and 4 to 10% with four or more. With P. ovalis, 2,250 cells of 40 strains were counted and showed 33% with one flagellum, 33% with two flagella, 21% with three flagella, and 13% with four or more flagella. The authors concluded there is a “striking difference” between P. aeruginosa and the other two species, and proposed that the number of flagella be expressed as the “flagellar index,” defined as the percentage of flagellated cells with more than one flagellum. The index for P. aeruginosa was 3; for P. ovalis, 67; and for P. fluorescens, 52 and 50. Where the culture is described as having “polar flagella,” did the author not know how many?

Similar disregard for flagellar morphology is also found in many other taxonomic papers, for example: Beers et al. (1) in the Journal of General Microbiology give no details on flagellation at all but simply describe their cultures as motile or nonmotile; Colwell (2) in the Journal of General Microbiology describes P. aeruginosa as “polar flagellate,” apparently indifferent to the number and shape of the flagella; Colwell and Liston (3) and Liston, Wiebe, and Colwell (12) in the Journal of Bacteriology describe their cultures simply as polar flagellate or not. Malik, Rodochova, and Lysenko (14) state: “Achromobacter species are non-motile bacteria . . . ." Also they state: “Even though the value of the arrangement of the flagella as a taxonomic feature is debatable, it is still used as a characteristic for differentiation of Pseudomonas from other gram negative bacteria.” Rhodes (17) states: “The results suggest that P. aeruginosa may be regarded as a variety of P. fluorescens.” Since these two species are generally regarded as being different physiologically, taxonomically, and culturally, the author must at least regard the two species as having identical flagellation.

Anyone who has looked at well-stained slides of various kinds of bacteria in the microscope must have been impressed with the definitive characteristics of the flagella. Organisms such as Pseudomonas diminuta and caulobacters, with the very short flagellar wavelength, cannot possibly be confused with pseudomonads such as P. aeruginosa. Also readily distinguished from P. aeruginosa is P. fluorescens, P. reptilivora, P. aureo-
faciens, and most phytopathogenic pseudomonads. Mixed polar-peritrichous flagellation cannot be determined with certainty without attention to flagellar wavelength. Both the shape and the arrangement of the flagella are of great diagnostic importance. The subpolar multitrichous flagellation of Sphaerotilus natans is highly definitive, and so is the lateral tuft of flagella of Selenomonas spp.

I fortunately am not entirely alone in my emphasis on flagellar morphology as a definitive characteristic of major importance. A somewhat unexpected support is found in the paper by Marmur, Falkow, and Mandel (15) in the Annual Review of Microbiology: "The properties (e.g. morphological) which represent the combined expression of many genetic loci will still remain, in the broad sense, the cornerstone of microbial classification." The history of bacteriology is replete with examples, some of which I have pointed out, of the confusion which results when flagellar morphology is neglected or minimized. Proper illustrations are a must for all descriptions of bacteria, and it is hoped that both authors and editors will keep this in mind.

Numerical Taxonomy

The use of electronic computers in bacterial taxonomy was first effectively introduced by P. H. A. Sneath in 1957. Following the initial papers by Sneath (20-22), others have been published by various authors. The general idea is to classify bacteria on the basis of overall similarity by use of a large number of features. The method is not intended for the identification of bacteria but for the production of taxonomic groups which may be labeled species, genera, etc. One must still resort to a number of definitive features for practical identification of individual cultures.

The prototype of studies on numerical taxonomy is the paper by Sneath dealing with Chromobacterium (22). The data are based on a detailed study of a large number of strains of Chromobacterium (20, 22); 105 features were selected and used to program the computer. Although the selected features obviously must have equal weight, the "characteristics" on which the features were based were not given equal weight.

In Sneath's data we find morphological characteristics separated into 24 features: length of rods, five features; breadth of rods, four features; shape and arrangement of rods, six features; flagellar arrangement, three features; Gram reaction, one feature; fat, two features. The size of the rods was thus given nine features; flagellation was given three features; and Gram reaction, one feature. If the morphological characteristics of a heterogeneous collection of bacteria were similarly programmed, the resulting groups would be very odd indeed. Obviously, this program cannot be applied to a heterogeneous group of bacteria unless we are willing to scrap completely our conventional taxonomy. Even if we select our group to include only gram-negative heterotrophic rods, the division into polar flagellates (Pseudomonas, Aeromonas, Vibrio, etc.) and peritrichous flagellates (Alcaligenes, Salmonella, Listeria, etc.) would likely disappear. The genus Chromobacterium is a selected group with several definitive characteristics: gram-negative, purple pigmentation, mixed polar-peritrichous flagellation. The group undoubtedly was originally designated as a genus because of the purple pigmentation. Should gram-positive purple bacteria exist, the one feature given to the Gram reaction would not exclude them from the genus. With only three features given to flagellation, this characteristic does not carry much weight.

Although gross differences in somatic size undoubtedly have taxonomic significance, small differences must be treated with caution. It is common knowledge that the size of the soma is not the same throughout the growth cycle and, at the same stage in the growth cycle, varies with the pH, the temperature, and the nature of the medium. Under specific cultural conditions, individual strains of a species may have different growth rates and, after a specific incubation time, may be in different phases of the growth cycle. Differences in nutritional requirements may be considerable for different species of a genus, and even for individual strains within a species. A single medium may thus be more optimal for one culture than for another and thus affect the somatic size. Halophilic bacteria may produce elongated forms and filaments with as little as 0.5% of NaCl (11), and halophilic bacteria may likewise produce elongated and filamentous forms at lowered salt concentrations. Shands (18) showed that a strain of Salmonella typhimurium, when grown at a rapid rate in a rich medium, was five times larger in the exponential phase than when grown at a slow rate in a chemostat. Sneath's measurements of size for both mesophiles and psychrophiles were made with a single nutrient agar medium and a temperature of 25 C. After 18 hr of incubation, the mesophiles averaged 0.75 by 1.9 , and the psychrophiles, 0.95 by 3.7 . One mesophile measured 1 by 5 , and one psychrophi, 0.8 by 2 . After 4 days of incubation, there was much less difference in size: mesophiles averaged 0.75 by 2 , and the psychrophiles, 0.85 by 2.5 . When one remembers that Sneath's mesophiles were mainly fermentative, facultative
bacteria and his psychrophiles were oxidative, aerobic bacteria, the significance of the size differences in the young cultures on a single medium and at the same temperature seems highly questionable, and nine features given to somatic size unjustifiable.

Under the heading of Metabolism and Nutrition are 22 features: anaerobic growth, one feature; temperature relationships, nine features; citrate slants alkaline, two features; NaCl tolerance, two features; pigmentation, seven features. The heavy weighting here is on temperature relationships and pigmentation. The weighting of temperature relationships with seven features seems rather extreme, but certainty helped to produce the two taxonomic groups, mesophiles and psychrophiles, which Sneath has proposed as the two species of *Chromobacterium*. Sneath’s differentiation of mesophiles and psychrophiles is based mainly on ability to grow at 37°C. From his data, we see that 6 of his 16 strains of mesophiles had an optimal growth temperature of 30°C, and 14 of his 23 strains of psychrophiles also had an optimal growth temperature of 30°C. Personally, I have studied many cultures of bacteria which are morphologically and physiologically alike but differed in their ability to grow at 37°C. While not discounting the taxonomic significance of temperature relationships, to give nine features to this characteristic seems inappropriate. Anaerobic growth has proved to be a very important taxonomic characteristic and certainly deserves more than one feature. The *Aeromonas-Vibrio* genera may be separated from the *Pseudomonas* genus on this characteristic alone.

Under the heading Biochemical Reactions are 34 features. Fermentation of glucose is scored as one feature and oxidative metabolism of glucose as one feature. The differentiation between aerobic and anaerobic metabolism of glucose has been one of the major advances in bacterial taxonomy: *Aeromonas* versus *Pseudomonas*; *Achromobacter* versus the *Enterobacteriaceae*; micrococci versus staphylococci. Sneath assigns only two features to this characteristic. Eleven features are assigned to acid production in peptone-water from carbohydrates. To assign any feature to acid production in peptone media where carbohydrate oxidizers are involved is very questionable. In fact, in protocol 8, under psychrophiles, we find more question marks than any other marks. No distinction is made between acid from fermentation and acid from oxidation. An organism which ferments, for example, sucrose is thus considered identical in this respect to an organism which oxidizes sucrose. It seems to me that separate features must be assigned to fermentative acid and to oxidative acid from each of the carbohydrates tested. Since it is not possible by the usual methods to determine oxidative metabolism in the presence of fermentative metabolism, with the fermentative feature positive the oxidative feature would have to be marked no score. Acid production from carbohydrates, even when a distinction is made between fermentative and oxidative acid, may not be a good feature, unless one is dealing with rather closely related bacteria. Different types of bacteria may produce quite different acids. Acid production from lactose by a homofermentative species of *Lactobacillus* should not be equated with acid production from lactose by a heterofermentative species of *Escherichia*. Much the same may be said for other tests which are based on production of acid and alkali.

From his study of *Chromobacterium*, Sneath concluded that his cultures formed two fairly distinct groups which could be designated as species: a psychophilic group, *Chromobacterium violaceum*, and a mesophilic group, *Chromobacterium lividum*. The two groups are largely the result of Sneath’s selection of features, with heavy emphasis on somatic morphology (15 features) and temperature relationships (9 features), and slight emphasis on the basic differences in carbohydrate metabolism. Four cultures in Sneath’s collection were mesophilic and showed oxidative metabolism of glucose, as did the psychophilic group, but were classified as *C. violaceum* along with the mesophilic fermenters. This is comparable to classifying pseudomonads in the same species with aeromonads, or achromobacters in the same species with salmonellae. By a different “weighting” of the various characteristics, the results could have been quite different: with the fermenters in one group and the oxidizers in another group; or three groups, as proposed by Leifson (10), with the mesophilic fermenters (*Chromobacterium manilae*), mesophilic oxidizers (*C. laurentium*), and psychophilic oxidizers (*C. violaceum*) in separate groups or species. My own experience has been that, with bacteria in general, temperature relationships are not of great taxonomic importance. I have studied many cultures which were almost identical except for differences in their temperature relationships. Fermentative versus oxidative metabolism of carbohydrates appears to be a much more important and definitive taxonomic characteristic.

Since the publication by Sneath, a number of papers have appeared on numerical taxonomy. In most of these papers, the heavy emphasis has been on somatic morphology, colony characteris-
tics, and characteristic growth in broth. With the groups studied, none of these characteristics is very definitive. In a paper by Colwell and Liston (3) on the taxonomic relationships among the pseudomonads, we find, with 77 features, 14% were given to somatic morphology, 13% to colony characteristics (including colors such as white, off-white, and gray), 7% to growth in broth, and 6% to litmus milk, making a total of 40%. Flagellation is given two features: motile and polar flagella. Apparently, the authors regarded as of no taxonomic importance the flagellar wavelength, the number of polar flagella, mixed flagellation, etc. The carbohydrate metabolism was determined in liquid media, except for glucose for which the O-F medium was used. Oxidative metabolism of carbohydrates by members of the genus Pseudomonas cannot be determined reliably in liquid peptone media. Several features are given to gas production from carbohydrates, which seems rather odd unless the computer program was designed to include fermentative types. If this were so, the authors would equate fermentative acid with oxidative acid, which is not justifiable.

In a paper by Rhodes (17), we find this rather instructive statement: "The position of the two Aeromonas isolates and their apparent fusion with the P. fluorescens group at the 83% level is misleading because their distinctly different non-pseudomonad properties, which relate to their characteristic fermentative metabolism, were characters not included in the present analysis." The genus Chromobacterium includes both fermentative and oxidative types. If the nature of the carbohydrate metabolism is so fundamental, a taxonomic characteristic in the differentiation of Pseudomonas and Aeromonas, why is it not equally fundamental in the differentiation of fermentative and oxidative members of the genus Chromobacterium? Sneath includes both fermenters and oxidizers in his Chromobacterium lindow species. Rhodes herself is rather inconsistent when she states in the same paper that the genus Pseudomonas is closely related to the genus Chromobacterium. As I have stated before, the present genus Chromobacterium could well be elevated to the rank of a family.

In a paper entitled "Taxonomy of Pseudomonas piscicida (Bein) Buck, Meyers and Leifson," by Hansen, Weeks, and Colwell (6), we have a striking example of how far afield the computer taxonomy method can lead. Twenty strains labeled P. piscicida were studied by use of 120 features. Their strain 14 of P. piscicida was found to be most similar to the median organism, with an S value of 91.67. Strain 14 was deposited with ATCC and given accession number 15802.

ATCC 15802 is nonpigmented on seawater-peptone media, not halophilic but grows very well in simple media without added salt, lophotrichous flagellate. The type strain of P. piscicida which was described and illustrated by Buck, Meyers, and Leifson and deposited in ATCC is: pigmented, halophilic, polar monotrichous flagellate. In other words, the most typical strain of P. piscicida, according to their analysis, is not a marine organism at all but a nonpigmented terrestrial lophotrichous pseudomonad. How this sort of an analysis can benefit the taxonomy of P. piscicida, or any group of bacteria for that matter, is difficult to comprehend. One is reminded of the classical argument about the number of teeth in a horse's mouth. Sometimes it pays to have a look, even at the flagellation. Others have arrived at a somewhat similar opinion, as for example the following statement by Marmur, Falkow, and Mandel (15) in the Annual Review of Microbiology: "While this approach has its uses in certain cases, numerical taxonomy is tedious in its practice, limited in its concepts, and has produced no new principles, nor has it as yet led to a meaningful improvement in the definition of taxonomic categories."

Literature Cited
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