Methods of Detection and Identification of Bacteria

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PREFACE

During the past decade, many new methods have been developed for the detection and identification of bacteria in clinical specimens and biological samples. The rapidly increasing work load in the laboratory (particularly in clinical microbiology), recent advances in electronic technology, and successful application of technological know-how in other clinical laboratories (such as clinical chemistry) have inspired many bacteriologists to evaluate the usefulness of newer methods for the identification of bacteria. Explorations of outer space coupled with a curiosity to determine the existence of life beyond the boundaries of the planet earth have also necessitated the invention of rapid, ultrasensitive, and specific methods for the determination of bacteria. The purpose of this book is to summarize the methods applicable to bacteriology, including conventional methods that are currently used as well as new approaches and automated methods in diagnostic, public health, and industrial bacteriology laboratories.

Most of the newer methods of detection and identification of bacteria utilize the basic properties of bacterial cells, such as their size, staining characteristics, colonial morphology, chemical constituents, biochemical reactions, and immunologic properties. Therefore, a comprehensive review of current methods of detection, enumeration, characterization, and identification of bacteria is presented. This is followed by a discussion of the newer techniques applied to bacterial identification, utilizing principles of morphologic, biochemical, and serological methods. The next section deals with automated methods, with a view towards their possible applications in the bacteriology laboratory. The last chapter includes a discussion on the use of computers for the identification of bacteria, storage and retrieval of taxonomic information, and data processing in the bacteriology laboratory. The application of computers in bacteriology may be useful in coping with the increasing work load and achieving greater precision and accuracy in methodology.

I hope this book will be useful for everyone involved in the bacteriology laboratory.

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Philadelphia, Pennsylvania
July 12, 1976

THE AUTHOR

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B. M. M.

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Chapter 1
INTRODUCTION

Mary J. Bonner and Brij M. Mitruka

DIFFERENTIATION OF PROCARYOTIC AND EUCARYOTIC CELLS

Concepts proffered in Darwin's *Origin of the Species*[1] provided the framework for the description of a third kingdom to serve as the transition form between the plant and animal kingdoms. Haeckel[2] termed this kingdom the Protista. Chatton[3] recognized two general patterns of cellular organization in Protista, the eucaryotes (Greek, true nucleus), which include protozoa, fungi, and most algae, and the procaryotes, which include all bacteria and the small group of blue-green algae (Cyanophyceae). While the original division of Protista was based on the simple organization of the procaryotic bacteria, the electron microscope has revealed a fundamental division based on complexity of organization.

Bacteria form a heterogeneous group of procaryotic cells anatomically, physiologically, and biochemically. In general, the main distinguishing features of the procaryotic cells (bacteria and related organisms such as rickettsiae, chlamydiae, and mycoplasmas) are as follows.

1. Their nucleus is a simple homogeneous body without a nuclear membrane separating it from the cytoplasm. They lack nucleolus, a spindle, and a number of separate nonidentical chromosomes.

2. Procaryotes lack the internal membranes isolating the respiratory and photosynthetic enzyme systems in specific organelles comparable to the membrane-bound mitochondria and the chloroplasts of eucaryotic cells. Thus, the respiratory enzymes in bacteria are located mainly in the peripheral cytoplasmic membrane, and their effective functioning is dependent on the integrity of the cell protoplast as a whole.

3. Procaryotes have a rigid cell wall structure containing a specific mucopeptide not found in eucaryotic cells.

4. Morphologically, bacteria are characterized by their small cell size (usually between 0.4 and 1.5 μm in short diameter); characteristic shape, which may be spherical (coccus), rod shaped (bacillus), comma shaped (vibrio), spiral (spirillum and spirochete), or filamentous (actinomyces); and arrangement, such as clusters, chains, rods, filaments, or mycelia.

5. Although unicellular bacteria may grow attached to one another so as to appear multicellular (in clusters, chains, rods, etc.), each cell is physiologically self-sufficient and, if isolated artificially, able to nourish itself, grow, and reproduce the species by binary fission.

6. Another distinctive feature of the procaryotic cell is that its ribosomes are small[4,5] (10 to 20 nm) with a sedimentation constant of 70S as compared to the larger 80S ribosome of eucaryotes. The 70S ribosome is composed of 30S and 50S subunits, whereas the 80S ribosome of the eucaryotic cell is composed of 40S and 60S subunits.

7. The basic chemical composition of all microorganisms is essentially similar, i.e., made up of compounds of lower molecular weight which are about 10% of dry weight in procaryotes and 15% in eucaryotes. Protein, nucleic acid, and lipid contents are also slightly lower in procaryotic cells as compared to eucaryotic cells.

The procaryotic bacteria show a considerably narrow range of structural and biochemical variations as compared to those in eucaryotic cells. Thus, evolutionary specialization among bacteria is expressed in metabolic rather than structural terms. However, there is great metabolic variation among bacteria; for example, representatives of all four primary nutritional categories (photoautotrophs, photoheterotrophs, chemoautotrophs, and chemoheterotrophs) occur in bacteria. Almost every type of metabolic activity present in eucaryotic cells can be found in bacteria. The physiological diversity of bacteria, which explains both their numerical abundance and their ubiquity throughout the biosphere, is reflected in the role they play as agents in the cycles of carbon, nitrogen, sulfur, oxygen, and phosphorus.

Comparative cytology using the light microscope and classical staining methods, extension of comparative cytology to the ultrastructural level

with the electron microscope (EM), and advances in biochemical techniques have established the uniqueness of bacteria.[6,7] By using these three basic experimental approaches, it was recognized that bacteria have a wide range of anaerobic energy-yielding reactions, synthesize unique cell wall polymers (except for the "L"-form *Mycoplasma* and those without walls and exceptional bacteria such as *Halobacterium*), fix nitrogen, and accumulate poly-β-hydroxybutyrate as a reserve material. Such properties are virtually or completely absent from eucaryotes.

The cytological recognition of procaryotic organization supported by biochemical and physiological data provided the foundation for a coherent, systematic view of the nature of bacteria such as that described in *Bergey's Manual of Determinative Bacteriology.*[14]

TAXONOMY OF BACTERIA

Bacteria are usually classified according to the Linnean binomial scheme of genus and species, although many kinds of systematic compilations are possible. Detailed classification of bacteria utilizes results of cytological, biochemical, and physiological tests as well as specialized techniques of strain identification (such as serotyping, biotyping, bacteriophage typing, and genetic analyses including measurement of guanine and cytosine (GC) contents and DNA-DNA homology analysis).[7-13]

Phylogenetic Scheme

The most widely used system of classification and nomenclature in the United States is *Bergey's Manual of Determinative Bacteriology.*[14] The first edition appeared in 1923 and the eighth edition in 1974. Bacteria are placed into the following 19 parts:

Kingdom – Procaryotae
- Division I – Cyanobacteria
- Division II – Bacteria
  - Part 1 – Phototrophic bacteria
  - Part 2 – Gliding bacteria
  - Part 3 – Sheathed bacteria
  - Part 4 – Budding and/or appendaged bacteria
  - Part 5 – Spirochetes
  - Part 6 – Spiral and curved bacteria
  - Part 7 – Gram-negative aerobic rods and cocci
  - Part 8 – Gram-negative facultatively anaerobic rods
  - Part 9 – Gram-negative anaerobic bacteria
  - Part 10 – Gram-negative cocci and coccobacilli
  - Part 11 – Gram-negative anaerobic cocci
  - Part 12 – Gram-negative chemolithotrophic bacteria
  - Part 13 – Methane-producing bacteria
  - Part 14 – Gram-positive cocci
  - Part 15 – Endospore-forming rods and cocci
  - Part 16 – Gram-positive, asporogenous, rod-shaped bacteria
  - Part 17 – Actinomycetes and related organisms
  - Part 18 – Rickettsias
  - Part 19 – Mycoplasmas

Each part is also divided, where appropriate, into classes, orders, families, tribes, genera, and species.

In the older phylogenetic scheme of classification, the class Schizomycetaceae included all true bacteria, filamentous bacteria (including mycobacteria), spirochetes, and mycoplasmas. For example, in this scheme *Escherichia coli* was placed as follows:

Kingdom – Monera
- Class (-aceae) – Schizomycetaceae
  - Order (-ales) – Eubacteriales
    - Family (-aceae) – Enterobacteriaceae
      - Genus – *Escherichia*
        - Species – *coli*

However, since the publication of *Bergey's* eighth edition, *Escherichia coli* is placed as follows:

Kingdom – Procaryotae
- Division II – Bacteria
  - Part 8 – Gram-negative facultatively anaerobic rods
    - Family I – Enterobacteriaceae
      - Genus I – *Escherichia*
        - Species – *coli*

As specified in the International Code of

Nomenclature of Bacteria,[15] scientific names of all taxa, or those words used to refer to any taxonomic group, are either to be taken from Latin or are Latinized if taken from other languages. The major categories of taxa are

Category	Name ending
Individual	
Species	
Series	
Section	
Genus	
Tribe	-eae
Family	-aceae
Order	-ales
Class	
Division	

A nomenclatural type is recognized as the constituent element of a taxon to which the name of the taxon is permanently attached, for example, the type species of a genus. Provisions are made for changes in rules and for rule interpretation through the International Committee of Nomenclature of Bacteria and its Judicial Commission. These bodies are organized by the International Association of Microbiological Societies.

Well-known, trivial, or commonly used names (such as "tubercle bacillus" for *Mycobacterium tuberculosis* and "typhoid bacillus" for *Salmonella typhi*) appear frequently in medical literature. In the phylogenetic classification, the higher groupings of order and family are distinguished by characteristics such as cell shape, Gram reaction, spore formation, and flagellation, whereas genus and species are distinguished by characteristics such as fermentation reactions, nutritional requirements, and pathogenicity. The fundamental weakness of such a system is the arbitrary importance attached to the distinguishing characteristics. Other schemes for classification are currently being developed, usually to meet specific requirements, and some of these are described in the following sections.

Genetic Basis of Classification

There are two approaches to classification on a genetic basis. One approach is based on the nucleic acid homology of bacteria; the other approach groups bacteria together according to the biochemical manifestation of gene-controlled stable metabolic patterns, cell polymers, and organelle structures. Surface polymers (including capsules, teichoic acids, and O antigens) can be used for comparison of bacterial relatedness.

In the genetic approaches to bacterial taxonomy, certain techniques give insight into the genotypic properties of bacteria, thereby complementing the hitherto exclusively phenotypic characterization of these organisms. Two different kinds of analyses performed on isolated nucleic acids (the analysis of the base composition of DNA[16-18] and the study of chemical hybridization between DNA and DNA or DNA and RNA) furnish information about the genotype.[19-21] The genetic information encoded in the DNA base sequence changes in different environments by processes such as mutation, recombination, transduction, and selection. The genes of the organisms change in size, nucleotide base composition, and nucleotide base sequence. Base composition has been shown by both chemical and physiochemical methods to be constant and characteristic in each organism (Table 1.1). Base composition is generally expressed in terms of the mole fraction of guanine plus cytosine (G + C/G + C + A + T) expressed as a percentage. The G + C ratio or DNA base composition may be determined by several methods, one of which is denaturation or melting temperature of DNA. An equation relating G + C content and melting temperature has been derived by Marmur and Doty.[16] Values of GC content for different organisms vary from 30 to 75%; this reflects the differences in the amino acid composition of the cellular proteins of different organisms, some having a preponderance coded by A + T-rich triplets and others by G + C-rich triplets. A substantial divergence between two organisms with respect to mean DNA base composition reflects a large number of individual differences between the specific base sequences of their respective DNAs. This in turn indicates a major genetic divergence and, hence, a wide evolutionary separation. However, two organisms with identical mean DNA base composition may differ greatly in genetic constitution. Although the values for the species in a particular genus differ, the total for most bacterial genera is fairly narrow and can be considered a useful character for definition of a bacterial genus. Organisms that are clearly related will have close G + C ratios, but the converse does not necessarily hold true. Data concerning G + C ratios tend to confirm existing classifications. Base ratio data also point out the organisms that are not adequately differentiated by present laboratory tests.

TABLE 1.1

Nucleotide Base Composition of the DNA of Various Bacteria

Organism	% GC
Spirillum linum	28–30
Clostridium perfringens, C. tetani, Fusobacterium fusiforme	30–32
Staphylococcus aureus	32–34
Bacillus alvei, B. cereus, B. anthracis, B. thuringiensis, Clostridium kluyverii, Mycoplasma gallisepticum, Streptococcus faecalis, Treponema pallidum, Veillonella parvula	34–36
Diplococcus pneumoniae, Streptococcus salivarius, Streptococcus pyogenes, S. bovis, S. viridans, Leuconostoc mesenteroides, Lactobacillus acidophilus, Listeria monocytogenes, Proteus vulgaris, P. mirabilis, P. rettgeri, Haemophilus suis, H. influenzae. H. parainfluenzae, H. aegypti, Bacillus megaterium	38–40
Moraxella bovis, Neisseria catarrhalis, Leptospira biflexa	40–42
Bacillus subtilis, B. polymyxa, B. stearothermophilus, Coxiella burnetii, Vibrio metschnikovii	42–44
Vibrio comma, Pasteurella pestis, Corynebacterium acnes	46–48
Neisseria perflava, N. gonorrhoeae, N. flava	48–50
Neisseria meningitidis, N. sicca, N. subflava, Proteus morganii, Escherichia coli, Citrobacter freundii, Shigella dysenteriae, Salmonella typhosa, S. typhimurium, S. enteritidis, S. arizona, S. ballerup	50–52
Klebsiella aerogenes, Corynebacterium diphtheriae	52–54
Klebsiella pneumoniae, K. rhinoscleromatis, Azotobacter agilis, Alcaligenes faecalis, Brucella abortus, Nitrosomonas sp.	54–56
Azotobacter vinelandii, Lactobacillus bifidis	56–58
Agrobacterium tumefaciens, Serratia marcescens	58–60
Pseudomonas fluorescens, Rhizobium japonicum, Rhodospirillum rubrum	60–62
Xanthomonas phaseoli	62–64
Desulfovibrio desulfuricans	64–66
Pseudomonas aeruginosa, Mycobacterium phlei, M. tuberculosis	66–68
Mycobacterium smegmatis, Micrococcus lysodeikticus, Nocardia sp.	70–80

Marmur et al.[10] arranged individual organisms into groups on the basis of the homology of their DNA base sequences. The basis of DNA homology classification is the fact that double strands re-form from separated strands during controlled cooling of a heated preparation of DNA. This "annealing" process can be readily demonstrated with suitably heated homologous DNA extracted from a single species, but it can also occur when a mixture of DNA from two related species is used; in the latter case, hybrid pairs of DNA strands are produced. These hybrid pairings frequently occur between complementary regions of two bits of DNA, and the degree of hybridization can be assessed if labeled DNA preparations are used. mRNA binding studies can also give information

to complement these observations providing genetic evidence of relatedness among bacteria. Measurement of DNA homology has recently been quantified by several procedures which determine the extent of formation of molecular hybrids from two DNA strands of different origin. The techniques of nucleic acid hybridization show relatedness between different bacterial strains (Table 1.2).

Biochemical Basis of Classification

Metabolic reactions and the chemical composition of the cell wall are characteristic of various bacterial species. Although the mucopeptide framework (repeating units of *N*-acetylglucosamine and *N*-acetylmuramic acid cross-linked by peptide structures) in the cell wall of bacteria is common to all, there are structural differences between Gram-positive and Gram-negative bacteria. Individual differences of taxonomic value in chemical constituents of bacteria are also present; for example, Gram-positive bacteria contain considerable amounts of glycerol teichoic acid and ribitol teichoic acid. Also found are a wide variety of monosaccharides that are, by and large, group specific, for example, rhamnose for *Streptococcus*, *Lactobacillus*, and *Clostridium* and arabinose for *Corynebacterium*, *Mycobacterium*, and *Nocardia*.

Gram-negative bacteria, on the other hand, contain a group-specific polysaccharide composed of glucose, galactose, *N*-acetylglucosamine, 2-keto-3-deoxyoctonate, and a haptose moiety together with a highly specific side chain bearing a type-specific arrangement of sugar. Various chromatographic and spectroscopic methods are employed to analyze chemical constituents of bacteria in order to distinguish genera, species, and strains based on their biochemical properties (see Chapter 3).

TABLE 1.2

DNA Homologies among Certain Bacteria

DNA source	% relatedness to *E. coli*
Escherichia coli	100
Shigella dysenteriae	71
Aerobacter aerogenes	45
Salmonella typhimurium	35
Proteus vulgaris	14
Serratia marcescens	7
Pseudomonas aeruginosa	1
Bacillus subtilis	1
Brucella neotomae	0
	% relatedness to *N. meningitidis*
Neisseria meningitidis	100
Neisseria gonorrhoeae	80
Neisseria perflava	55
Neisseria subflava	48
Neisseria catarrhalis	15
Neisseria caviae	10
Mima sp.	5
Herellea	5
Escherichia coli	3
Monkey kidney	0.1

Data from Brenner, D. J., Martin, M. A., and Hoyer, B. H., *J. Bacteriol.*, 94, 486, 1967; McCarthy, B. J. and Bolton, E. T., *Proc. Natl. Acad. Sci. U.S.A.*, 50, 156, 1963; Kingsbury, D. T., *J. Bacteriol.*, 94, 870, 1967.

Numerical Taxonomy or Adansonian System

Because of the difficulties in constructing phylogenetic classifications which are based on a few arbitrarily weighted characteristics, descriptive taxonomy has been revived for many strains in the form of computerized numerical comparisons of large numbers of diagnostic features. Computer taxonomy has been developed for groups of bacteria in which large numbers of strains exist. They are described in terms of 100 or more clear-cut taxonomic properties such as presence or absence of certain enzymes and presence or absence of certain morphologic structures.[22,23] Punch cards are prepared for each strain. The computer compares the cards and prints out a list of the strains so that each strain is followed by the strain with which it shares the most characteristics. When this is done, the list often reveals several broad subgroups or strains, with each subgroup characterized by a large number of shared characteristics (see Chapter 6). The median strain within each subgroup can then be considered a type species.

Based on two main criteria, the mechanism of motility and the character of the cell wall, bacteria can be classified into four major groups.

1. Eubacteria, which have thick rigid cell walls. Motility, when present, is by means of flagella.

2. Spirochetes, which have thick rigid cell walls. Motility is by means of contraction of an axial filament wound helically about the cell and anchored at both ends.

3. Gliding bacteria, some of which have thick rigid walls and others with thin flexible walls. Although the organelles of motility have not been detected in these organisms, they move smoothly along solid surfaces by means of an unknown mechanism.

4. Mycoplasma, which do not have cell walls but do have a triple-layered unit membrane and are highly pleomorphic.

Artificial Classification or Simplified Working System

In this scheme, descriptive properties are arranged so that an organism may be readily identified. Organisms are grouped together in a "key;" they are not necessarily related phylogenetically, but are listed together because they share certain easily recognizable characteristics. For example, it is reasonable to include a group of organisms which form red pigments in the "key" type of classification even though this would necessitate the inclusion of such unrelated forms as *Serratia marcescens* and purple sulfur bacteria. Another approach to the classification of bacteria is to avoid the conflicting arguments of the taxonomists and use a simplified working system such as that used by medical bacteriologists (Table 1.3).

TABLE 1.3

Bacteria of Medical Importance

Part 5	
Spirochetes	
Order I. Spirochaetales	Slender, flexuous, helically coiled, unicellular, 3–500 μm in length; multiplication by transverse fission; motility may be rapid rotation about long axis of helix, flexion, or serpentine; no endospores; larger cells Gram negative; may have inclusions; aerobic, facultatively anaerobic, or anaerobic; chemoheterotrophic; free living, commensal, or parasitic
Family I. Spriochaetaceae	
Genus III. *Treponema*	
Genus IV. *Borrelia*	
Genus V. *Leptospira*	
Part 7[a]	
Gram-negative aerobic rods and cocci	
Family I. Pseudomonodaceae	Family I – Straight or curved rods, motile by polar flagella, Gram negative, metabolism respiratory, never fermentative, strict aerobes, catalase +; oxidase usually +; growth 4 to 43°C
Genus I. *Pseudomonas*	
Part 8[b]	
Gram-negative facultatively anaerobic rods	
Family I. Enterobacteriaceae	Family I – Small Gram-negative rods; motile by peritrichate flagella or nonmotile; capsulated or noncapsulated; no spore formation; not acid fast; aerobic and facultatively anaerobic; chemoorganotrophic; metabolism respiratory and fermentative; acid produced from carbohydrates and alcohols; catalase + except one serotype of *Shigella*; nitrates reduced to nitrites except some strains of *Erwinia*; type genus *Escherichia*, Castellani and Chalmers, 1919, 941
Genus I. *Escherichia*	
Genus II. *Edwardsiella*	
Genus III. *Citrobacter*	
Genus IV. *Salmonella*	
Genus V. *Shigella*	
Genus VI. *Klebsiella*	
Genus VII. *Enterobacter*	
Genus VIII. *Hafnia*	
Genus IX. *Serratia*	
Genus X. *Proteus*	
Genus XI. *Yersinia*	
Genus XII. *Erwinia*	
Family II. Vibrionaceae	Family II – Rigid Gram-negative rods, straight or curved; motile by polar flagella and some lateral flagella; chemoorganotrophs, metabolism both fermentative and respiratory; oxidase positive; facultative anaerobes; type genus *Vibrio*, Pacini 1854, 411
Genus I. *Vibrio*	
Genus II. *Aeromonas*	

[a]Genera of uncertain affiliation include *Alcaligenes, Acetobacter, Brucella, Bordetella,* and *Francisella.*
[b]Genera of uncertain affiliation include *Haemophilus* and *Pasteurella.*

TABLE 1.3 (continued)

Bacteria of Medical Importance

Part 9	
Gram-negative anaerobic bacteria	Gram negative; uniform or pleomorphic rods; nonmotile or motile with peritrichous flagella; no spore formation; chemoorganotrophs; obligate anaerobes
Family I. Bacteroidaceae	
Genus I. *Bacteroides*	
Genus II. *Fusobacterium*	
Genus III. *Leptotrichia*	
Part 10	
Gram-negative cocci and coccobacilli	Spherical in pairs or masses with adjacent sides flattened, may also be rod shaped in pairs or short chains; no flagellus; some twitching motility; Gram negative; most species catalase + cytochrome oxidase +; aerobic; some species have complex growth requirements following isolation and may later grow in simple defined media; type genus *Neisseria*, Trevisan 1885, 105
Family I. Neisseriaceae	
Genus I. *Neisseria*	
Genus II. *Moraxella*	
Part 11	
Gram-negative anaerobic cocci	Cocci with varying diameter (0.3–2.5 μm); pairs, single cells, chains, or masses; no endospores; no flagellum, nonmotile; Gram negative but resist decolorization; Chemoorganotrophic; complex nutritional requirements; anaerobic; cytochrome oxidase –; catalase –; some strains have pseudocatalase; type genus *Veillonella*, Privot 1933, 118
Family I. Veillonellaceae	
Genus I. *Veillonella*	
Part 14	
Gram-positive cocci	
a. Aerobic and/or facultatively anaerobic	
Family I. Micrococcaceae	Family I – Cells spherical; 0.5 μm in diameter; divide in more than one plane, yielding packets; motile or nonmotile; Gram positive; no endospores, chemoorganotrophs; metabolism respiratory or fermentative; acid but no gas produced from glucose when utilized; catalase +; type genus *Micrococcus*, Cohn 1972, 151
Genus I. *Micrococcus*	
Genus II. *Staphylococcus*	
Genus III. *Planococcus*	
Family II. Streptococcaceae	Family II – Cells spherical or ovoid, chains, or tetrads; rarely motile; no endospores; Gram positive; chemoorganotrophs; metabolism fermentative; lactic, acetic, formic acids, ethanol, CO_2 produced from carbohydrates; catalase variable, facultatively anaerobic; type genus *Streptococcus*, Rosenback 1884, 22
Genus I. *Streptococcus*	
Genus II. *Leuconostoc*	
b. Anaerobic	
Family III. Peptococcoceae	Family III – Cocci (0.5–2.5 μm) singly, pairs, tetrads, masses, cubic packets; no flagellum; no endospores; nonmotile; Gram positive; chemoorganotrophic; carbohydrate fermentation ±
Genus I. *Peptococcus*	
Genus II. *Peptostreptococcus*	
Genus IV. *Sarcina*	
Part 15	
Endospore-forming rods and cocci	Rod-shaped cells except in one genus, spherical endospores; mostly Gram positive; motile by lateral or peritrichous flagella or nonmotile; aerobic, facultative, or anaerobic
Family I. Bacillaceae	
Genus I. *Bacillus*	
Genus III. *Clostridium*	

TABLE 1.3 (continued)

Bacteria of Medical Importance

Part 16[c] Gram-positive, asporogenous rod-shaped bacteria Family I. Lactobacillaceae Genus I. *Lactobacillus*	**Straight or curved rods, single or in chains; rarely motile; Gram positive; anaerobic or facultative; marked lactate production; catalase –**
Part 17 Actinomycetes and related organisms Coryneform group of bacteria Genus I. *Corynebacterium*	**Straight to slightly curved rods; division yields palisade arrangements of cells; usually nonmotile; Gram positive; not acid fast; chemoorganotrophs; carbohydrate metabolism; mixed fermentative and respiratory; aerobic and facultatively anaerobic; catalase +; type species *Corynebacterium diphtheriae* (Kruse), Lehmann and Neumann 1896, 350**
Part 18 Rickettsias Order I. Rickettsiales Family I. Rickettsiaceae Tribe I. Rickettsiae Genus I. *Rickettsia* Genus II. *Coxiella*	**Usually rod shaped, coccoid, pleomorphic; no flagellum; typical bacterial cell walls; *Gram negative; multiply only inside* host cells**
Part 19 Mycoplasmas Class I. Mollicutes Order I. Mycoplasmatales Family I. Mycoplasmataceae Genus I. *Mycoplasma*	**Small cells sometimes 200 nm, pleomorphic; coccoid to filamentous; bounded single triple-layered nonmotile; Gram negative; minute colonies; grow into media; most species completely resistant to penicillin**

[c]Genera of uncertain affiliation include *Listeria* and *Erysipelothrix*.

Adapted from Stonier et al.,[30] Joklik and Smith,[31] Cruickshank et al.,[32] Davis et al.,[33] and Buchanan and Gibbons.[14]

DETECTION, ENUMERATION, AND IDENTIFICATION OF BACTERIA

Many methods of detection, enumeration, and identification of bacteria have been established and are routinely used in diagnostic bacteriology in hospitals; public health laboratories; and dairy, food, and soil microbiology laboratories. If the concentration of bacteria in a given sample is high enough and if sufficient time is available for a detailed examination, the detection, specific identification, and quantitative assessment of the bacteria does not present much of a problem to the bacteriologist. However, immediate or rapid detection or specific identification of a small number of bacteria in a sample is much more difficult and requires specially developed methods.[24] Recent trends in the development of newer and better methods of bacterial detection and identification exploit many of the known chemical, physical, and physiological characteristics of bacteria. Some of these methods are listed below.

1. Methods of detection and enumeration of bacteria

A. Selective media, preformed media, and reagents (for the determination of growth and metabolism)

B. Staining methods

C. Coulter Counter® (for particle counting)

D. Partichrome analyzer (for the detection and counting of microscopic particles)

E. πMC system (for the detection of particles in a microscopic field)

F. Membrane filtration

G. Velocimeter (a sound velocity probe)