

1 **Classification of Bacteria: From Unicellular Plants to the Age of Genomics**

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6 **Introduction**

7 The history of the classification of bacteria clearly demonstrates that changes were caused by
8 the availability of new technologies. The late 19th century was the beginning of the bacterial
9 taxonomy. Ferdinand Cohn in 1872 was the first to classify six genera of bacteria (as member
10 of the plants) mainly based on their morphology. At the beginning of the 20th century more
11 and more physiological and biochemical data were used in addition to morphology as
12 important markers for the classification and identification of microorganisms. The first edition
13 of Bergey's Manual of Determinative Bacteriology (Bergey et al, 1923) classified the bacteria
14 on the basis of these phenotypic properties as "typically unicellular plants", the so-called
15 Schizomycetes. Even until 1957 bacteria were classified as members of plants. In the 8th
16 edition of Bergey's Manual (Buchanan and Gibbons, 1974) bacteria were arranged in groups
17 mainly based on Gram-stain, morphology and oxygen requirement, but at least bacteria were
18 no longer considered as plants and recognized meanwhile as members of the kingdom
19 Procaryotae.

20 **Numerical taxonomy**

21 Numerical taxonomy improved the phenotypic identification by increasing the number of
22 tests used and by calculating coefficients of phenetic similarities between strains and species
23 (Sneath and Sokal, 1973). For numerical studies, the results are tabulated in a table of t
24 organisms versus n characters and the term OTU (operational taxonomic unit) is used for an
25 individual strain. The characters are equally weighted and should come from the various
26 different categories of properties (morphology, physiology, biochemistry, etc.). The number
27 of common characteristics is considered as a quantitative measure of taxonomic relatedness
28 but this does not mean that the organisms are also phylogenetically related.

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31 **Chemotaxonomy**

32 The chemical composition of cell constituents is a useful property for improving the classifi-
33 cation and identification of prokaryotes. Chemotaxonomic methods are widely used, in
34 particular, for those groups of prokaryotes where morphological and physiological characters
35 have largely failed or have not been sufficient to provide a satisfactory classification.

36 The DNA base composition, guanine-cytosine (GC) content of DNA, is one of the required
37 characteristics of the minimum list of data for a description of a new species. However, it is
38 only an exclusionary determinant in the classification of bacteria, in that two strains differing
39 in more than 10 mol% should not be considered members of the same genus, whereas on the
40 other hand, a similar DNA base composition does not necessarily imply that the two strains
41 are closely related.

42 The occurrence of alkyl glycerol ether lipids instead of fatty acid ester lipids is a very
43 characteristic property of archaea and can be used for distinguishing them from bacteria and
44 eukaryotes. Bacteria contain a wide variety of fatty acids (unbranched or branched fatty acids,
45 hydroxy fatty acids, cyclopropane fatty acids saturated and unsaturated ones). Most fatty
46 acids of bacteria are in the range of C₁₂ to C₂₀. Fatty acid patterns can be determined rather
47 easily and quickly, and automatic identification is even possible. However, the bacteria have
48 to be cultivated under carefully controlled conditions since fatty acid patterns may alter in
49 response to exogenous and endogenous parameters, such as growth temperature, composition
50 of pH of medium, or age of the culture.

51 The ultrastructure and chemical composition of cell walls of Gram-positive and Gram-
52 negative bacteria is quite different. The cell wall of Gram-positive bacteria reveals in profile
53 one thick and more or less homogeneous layer, whereas Gram-negative bacteria have a
54 thinner, distinctly layered cell wall with an outer membrane resembling the typical trilaminar
55 cytoplasmic membrane in profile. The polymers found in the cell walls of these two groups of
56 bacteria are chemically quite different. The walls of Gram-negative cells are mainly
57 composed of lipopolysaccharide, phospholipid, protein, lipoprotein, and relatively little
58 peptidoglycan .

59 The Gram-positive cells contain as major components peptidoglycan, polysaccharides or
60 teichoic acid (or both), or teichuronic acid. Thus, in contrast to the Gram-negative, the Gram-
61 positive bacteria contain hardly any lipids in their cell walls. There is, however, one

62 exception: acid-fast bacteria. They are resistant to decolorization with acidic ethanol after
63 staining with fuchsin (Ziehl-Nielsen staining). These acid-fast bacteria (*Mycobacterium*,
64 *Nocardia*, and *Corynebacterium sensu stricto*) are Gram-positive bacteria which contain
65 large amounts of lipids in their cell walls; in particular, mycolic acids (high-molecular-weight,
66 3-hydroxy acids with a long alkyl branch in position 2). Peptidoglycan (murein) is the only
67 cell wall polymer found in both Gram-positive and Gram-negative bacteria. It is a
68 heteropolymer consisting of glycan strands that are cross-linked through short peptides. The
69 glycan strand is made up of alternating β -1,4-linked residues of N-acetylglucosamine and N-
70 acetylmuramic acid, a derivative of glucosamine and unique constituent of peptidoglycan.
71 The peptide moiety is linked to N-acetylmuramic acid and contains both L- and D-amino
72 acids. The peptidoglycan of Gram-negative bacteria is remarkably uniform (Schleifer and
73 Kandler, 1972). Gram-positive bacteria contain a multilayered peptidoglycan and reveal, in
74 contrast to Gram-negative organisms, a great variation in the chemical composition of their
75 peptidoglycans. Based on the mode of cross-linkage, two main groups of cross-linkage, A and
76 B, can be distinguished (Schleifer and Kandler, 1972). A given peptidoglycan structure is a
77 fairly stable character and fulfils the most important prerequisites of a useful taxonomic
78 marker.

79 **Phylogenetic and genotypic information**

80 Most of the chemotaxonomic data are of only limited use for providing insight into the
81 relatedness of bacteria. However, in the late 1970s and beginning of 1980s a breakthrough
82 was achieved by Carl Woese and coworkers when they were able to derive a tree of life
83 consisting of three distinctly different branches (Bacteria, Archaea and Eukarya) by
84 comparing first partial and later on complete small subunit rRNA gene sequences (Woese,
85 1987). This study revolutionized bacterial taxonomy and for the first time bacteriologists were
86 able to classify prokaryotes on the basis of their phylogenetic relatedness. 16S rRNA gene
87 sequence comparisons are currently considered as gold standard for deducing the
88 phylogenetic relationship of prokaryotes. These genes fulfil all properties of useful molecular
89 markers (ubiquitous, functional constant, conserved, homologous). They are stable markers
90 and less subjected to lateral gene transfer. There is also a good congruence of branching
91 pattern of phylogenetic trees derived from conserved mostly informational genes involved in
92 translation (e.g. EF-Tu) and transcription (rpoB, rpoC), respectively. Moreover, genome
93 based studies are consistent with the rRNA data (Ciccarelli et al., 2006). However, species

94 definition using rRNA sequences is often not possible because the molecule is too conserved
95 to distinguish closely related species. DNA-DNA hybridization

96 Attempts are underway to test whether data from genome comparison can be used for
97 taxonomic purposes. Goris et al (2007) have compared completely sequenced genomes and
98 their corresponding hybridization values. Pairwise comparison of complete genome
99 sequences, showed that the average nucleotide identity (ANI) of shared orthologous genes
100 correlated well with 16S rRNA sequence identity and DNA-DNA similarity values. 70%
101 DNA-DNA similarity corresponds to 95% ANI and 69% conserved DNA, respectively.

102 Recently, multilocus sequence analysis (MLSA) has been employed to classify bacteria
103 (Gevers et al., 2005). MLSA is a method for the genotypic characterization of a diverse group
104 of prokaryotes by comparing sequences of multiple house-keeping protein-coding genes. The
105 best approach is to concatenate the sequences of 7 genes from a set of strains and to use the
106 concatenated sequences to construct a dendrogram which can identify deeply branching
107 clusters and help to delineate genotypic clustering within a genus. However, the proper
108 number of genes and the size of sequenced fragments of each gene have not been system-
109 atically explored.

110 **Current Classification**

111 It has to be emphasized that there is still no officially recognized system for the classification
112 of prokaryotes. The currently applied classification systems rely - for practical reasons - on
113 methods and do not depend on theoretical concepts. Prokaryotes are currently classified by a
114 pragmatic polyphasic approach. This approach includes phenotypic, chemotaxonomic,
115 genotypic data and phylogenetic information. 16S rRNA gene sequencing is applied for
116 determining the phylogenetic position of the organisms. Based on these results, organisms are
117 selected for DNA-DNA hybridization (DDH) studies and species have been defined using the
118 70% DDH cut-off criterion (Wayne et al., 1987). Each taxon should be described and
119 differentiated from related taxa by its phenotypic, chemotaxonomic and genotypic
120 characteristics. It should be kept in mind that the end-users need a pragmatic classification
121 system that can serve as a tool in routine identification. A classification that is of little use to
122 microbiologists, no matter how sophisticated a scheme is, will soon be ignored or
123 significantly modified (Staley and Krieg, 1984).

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125 **Classification of not yet culturable prokaryotes**

126 The provisional status *Candidatus* has been established for certain putative taxa that could not
127 be described in sufficient detail as a novel taxon (Murray and Schleifer, 1994; Murray and
128 Stackebrandt, 1995). The designation *Candidatus* is currently not formally recognized in the
129 *International Code of Nomenclature of Bacteria*. The category *Candidatus* should be used for
130 describing uncultured prokaryotic cells for which their phylogenetic relatedness has been
131 determined and their authenticity revealed by *in situ* probing (e.g. fluorescence *in situ*
132 hybridization, FISH) or similar techniques. In addition to the genomic information all
133 phenotypic information, including structural, metabolic, physiological and reproductive
134 features should be included in the description. Currently, there are more than 200 bacteria and
135 archaea, respectively, are described as *Candidatus*. They are in particular endosymbionts or
136 parasites of Eukaryotes, many of them belonging to the phyla Mollicutes, Chlamydiales and
137 Rickettsiales, respectively.

138 **Outlook**

139 It is now apparent that prokaryotes reveal a mosaic genome structure. It consists of conserved
140 core components (core genome) made up of essential genes and dispensable, often strain
141 specific genes (character and accessory genes), where at least some of them were acquired by
142 lateral gene transfer. The core genome comprises a set of genes common to all genomes of a
143 phylogenetically coherent group. It preferentially contains informational or house-keeping
144 genes that are rather stable and less prone to lateral gene transfer. Some genes of the core
145 genome may be ideal phylogenetic markers for the genotypic classification of distantly
146 related prokaryotes, whereas for the classification of lower taxa, such as the delineation of
147 species, genes that code for characteristic differences may be the ideal candidates.

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