A MECHANICAL KEY FOR THE GENERIC IDENTIFICATION OF BACTERIA

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THE NEED FOR ADEQUATE DESCRIPTIONS

Many of the descriptions of bacteria in Bergey's Manual of Determinative Bacteriology (1939, 1948) are decidedly poor when viewed from present-day standards. Some will be difficult to improve since a number of the original cultures have probably been lost. The original descriptions which still remain on record present us with an awkward problem in establishing priorities. Some of these descriptions are so inadequate that one description could be equally well applied to many new isolates. The original authors cannot be blamed for the inadequacy of these descriptions which no doubt conformed to the standard of the day and it would be a breach of ethics to refuse recognition of these descriptions. Nevertheless present-day workers cannot regain the original cultures in some instances to subject then to further examination and would-be key formers are handicapped by the lack of this information. Thus one cause of the chaotic state of bacterial nomenclature is the lack of "type" specimens regarded as essential by systematic botanists. There is only one remedy for this, namely the redescription of all available cultures according to a certain code which should be applied to all bacteria alike. On the basis of these descriptions the organisms should be renamed, for the most part with the names they now possess. Priorities should be based on these names and all descriptions and names for which there are no procurable cultures should, by common consent, be discarded.

Such a move would necessarily have to be backed by a definite decision on a code for description and standardization of methods whereby characters employed in the code are determined. Although an attempt has been made on an international basis to maintain uniformity in methods of nomenclature, no attempt has been made to standardize the methods by which tests are carried out to determine the characteristics of a species. The Society of American Bacteriologists through its Committee on Bacteriological Technic has suggested a scheme whereby all organisms should be described and in its "Manual of Methods of Pure Culture Study of Bacteria" has made an attempt to standardize the methods by which the tests are carried out. The scheme is considered by some to be too extensive and quite unnecessary for some groups of organisms. I cannot agree with this attitude. It is easier to discard evidence considered unnecessary than it is to repeat an investigation through lack of it.

A descriptive code need not be static. It could be expanded or contracted as the need arises. Expansion would necessitate further investigation of described species. Necessary alterations can easily be made to mechanical keys.

A uniform code should be enforced and publication of descriptions should be contingent on complete descriptions being submitted, even if this entailed work-

ers in poorly equipped laboratories having to submit cultures to recognized institutions for complete description. The main objection to this procedure would be overcome if the worker submitting the culture retained the right of discovery and the right to name the organism.

THE DEFINITION OF SPECIES AND GENERA

A species is defined only by those characteristics which, through their constancy and stability have served in the past to assign it to a genus, together with those characteristics which have served to separate it from other species in the same genus. A distinct advantage would be gained if a list of these characteristics were appended to the name of each bacterium prior to the general description. Attention would then be focused on these essential characteristics and a better appreciation of the extent and importance of variations be gained.

Characters which are not employed in differentiation of the species could be employed for the subdivision of species into types.

There is also need for more precise definitions for genera. In the hands of the authors of most of our textbooks the term "definition" has entirely lost its meaning. Many of the definitions contain very little which is definite. They approach more towards condensed, and often confusing, descriptions which attempt to embrace all the possibilities which one may encounter among the species in the genus rather than a precise statement of the characters which can be uniformly found among all or the majority of species within that genus and on the basis of which one might expect the genus to be distinguished from other genera.

Let us take for example the definition of the genus *Streptococcus* from Topley and Wilson's Principles of Bacteriology and Immunity (1947). It reads as follows:

DEFINITION. *Streptococcus.* (Author's italics.)

Spherical or ovoid cells, arranged in short or long chains or in pairs. *Usually* non-motile. Non-sporing. *Most* species Gram-positive. *Some* species form capsules. Growth *tends to be* relatively slight on artificial media and *some* species grow poorly in the absence of native protein. *Several* species produce characteristic changes in media containing blood. *Various* carbohydrates are fermented with the production of acid. *Most* species fail to liquefy gelatin. *Most* species are aerobic and facultatively anaerobic. *Some* are anaerobic. *Many* species are normally pathogenic on man or animals; *some* species are highly pathogenic and *some* produce soluble toxins.

This so-called "definition" contains two statements, the first and third, which can reasonably be applied to all species and therefore could be said to characterize the genus if the genus *Leuconostoc* is not recognized.

The above "definition" would be more aptly termed a "description."

There is a great need for precise definitions in systematic studies. Adoption of precise definitions for species should automatically lead to more precise definition of genera to which they belong and the characters used in the definition of genera would, like those used in defining species, be definitely determinable. Inclusion of species in genera on purely speculative ground should be entirely eliminated. It would be infinitely better if a new genus was created for species which do not

conform to the general pattern, particularly if one views the genera as "form genera" without any phylogenetical significance.

Variation will no doubt be raised as a barrier to this unquestionably idealistic approach to bacterial classification. In the opinion of some workers variation is so prevalent that definition of species would be impossible. But despite this we cannot lose sight of the fact that over the past half century a great number of organisms have been described and have definitely attained species rank. Recognition of them as species has come through the gradual recognition of the constancy of certain characteristics which they display. Variants have been recognized only because certain characters were selected as representative of a species, that is, a certain standard was created. It is a fundamental fact that without such standards there can be no variants, otherwise all variants would themselves assume the status of standards. For this reason the acceptance of a suitably worded list of the stable characters of a species as a "definition" for the species is entirely justified.

THE CLASSIFICATION OF BACTERIA; FORMATION OF HIGHER GROUPS

The adoption of the botanical system of nomenclature made possible the segregation of bacteria into Species, Genera, Tribes, Families and Orders. A number of classifications have been suggested. These have been reviewed by van Niel (1946) and have been discussed briefly in Bergey's Manual. Van Niel questions the wisdom of attempting to form a "classification" on the evidence which is at present available but in so doing he does not discount the fact that there is strong evidence for phylogenetical relationships between some groups of bacteria. A close study of the number of determinable characters which could represent all species within each genus would reveal this number to be very small. The number of characters which are common to all genera within a Tribe must inevitably be smaller, and would continue to diminish as groupings become broader. Where groupings have become impossible on purely determinable characters resort has to be made to the use of certain assumptions which originate from the study, not of single organisms, but of large groups of organisms. On the basis of these assumptions some bacteria may be placed in higher groupings on a character or characters which they do not possess but which one is led by assumption to believe that they probably did possess by virtue of obvious relationships to other bacteria which do possess these characters. This was the case with *Corynebacterium pseudodiphthericum* (hofmanni) which in the fifth edition of the Manual was placed in the Order *Actinomycetales*, because of its relationship to certain other species in the same genus reputed to show positive evidence of branching—a salient character of the Order *Actinomycetales*. But how one was expected to identify the organism by first determining a character which it did not possess is difficult to understand.

This is the obvious result of an attempt to form a key for *identification* which operates in the reverse direction to that in which the *classification* was constructed. In the development of our system of classification the discovery and naming of species with a generic and specific name came first. Grouping into

Genera was followed by grouping of Genera into Tribes and Tribes into Families and Families into Orders. In developing the key in the reverse order, the authors of the keys in the Manual were forced to use initially for identification characters which by their very nature are largely indeterminable.

Van Niel struck at the heart of this matter and advocated the complete separation of a system of identification from a system of classification. He suggested the formation of a number of mechanical keys for the identification of bacteria. I am strongly in favor of van Niel's suggestion. It is the only way to overcome the vexatious problem of identifying organisms. Van Niel did not elaborate greatly on the type of keys he envisaged. I therefore suggest that initially keys should be formed for the identification of species into genera. Subsequent keys could be formed for identification of species in each genus on the lines already followed in the Manual. Concurrent with the production of generic keys and keys to species for purposes of identification, systematists could also direct attention to the formation of keys to *show how* Tribes are formed from Genera, Families from Tribes and so on. Such keys should assume a different form from those used for identifying bacteria and should be largely discussional. They should *aim to educate* all workers in this fascinating field of biological relationships.

A key to the genera of bacteria is presented in this paper. Genera included are those in the Suborder *Eubacteriineae* of the Order *Eubacteriales* and those in the Order *Actinomycetales* in the sixth edition of Bergey's Manual of Determinative Bacteriology. For the convenience of all workers, the generic terminologies employed in the fifth and sixth editions of the Manual and that employed in the third edition of Topley and Wilson's "Principles of Bacteriology and Immunity" have all been included. The only genus omitted is the miscellaneous genus *Bacterium* as used in the Manual.

The bacteria in the Suborder *Eubacteriineae* are characterized by the possession of rigid cell walls, the absence of photosynthetic pigments and the absence of a stalk which would fix them to their substrate. Those in the Order *Actinomycetales* are similar except that they show marked or rudimentary branching. Unfortunately not all species included in the Order do branch and the absence of the character renders their identification difficult. For this reason all species in the order have been treated with those in the Suborder *Eubacteriineae* in the key.

It must be emphasized that the only advantage that can be claimed for the key is that it is workable. It cannot improve in any way on the original material. It was quite apparent at the outset that it was impossible to obtain the type of "definition" for individual genera advocated in the earlier part of this paper. It was impossible to obtain a combination of characters for every genus which would separate it from other genera. Therefore in preparing the key the only other course was adopted. The reactions of all bacteria given species rank in the Manual were tabulated and characters were selected which appeared to be uniform for each separate genus. In some instances this was found impossible and genera had to be subdivided and each subdivision treated separately. In some instances descriptions from Topley and Wilson's "Principles of Bacteriology and Immunity" were substituted for those in the Manual.

Characters employed in the key were selected solely on their suitability for separating organisms and the ease with which they could be determined. No other importance was attached to any of the tests. As far as possible an attempt was made to keep the sequence of characters in line with accepted laboratory practice.

Quite frequently information on the reaction of species to tests employed in the key was not available. In such cases the species has been treated as being either positive or negative and separation has been effected in both sections of the key. The same procedure was adopted where species were known to be variable within a genus to a particular character, e.g., with motility in *Escherichia coli*. The absence of information has greatly increased the length of the key. It could be considerably reduced in size if the information was made available.

The key is of a type commonly employed by botanists. The numbers on the left hand side of the key cover, for the greater part, pairs of opposing characters such as "gram positive or gram negative." The character of the pair which applies to the organism in question is selected and the number immediately on the right indicates the next number on the left to be consulted. The numbers must be followed in strict sequence until a number on the right is replaced by a generic name. A single example is given.

For species of the genus *Escherichia* the number sequence is as follows: 1–7; 7–10; 10–44; 44–45; 45–46; 46–47; 47–48; 48–49; 49–50; 50–52; 52–54; 54–57; 57–62; 62–63; 63–64; 64–65 or 64–71

65–66; 66–67; 67—*Escherichia;*
or, 71–72; 72–73; 73—*Escherichia.*

THE KEY

1. Organisms which can grow on a mineral salts medium with carbon dioxide as the sole source of carbon; strict autotrophs which will not grow on meat extract agar; do not oxidize hydrogen to water 2
   Other than above 7
2. Organisms which oxidize ammonia to nitrites 3
   Organisms which oxidize nitrite to nitrate 6
   Organisms which oxidize sulfur compounds (inorganic) ..... *Thiobacillus*
3. Zoogloea formed 4
   Zoogloea not formed 5
4. Zoogloea surrounded by a membrane, forming a cyst ..... *Nitrosocystis*
   Zoogloea not encysted ..... *Nitrosogloea*
5. Cells ellipsoidal ..... *Nitrosomonas*
   Cells spherical ..... *Nitrosococcus*
   Cells spiral ..... *Nitrosospira*
6. Zoogloea present ..... *Nitrocystis*
   Zoogloea absent ..... *Nitrobacter*
7. Organisms will grow on a mineral salts medium with carbon dioxide as a sole source of carbon but will also grow on meat extract agar 8
   Organisms will grow in a mineral salts medium if carbon monoxide or methane is present as a sole source of carbon 9
   Other than above 10
8. Organisms grow autotrophically oxidizing hydrogen to water ..... *Hydrogenomonas*
   Organisms grow autotrophically oxidizing sulfur compounds ..... *Thiobacillus*
9. Organisms will grow autotrophically using carbon monoxide as the sole source of carbon ..... *Carboxydomonas* (5th ed.)

(Note: the genus *Carboxydomonas* has been appended to the genus *Streptomyces* in the 6th edition of the Manual.)

Organisms will grow autotrophically using methane as a source of carbon and energy ..... *Methanomonas*

10. Gram positive 11
    Gram negative 44
11. Organisms will grow in contact with air without the addition of specific reducing substances to the medium 12
    Organisms will grow only in the absence of air, or in the presence of air if specific reducing substances are added to the medium 37
12. Rods and cocco-bacilli 13
    Cocci 33
13. Sporing rods ..... *Bacillus*
    Non-sporing rods 14
14. Organisms showing distinct branching 15
    Organisms not branched 17
15. Long intertwining branching forms showing little or no tendency to fragmentation of the pseudomycelium except at the tips where short conidia-like bodies are formed in large numbers in chains ..... *Actinomyces* (5th ed.)
    Streptomyces (6th ed.)
    Actinomyces (T & W.)
    Similar to above except that only one "conidium" is produced at the end of each side branch or "conidiophore" ..... *Proactinomyces* (5th ed.)
    Micromonospora (6th ed.)
    Actinomyces (T & W.)
    Branched rods which do not produce conidia 16
16. Organisms present in young cultures as branched filaments which rapidly disintegrate to produce short branched rods and some unbranched rods and coccal forms; non-acid fast or only weakly acid fast; obligate aerobes ..... *Actinomyces* (5th ed.)
    Nocardia (6th ed.)
    Actinomyces (T & W.)
    Branched rods with little or no tendency to form filaments; colonies dry and crumbly; acid fast ..... *Mycobacterium*
    Branched rods with only a slight tendency to form filaments; division by a sharp snapping action resulting in palisade formation; non-acid fast ..... *Corynebacterium*
17. Motile rods 18
    Non-motile rods 22
18. Carbohydrate fermented 19
    Carbohydrates not fermented ..... *Kurthia* (5th ed.)
    Zopfius (T & W.)
    placed in genus *Bacterium* (6th ed.)
19. Organisms pathogenic to plants 20
    Organisms not pathogenic to plants; pathogenic to warm-blooded animals causing monocytosis ..... *Listerella* (5th ed.)
    Listeria (6th ed.)
    Erysipelothrix (T & W.)
20. Yellow pigmented colonies ..... *Xanthomonas* (6th ed.)
    Colonies with no yellow pigments 21
21. Flagella polar ..... *Phytomonas* (5th ed.)
    Flagella peritrichiate ..... *Erwinia*
22. Unbranched rods; colonies dry and granular; organisms have twisted axes and tend to form in clumps; acid fast ..... *Mycobacterium*
    Not as above; non-acid fast 23

23. Litmus milk acid or acid and coagulated....24
Litmus milk alkaline or unchanged....29
24. Litmus milk acid and coagulated....25
Litmus milk acid only....26
25. Organisms beaded and club shaped; pleomorphic; pathogenic to guinea pigs, rabbits and mice; no growth in a glucose acetic acid broth with pH 4.5...*Corynebacterium*
Non-pleomorphic; non-pathogenic; growth in glucose acetic acid broth with pH 4.5 *Lactobacillus*
26. Colonies small, dewdrop, about 0.2 mm in diameter; in smooth colonies the organisms appear as short rods; in rough colonies they appear as long filaments; no growth in acetic acid glucose broth with pH 4.5; cause erysipelas in man and animals *Erysipelothrix*
Not as above....27
27. Catalase positive....28
Catalase negative....*Lactobacillus*
28. Small thin rods; granular staining; resist heating to 71 C for 2½ minutes . *Microbacterium*
Not as above....*Corynebacterium*
29. Growth in acetic acid glucose broth at pH 4.5; non-pleomorphic rods producing over 1% lactic acid from glucose without continuous neutralization....*Lactobacillus*
Not as above....30
30. Human mouth parasites; long tangled threads intermingled with a few short rods *Leptotrichia* (5th ed.)
appended to genus *Lactobacillus* (6th ed.)
Not as above....31
31. Small dewdrop colonies; organisms cause erysipelas in man and animals *Erysipelothrix*
Not as above....32
32. Rods straight or curved; arranged singly; non-pleomorphic; litmus milk alkaline and peptonized; no carbohydrates fermented....*Alcaligenes*
treated in the miscellaneous section by T & W.
Rods usually in palisades; division by a characteristic snapping motion; pleomorphism common; litmus milk may be rendered acid or alkaline but is not peptonized *Corynebacterium*
33. Organisms arranged in cubical packets....*Sarcina*
Organisms arranged in chains or in diplococci in liquid media....34
Organisms arranged in clusters other than cubical packets....36
34. Kidney shaped or hemispherical organisms arranged in diplococci in clumps. Colonies high, convex, and granular and usually difficult to emulsify; species cause gonorrhea, meningitis; others normal inhabitants of the throat....*Neisseria*
Spherical or oval organisms in chains or in diplococci which do not form clusters....35
35. Organisms producing a slimy pellicle in sucrose broth; in which the organisms are heavily capsulated and form zoogloea....*Leuconostoc*
Other than above....*Streptococcus*
including the genus *Diplococcus*
36. Organisms divide in two directions producing plates of organisms all lying in the one plane....*Staphylococcus* (5th ed.)
Micrococcus (6th ed.)
Staphylococcus (T & W.)
including the genus *Gaffkya*
Organisms apparently divide in more than two directions producing clusters or organisms lying in more than one plane....*Micrococcus*
37. Cocci....33
Rods....38

38. Branching rods....39
Not branching rods....40
39. Long branching filaments which show no tendency to disintegrate; produce a single "conidium" at the ends of short side branches or "conidiophores"
Actinomyces (5th ed.)
Micromonospora (6th ed.)
Actinomyces (T & W.)
Long branching filaments showing no tendency to disintegrate except at the tips of aerial "hyphae" where chains of "conidia" are produced; giving the colony a dry powdery surface....*Actinomyces* (5th ed.)
Streptomyces (6th ed.)
Actinomyces (T & W.)
Short branching rods. In very young cultures branching filaments are formed which rapidly disintegrate to form short branched rods and some unbranched rods and cocci; no conidia formed....*Actinomyces* (5th ed.)
Actinomyces (6th ed.)
Actinomyces (T & W.)
40. Sporing rods....*Clostridium*
Non-sporing rods....41
41. Plant pathogens; motile....42
Not plant pathogens; non-motile....43
42. Flagella peritrichiate....*Erwinia*
Bacterium (T & W.)
Flagella polar....*Phytomonas* (5th ed.)
43. Catalase positive; propionic acid produced from lactic acid and from glucose
Propionibacterium
Catalase negative; butyric acid produced from lactic acid and from glucose
Butyribacterium (6th ed.)
Catalase negative; lactic acid accumulates as an end product of glucose breakdown
Lactobacillus
44. Organisms grow in contact with oxygen without the addition of specific reducing substances to the medium....45
Organisms will not grow in contact with oxygen unless some specific reducing substances are added to the medium....118
45. Organisms isolated from and capable of producing nodules on the roots of leguminous plants....*Rhizobium*
Not as above....46
46. Organisms capable of continued growth in a medium devoid of inorganic or organic nitrogen compounds; large rod-shaped organisms which change to spherical forms in ageing cultures....*Azotobacter*
Not as above....47
47. Kidney shaped or hemispherical organisms arranged in pairs in clusters; organisms found commonly in the throat....*Neisseria*
Rods and cocco-bacilli....48
48. Sporing rods....*Bacillus*
Non-sporing rods....49
49. Organisms grow well in a yeast water medium containing up to 10% alcohol, oxidizing the latter to acetic acid....*Acetobacter*
Not as above....50
50. Colonies with a purple pigment....*Chromobacterium*
Colonies with a red pigment....51
Colonies other than the above....52
51. Motile rods; do not attack alkylamines....*Serratia*
Chromobacterium (T & W.)
Motile rods capable of attacking alkylamines....*Protaminobacter*

52. Organisms producing water soluble green, blue, or yellow pigments which diffuse into the medium....53
Not as above; pigments, if produced, are not water soluble....54
53. Plant pathogens....*Phytomonas* (5th ed.)
Pseudomonas (6th ed.)
Not plant pathogens....*Pseudomonas*
54. Organisms isolated from disease lesions in plants....55
Not as above....57
55. Yellow pigment produced which is not soluble in water....*Phytomonas* (5th ed.)
Xanthomonas (6th ed.)
No yellow pigment produced....56
56. Flagella polar....*Phytomonas* (5th ed.)
some species in the the genus *Agrobacterium* (6th ed.)
other species apparently in the genus *Pseudomonas* (6th ed.)
Flagella peritrichiate....*Erwinia*
Bacterium (T & W.)
57. Curved rods, single or united in chains....58
Straight rods and cocco-bacilli....62
58. Rods curved; organisms capable of using phenolic compounds as a source of carbon; gas produced in 0.1% nitrate broth but no nitrites are produced; no carbohydrates fermented....*Mycoplana*
Not as above....59
59. Short stout rods, motile with a single flagellum....60
Long thin rods....61
60. Cellulose oxidized to oxycellulose; no growth on meat infusion agar; cells curved with pointed ends....*Cellfacicula*
Cellulose is not oxidized....*Vibrio*
Note: The genus *Thiospira* (6th ed.) is not treated.
61. Motile with a single flagellum; cellulose oxidized....*Cellvibrio*
Motile with several polar flagella; cellulose is not oxidized....*Spirillum*
(except *S. virginianum*)
62. Lactose fermented within seven days....63
Lactose not fermented within seven days....84
63. Growth on MacConkey's agar in three days at 37 C....64
Not as above....76
64. Motile at 37 C....65
Non-motile at 37 C....71
65. Acid and gas produced from glucose....66
Acid only produced from glucose....68
66. Colonies yellow....*Flavobacterium*
Chromobacterium (T & W.)
Colonies not pigmented....67
67. Methyl red positive; Voges Proskauer negative....*Escherichia*
Bacterium (T & W.)
Methyl red negative; Voges Proskauer positive....*Aerobacter*
Bacterium (T & W.)
68. Indole produced....*Eberthella* (5th ed.)
appended to the *Salmonella* (6th ed.)
Indole not produced....69
69. Small translucent colonies; greyish yellow; mucoid; small slender rods in smooth colonies; ovoid rods with bipolar staining in rough colonies; cause melioidosis in man and animals....*Malleomyces*
Pfeifferella (T & W.)
Not as above....70

70. Yellow pigmented colonies ..... *Flavobacterium*
Chromobacterium (T & W.)
Colonies not pigmented ..... *Achromobacter*
71. Acid and gas from glucose 72
Acid only from glucose 74
72. Methyl red positive; Voges Proskauer negative 73
Methyl red negative; Voges Proskauer positive ..... *Aerobacter*
Bacterium (T & W.)
73. Colonies not mucoid; organisms not capsulated ..... *Escherichia*
Bacterium (T & W.)
Colonies mucoid; organisms capsulated; pathogenic causing throat infections in man and animals ..... *Klebsiella*
Bacterium (T & W.)
74. Yellow pigmented colonies ..... *Flavobacterium*
Chromobacterium (T & W.)
Colonies not pigmented 75
75. Enteric organisms; optimum temperature 37 C; pathogenic; agglutinated with shigella antisera ..... *Shigella*
Non-enteric organisms; optimum temperature below 37 C; water or soil organisms not agglutinated by any shigella antisera ..... *Achromobacter*
76. Motile 77
Non-motile 80
77. Small cocco-bacilli; capsulated; colony mucoid; isolated from the conjunctiva
Noguchia
Not as above 78
78. Cellulose hydrolyzed ..... *Cellulomonas*
Cellulose is not hydrolyzed 79
79. Yellow colonies ..... *Flavobacterium*
Chromobacterium (T & W.)
Non-pigmented colonies ..... *Achromobacter*
80. Small ovoid cocco-bacilli usually showing bipolar staining; cause hemorrhagic septicemia in animals ..... *Pasteurella*
Not as above 81
81. Colonies adherent to the agar; resemble sulfur granules but are not yellow; organisms may show bipolar staining; strict parasites causing actinobacillosis in man and animals ..... *Actinobacillus*
Not as above 82
82. Cellulose hydrolyzed ..... *Cellulomonas*
Cellulose not hydrolyzed 83
83. Yellow colonies ..... *Flavobacterium*
Chromobacterium (T & W.)
Colonies not pigmented ..... *Achromobacter*
84. Non-motile at 37 C or at 22 C 85
Motile at one of these temperatures 108
85. Grows on MacConkey's agar in three days 86
No growth on MacConkey's agar in three days 95
86. Acid or acid and gas from glucose 87
No acid or gas from glucose 93
87. Acid and gas from glucose 88
Acid only from glucose 89
88. Organisms capsulated; colonies mucoid; organisms pathogenic causing throat infections in man and animals ..... *Klebsiella*
Colonies not mucoid; organisms not capsulated; agglutinated by salmonella group D "O" antiserum ..... *Salmonella*

89. Small ovoid cocco-bacilli; may show bipolar staining; colonies on agar very small, glistening and colorless; organisms cause plague in man and animals...*Pasteurella*
Not as above....90
90. Colonies pale grey; translucent....91
Colonies with a yellow pigment....92
91. Organisms agglutinated by shigella antisera; enteric pathogen....*Shigella*
Not as above....*Achromobacter*
92. Organisms attack alkylamines....*Protaminobacter*
Not as above....*Flavobacterium*
Chromobacterium (T & W.)
93. Colonies with a yellow pigment....*Flavobacterium*
Colonies not pigmented....94
94. Litmus milk strongly alkaline; no carbohydrates fermented....*Alcaligenes*
treated in the Miscellaneous groups (T & W.)
Litmus milk acid or unchanged....*Achromobacter*
95. Acid produced from glucose....96
Glucose not fermented....100
96. Colonies on blood agar very small, transparent, dew-drop or bisected pear; no growth on meat infusion agar; organisms usually small cocco-bacilli but may occur as long spiral filaments on first isolation; inhabitants of the respiratory tract but may be found in large numbers in the cerebrospinal fluid in cases of meningitis in children
Haemophilus
Not as above; will grow on meat infusion agar....97
97. Slender rods; 1.0 to 3.0 μ long often arranged in palisades; pleomorphic; will grow on meat infusion agar in 48 hours producing a smooth entire, glistening butyrous colony about 1 mm in diameter; bipolar staining common; café au lait on potato; Strauss reaction in guinea pigs; strict parasites causing glanders especially in man and horses....*Malleomyces*
Small ovoid cocco-bacilli; 0.7 to 2.0 μ long; arranged singly, in pairs and in small bundles; may be pleomorphic and show bipolar staining; will grow on meat infusion agar in 24 hours at 37 C producing a small entire smooth glistening colony which may extend from 1 to 6 mm in diameter in 5 days incubation; no growth on potato; parasites producing hemorrhagic septicemia in animals and birds....*Pasteurella*
Not as above....98
98. Organisms attacking alkylamines....*Protaminobacter*
Not as above....99
99. Colonies yellow....*Flavobacterium*
Chromobacterium (T & W.)
Not as above....*Achromobacter*
100. Litmus milk acid or alkaline....104
Litmus milk unchanged....101
101. Yellow colonies....*Flavobacterium*
Chromobacterium (T & W.)
Colonies not pigmented....102
102. Colonies 1 to 2 mm in diameter in 24 hours; pale grey, translucent; growth on meat infusion agar good; normal inhabitants of water; saprophytic....*Achromobacter*
Colonies on meat infusion or blood agar 0.5 to 1.0 mm in diameter; often no growth on meat infusion agar on first isolation; cocco-bacilli; parasitic on man or animals. 103
103. Organisms causing subacute infectious conjunctivitis or angular conjunctivitis; occur predominantly as diplobacilli; Loeffler's blood serum slowly liquefied
Moraxella (6th ed.)
Haemophilus (5th ed.)
Isolated from the respiratory tract, meningeal fluid or eye exudates; small cocco-bacilli which require blood or ascitic fluid or other body fluids and sometimes special growth

factors for primary isolation after which some species can be trained to grow on meat infusion media; organisms associated with influenza, whooping cough or meningitis; inspissated serum is not liquefied..... *Haemophilus*

Organisms associated with undulant fever in man; septicemic infections and abortion in animals; growth good on a liver extract agar; increased CO_2 tension often necessary for isolation..... *Brucella*

104. Organisms cause acute ophthalmia (pink eye) in cattle; small gram negative rods predominantly arranged in diplobacilli; enriched media necessary for growth; Loeffler's serum liquefied..... *Moraxella*

Not as above..... 105

105. Good growth on ordinary meat infusion agar; yellow pigment produced which is insoluble in water..... *Flavobacterium*
Chromobacterium (T & W.)

Not as above; no yellow pigment..... 106

106. Litmus milk alkaline..... 107

Litmus milk acid..... *Achromobacter*

107. Organisms associated with undulant fever in man and septicemic infections with or without abortion in animals..... *Brucella*

Not as above..... *Alcaligenes*

108. Acid and gas produced from glucose..... 109

Acid only from glucose..... 110

Glucose not fermented..... 114

109. Organisms produce a swarming growth on moist agar; gelatin is liquefied or indole is produced, or both..... *Proteus*

Discrete colonies on moist agar; gelatin is not liquefied and indole is not produced; sucrose and salicin are not fermented; enteric pathogens of man, animals and birds
Salmonella

110. Colonies mucoid; organisms capsulated; isolated from the eye (Rhesus monkeys); parasitic..... *Noguchia*

Not as above..... 111

111. Small translucent greyish yellow colonies; mucoid; small slender rods in smooth colonies; ovoid rods with bipolar staining in rough colonies; motile at 37 C; organisms cause melioidosis in man and animals..... *Malleomyces*
Pfeifferella (T & W.)

Small umbonate granular colonies; translucent with a dull finely granular "beaten copper" surface; entire; butyrous; organisms cause pseudotuberculosis in rodents
Pasteurella

Not as above..... 112

112. Yellow pigmented colonies..... *Flavobacterium*
Chromobacterium (T & W.)

Not as above..... 113

113. Pathogenic; organism responsible for typhoid fever in man; agglutinated by Salmonella group D antiserum..... *Eberthella* (5th ed.)
Salmonella (6th ed.; T & W.)

Non-pathogenic; not agglutinated as above..... *Achromobacter*

114. Yellow pigmented colonies..... *Flavobacterium*
Chromobacterium (T & W.)

Not as above..... 115

115. Litmus milk rendered strongly alkaline..... 116

Litmus milk rendered acid or unchanged..... 117

116. Organisms causing broncho-pneumonia in rodents and sometimes associated with canine distemper..... *Brucella*

Other than above..... *Alcaligenes*
(including *Agrobacterium radiobacter*)

117. Small circular translucent colonies; mucoid; organisms causing conjunctival folliculosis in rabbits ..... *Noguchia*
Not as above ..... *Achromobacter*
118. Cocci 119
Rods and cocco-bacilli 120
119. Kidney shaped diplococci ..... *Neisseria*
Cocci in clusters; average diameter 0.3 μ ..... *Veillonella*
120. Minute rod-shaped organisms which grow only in media containing sterile fresh tissue or ascitic fluid; found in the upper respiratory tract of man ..... *Dialister*
Not as above 121
121. Sporing rods ..... *Clostridium*
Non-sporing rods 122
122. Plant pathogens 123
Not plant pathogens 124
123. Peritrichiate flagella ..... *Erwinia*
Bacterium (T & W.)
Polar flagella ..... *Phytomonas* (5th ed.)
Pseudomonas (6th ed.)
124. Long tapered cells, 3 to 16 μ with distinct granules; grow poorly on ordinary meat infusion agar ..... *Fusobacterium*
Never more than 3 μ long; rounded ends; pleomorphic ..... *Bacteroides*

SUMMARY

A mechanical key for the identification of bacteria in the Suborder *Eubacterineae* and the Order *Actinomycetales* is submitted. Attention is drawn to the necessity for precise definitions for species and genera.

The key submitted in this paper was originally prepared as an alternative to the keys provided in the fifth edition of Bergey's Manual of Determinative Bacteriology for placing bacteria into their respective genera. It has since been amended to cover the sixth edition of the Manual and is being submitted in the hope that it will be generally tested and criticized and that its deficiencies will be rectified by suggestions from workers experienced in particular fields.

Directions for the procedure adopted in describing bacteria are available in the majority of textbooks but there are no directions for the application of the information to the identification of the organisms. The only attempt to supply this guidance which covers all types of bacteria and related microörganisms is to be found in Bergey's Manual of Determinative Bacteriology. The attempt has not been very successful. The fault in the Manual keys lies in the attempt to combine a system of classification with a system for identification. An alternative method, which effectively separates the two systems and should benefit both, is suggested in this paper. The key proposed will be capable of marked improvement when bacteriologists provide more adequate descriptions of many listed species and genera but in the meantime it should serve a useful purpose.

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