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Course Handout

Prokaryotic Systematics

Course Intended for Students:

3rd Year License- Microbiology

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Preface

Prokaryotic systematics course handout is intended for third-year Bachelor's students specializing in Microbiology. This subject is part of the Fundamental Teaching Unit (FTU1), carrying 6 credits with a coefficient of 03, for a weekly workload of 3 hours of lectures and 3 hours of practical work.

Prokaryotic systematics is a fundamental discipline in microbiology that aims to classify, identify, and name prokaryotic organisms based on their evolutionary relationships and phenotypic characteristics. With the rapid advancement of molecular biology and genomic technologies, the taxonomy of bacteria and archaea has undergone profound changes, shifting from classical phenotypic approaches, such as morphology, staining, and biochemical tests, to modern molecular techniques like 16S rRNA gene sequencing and genome analysis.

This course introduces the basic principles of microbial classification. It covers the historical development of taxonomy and the main groups of bacteria and archaea according to Bergey's Manual of Systematic Bacteriology, learning the defining characteristics, ecological roles, and importance of each group.

Semestre : 5

Unité d'enseignement Fondamentale (UEF 1)

Matière 1: Systématique des Procaryotes (Bactéries et Archaea)

Crédits : 6

Coefficient : 3

Objectifs de l'enseignement :

Cet enseignement est la suite et l'approfondissement des connaissances acquises en L2 (S4) : U.E. de Microbiologie générale. Il doit aboutir à un diagnostic bactériologique de l'ensemble des bactéries et des Archaea selon les données de la nouvelle édition du Bergey's Manual (Vol 1, 2, 3, 4 et 5). En plus des caractères classiques de détermination des procaryotes, l'apport de l'outil moléculaire sur lequel se base le Bergey pour l'identification des bactéries et des Archaea est d'une grande importance.

Connaissances préalables recommandées :

Sans pré-requis.

Contenu de la matière

I. Introduction à la systématique (Définitions, différentes approches taxonomiques)

II. Les différents groupes bactériens et archaeens : La présentation se base beaucoup plus sur la physiologie, la morphologie et l'écologie que sur la phylogénie avec par exemple les bactéries photosynthétiques sont présentées ensembles même si elles sont réparties dans plusieurs phyla.

III. Principes de la taxonomie chez les bactéries : les principales bases de la taxonomie actuelle en se basant sur "**Bergey's Manual of Systematic Bacteriology**"2013.

IV. Principaux types de classification : sont représentés par les différentes approches taxonomiques : **taxonomie moléculaire, Chimiotaxonomie, Taxonomie numérique, Taxonomie phénotypique.....**

V. Etudes des grands groupes bactériens :

1. Les bactéries photosynthétiques
2. Les bactéries autotrophes.
3. Les bactéries hétérotrophes à Gram négatif
4. Les bactéries hétérotrophes à Gram positif
5. Les actinomycètes

6. Les rickettsies et les chlamydiae

7. Les mycoplasmes

VI. Les grands phylums bactérien selon la classification du Bergey's Manual : biologie, taxonomie, morphologie et écologie

1. Phylum Proteobacteria :

- Classe 1: Alphaproteobacteria
- Classe 2: Betaproteobacteria
- Classe 3: Gammaproteobacteria
- Classe 4 : Epsilonproteobacteria

VII. Les cinq Phyla d'Archaea

Les deux premiers phyla seront étudiés plus en détail car ce sont les plus connus et ceux qui renferment le plus grand nombre de taxons :

- Les Euryarchaeota.
- Les Crenarchaeota
- Les Korarchaeota
- Les Nanoarchaeota
- Les Thaumarchaeota :

Travaux Dirigés:

TD1 : Techniques utilisées en Systématique bactérienne (classiques et moléculaires) avec une présentation de la PCR).

TD2 : Les principes de classification des archéobactéries, en donnant des exemples pour chaque groupe sous forme d'exposés et travaux personnels.

Travaux Pratiques :

TP 1 : Les entérobactéries : Coloration de Gram, Tests physiologiques (type respiratoire, Nitrate réductase, catalase, oxydase, Métabolisme des glucides sur Galerie API

TP2 : Les autres Bactéries à Gram négatif (*Pseudomonas*, *Vibrio*...) : Coloration de Gram, King A et B, Voie d'attaque des glucides, Antibiorésistance

TP3 : Les bactéries en forme de cocci à Gram positif : Coloration de Gram, Test physiologiques différentiels entre Streptocoques et Staphylocoques, Test présumptifs et confirmatifs de pathogénicité, Test de la staphylocoagulase.

TP4 : Les bacilles à Gram positif sporules : Gram avec observation de la spore (forme, position, déformante), tests Biochimiques (Indole, Gélatine, hémolyse)

Mode d'évaluation : Contrôle continu et Examen semestriel

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Prokaryotic systematics, which encompasses the identification, classification, and nomenclature of novel prokaryotic taxa, constitutes a fundamental basis for microbiological research across agricultural, ecological, industrial, medical, and veterinary fields. Prokaryotes are highly abundant in nature, with an estimated diversity ranging from 2.2 to 4.3 million species; however, only approximately 21,000 species have been formally characterized and validly published.

Historically, this discipline relied primarily on phenotypic characterization for species identification. Since the first edition of *Bergey's Manual of Determinative Bacteriology* in 1923, prokaryotic systematics has undergone significant evolution, incorporating numerical taxonomy in the 1960s and adopting the polyphasic taxonomy approach in the 1990s. This approach integrates multiple lines of evidence, including morphological, biochemical, and chemotaxonomic traits, as well as DNA–DNA hybridization and phylogenetic analysis based on 16S rRNA gene sequences.

Polyphasic taxonomy has long been considered the gold standard in prokaryotic classification. However, with the increasing accessibility of whole-genome sequencing technologies over the past decade, genomic approaches are now reshaping and advancing the field of prokaryotic systematics.

Chapter 1. Introduction to systematics

In the recent past, scientists grouped living things into five kingdoms (animals, plants, fungi, protists, and prokaryotes) based on several criteria, such as the absence or presence of a nucleus and other membrane-bound organelles, the absence or presence of cell walls, multicellularity, and so on. In the late 20th century, the pioneering work of Carl Woese and others compared sequences of small-subunit ribosomal RNA (SSU rRNA), which resulted in a more fundamental way to group organisms on Earth. Based on differences in the structure of cell membranes and in rRNA, Woese and his colleagues proposed that all life on Earth evolved along three lineages, called domains. The domain Bacteria comprises all organisms in the kingdom Bacteria, the domain Archaea comprises the rest of the prokaryotes, and the domain Eukarya comprises all eukaryotes, including organisms in the kingdoms Animalia, Plantae, Fungi, and Protista. Major differential features among bacteria, Archaea and Eukarya are described in the table 1.

Two of the three domains (Bacteria and Archaea) are prokaryotic. Prokaryotes were the first inhabitants on Earth, appearing 3.5 to 3.8 billion years ago. These organisms are abundant and ubiquitous; they are present everywhere. In addition to inhabiting moderate environments, they are found in extreme conditions: from boiling springs to permanently frozen environments in Antarctica; from salty environments like the Dead Sea to environments under tremendous pressure, such as the depths of the ocean; and from areas without oxygen, such as a waste management plant, to radioactively contaminated regions, such as Chernobyl. Prokaryotes reside in the human digestive system and on the skin, are responsible for certain illnesses, and serve an important role in the preparation of many foods.

Table 1. Major differential features among bacteria, Archaea and Eukarya (Trivedi, 2010)

Characteristic	Bacteria	Archaea	Eukarya
Morphological and Genetic			
Prokaryotic cell structure	Yes	Yes	No
DNA (covalently closed, circular)	Yes	Yes	No
Histone proteins	No	Yes	Yes
Membrane-enclosed nucleus	Absent	Absent	Present
Cell wall (muramic acid)	Present	Absent	Absent
Membrane lipids	Ester-linked	Ether-linked	Ester-linked
Ribosomes	70S	70S	80S
Initiator tRNA	Formylmethionine	Methionine	Methionine
Introns in most genes	No	No	Yes
Operons	Yes	Yes	No
mRNA capping and poly-A tail	No	No	Yes
Plasmids	Yes	Yes	Rare
Molecular Features			
Ribosome sensitivity to diphtheria toxin	No	Yes	Yes
RNA polymerases	One (4 subunits)	Several (8–12 subunits)	Three (12–14 subunits each)
Transcription factors required	No	Yes	Yes

Characteristic	Bacteria	Archaea	Eukarya
Sensitivity to antibiotics (chloramphenicol, streptomycin, kanamycin)	Yes	No	No
Physiological Features			
Methanogenesis	No	Yes	No
Dissimilative reduction (S^0 / $SO_4^{2-} \rightarrow H_2S$ or $Fe^{3+} \rightarrow Fe^{2+}$)	Yes	Yes	No
Nitrification	Yes	No	No
Denitrification	Yes	Yes	No
Nitrogen fixation	Yes	Yes	No
Chlorophyll-based photosynthesis	Yes	No	Yes (in chloroplasts)
Chemolithotrophy	Yes	Yes	No
Gas vesicles	Yes	Yes	No
Growth above 80°C	Yes	Yes	No
Examples	<i>Escherichia coli</i>	<i>Methanosarcina</i>	<i>Amoeba</i>

1. Prokaryotes

Prokaryotes are organisms with a primitive nucleus (from the Greek pro, first, and karyon, nucleus). Their cells are either isolated or form simple associations.

Prokaryotic cells completely lack a nuclear membrane, and their genetic material is suspended in the cytoplasm, in the form of a single, circular, double-stranded DNA molecule. This DNA contains all the genetic information necessary for the cell's growth and reproduction. It is replicated during cell division through a specific mechanism that does not involve mitosis. Cell division does not involve any change in cell texture. Most, if not all,

prokaryotic cells also contain small, circular, extrachromosomal, and transmissible DNA molecules: plasmids.

The cytoplasmic membrane is complex and can form vesicles, lamellae, or tubular inclusions within the cytoplasm. Respiratory and photosynthetic functions are associated with the cytoplasmic membrane, except in cyanobacteria. Ribosomes of the 70S type are dispersed throughout the cytoplasm, which is non-motile. Nutrients are assimilated in their molecular form. The presence of a cell wall is not mandatory. Cells can be immobile or motile, either by gliding or via flagella. These organisms, often ubiquitous, are most commonly unicellular but can also form colonies, mycelia, or filaments.

In the second edition of Bergey's Manual, prokaryotes are divided into two domains: Archaea and Bacteria. All of these organisms are composed of prokaryotic cells. Each domain is subdivided into phyla, each phylum into classes, and so on (Table 2).

Table 2. Classification of the prokaryotes (Torche, 2016).

Domains	2	Archaea	Bacteria
Phyla	34	5	29
Classes	57	9	48
Subclasses	6	0	6
Orders	119	15	104
Suborders	20	0	20
Families	292	26	266
Genera	~2,100	108	~2,000
Species	~7,300	~250	~7,000
Subspecies	~450	0	~450

1.1. Bacteria

Bacteria are microscopic, single-celled organisms (Figure 1). They are among the earliest known life forms on earth. There are millions of different kinds of bacteria, and they live in every conceivable environment all over the world. The domain Bacteria includes all pathogenic prokaryotes, many non-pathogenic prokaryotes found in soil and water, as well as photoautotrophs.

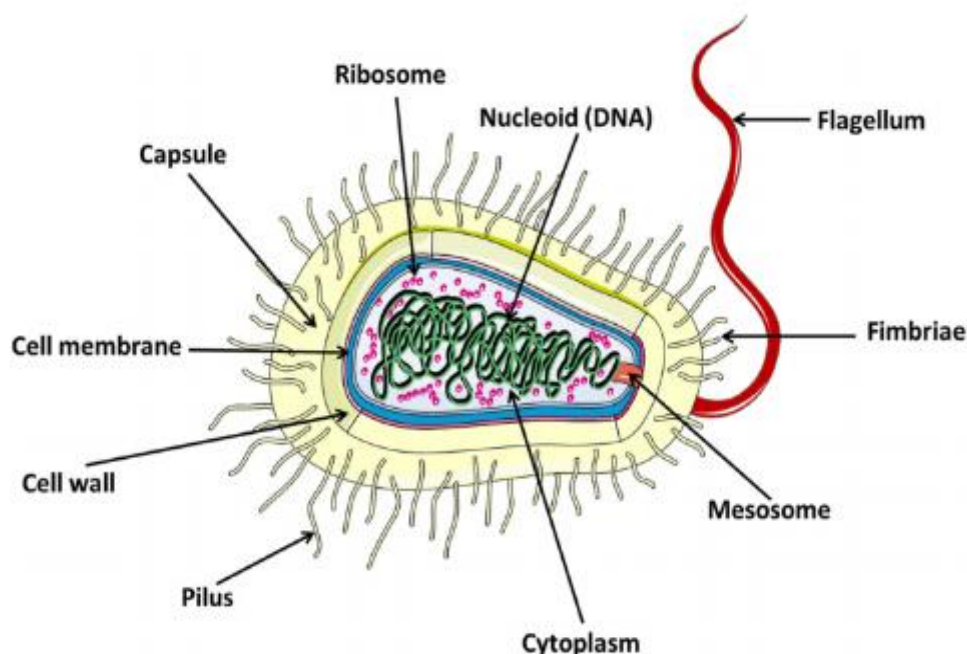


Figure 1. Structure of bacterial cell (Jiménez-Jiménez et al., 2022)

1.2. Archaea

Archaea are single-celled microorganisms with structure similar to bacteria. They are evolutionarily distinct from bacteria and eukaryotes and form the third domain of life.

The Archaea are predominantly terrestrial and aquatic microbes, occurring in anaerobic, hypersaline, or hydrothermally and geothermally heated environments; some also occur as symbionts in animal digestive tracts. They grow chemolithoautotrophically, organotrophically, or facultatively organotrophically. Archaea may be mesophiles or thermophiles, with some species growing at temperatures up to 110°C.

2. Definition of systematic

Systematic is the branch of biology concerned with the study of the diversity of life and its evolutionary relationships. Systematics utilizes various techniques, such as comparative anatomy, molecular biology, and phylogenetics, to classify and reconstruct the evolutionary tree of life. It is the overarching discipline that includes both classification and taxonomy.

Taxonomy: (taxis, arrangement, and nomose, name.) Put organism into categories or taxa (singular: taxon) to show degrees of similarities among organisms.

Taxonomy is the specific branch of systematics that focus on classification of organisms. Bacterial taxonomy consists of three separate, but interrelated areas: classification, nomenclature, and identification.

- **Classification** is the arrangement of organisms into groups (taxa) on the basis of similarities or relationships.
- **Nomenclature** is the assignment of names to the taxonomic groups according to international rules (International Code of Nomenclature of Bacteria). Scientific names are always italicized. The genus is always capitalized, while the species name is entirely in lowercase (*Enterococcus faecalis*).
- **Identification** is the practical use of a classification scheme to determine the identity of an isolate as a member of an established taxon or as a member of a previously unidentified species.

Taxonomy is important for several reasons:

First, it allows us to organize the vast amount of knowledge we have about organisms. In a sense, it functions like a giant classification system or library catalog, providing easy access to information.

Second, it enables us to make predictions and formulate hypotheses for future research based on knowledge of similar organisms.

Third, taxonomy groups microorganisms into meaningful and useful categories with precise

names, allowing microbiologists to study and communicate about them effectively.

Fourth, taxonomy is essential for the accurate identification of microorganisms.

3. Bacterial species concept

Species constitute the fundamental units of taxonomy and an ideal species definition would embody groups of genetically cohesive organisms reflecting their shared history, traits, and ecology.

- A group of bacteria derived from a single cell is called a **strain**.
- Closely related strains constitute a bacterial **species**.
- **Clone**: a population of cells derived from a single parent cell (All cells in the clone should be identical).

4. Intrasubspecific Ranks

Ranks below subspecies, such as biovars, serovars, phagovars, and pathovars, are often used to indicate groups of strains that can be distinguished by some special characters, such as antigenic makeup, reactions to bacteriophage, etc. Such ranks have no official standing in nomenclature, but often have great practical usefulness.

Table 3. Intrasubspecific designations (Garrity et al., 2005)

Preferred name	Synonym	Applied to strains having:
Morphovar	Morphotype	Special morphologic features
Phagovar	Phage type	Ability to be lysed by certain bacteriophages
Serovar	Serotype	Distinctive antigenic properties
Pathovar	Pathotype	Pathogenic properties for certain hosts
Biovar	Biotype	Special biochemical or physiologic properties

5. Hierarchical classification

The classification of microorganisms consists of placing them into hierarchical taxonomic levels. Microorganisms within each level or rank share a set of specific characteristics. The ranks are organized in a non-overlapping hierarchy, so that each one includes not only the characteristics that define the rank, but also a new set of more restrictive characteristics. The hierarchical classification system consists of the following taxa designations:

1. **Domain:** Is the highest taxonomic rank, which divides all living organisms into three domains (Bacteria, Archaea, and Eukarya)
2. **Kingdom:** It represents the broadest division within the tree of life, separating organisms into several large and distinct groups (contains similar divisions or phyla).
3. **Phylum:** It is the basic unit of taxonomy that includes classes of organisms based on their closed relationship in characters.
4. **Class:** It is a taxonomic rank that groups closely related orders.
5. **Order:** It is a taxonomic unit consists of one or more families that show significant similarities.
6. **Family:** Is a group of organisms that may contain multiple genera and consists of organisms with a common attribute. The name of a family is formed by adding the suffix -aceae to the root name of one of the group's genera (example: Staphylococcaceae).
7. **Genus** (plural, genera), contains different species that have several features in common.
8. **Species:** is a collection of bacterial strains that share common physiologic and genetic features and differ notably from other microbial species.

Table 4: Taxonomic ranks (Garrrity et al., 2005)

Formal rank	Example
Domain	<i>Bacteria</i>
Phylum	<i>Proteobacteria</i>
Class	<i>Alphaproteobacteria</i>
Order	<i>Legionellales</i>
Family	<i>Legionellaceae</i>
Genus	<i>Legionella</i>
Species	<i>Legionella pneumophila</i>
Subspecies	<i>Legionella supsp. supsp. pneumophila</i>

6. Different areas of systematics

6.1. Classification: Bacterial classification can be broadly divided into two main types: artificial and natural

6.1.1. Artificial classification

Artificial Classification is based on a key that groups a set of bacteria sharing the same phenotypic property, either physiological or metabolic, that is easily identifiable by its presence (+) or absence (-).

This classification arises from the fact that bacteriologists do not all have the same approach to microbiology (medical, agri-food, biotech, fundamental research, etc.). Each field tends to prioritize its own criteria for classification, which can result in a single bacterial species being assigned multiple names. Example: *Erwinia herbicola* (a plant saprophyte) = *Enterobacter agglomerans* (an intestinal bacterium).

6.1.2. Natural classification

Natural classification organizes organisms based on evolutionary relationships and genetic similarities.

In bacteria, a wide range of techniques is now available for analyzing their chemical composition, particularly their genetic and molecular structures. These approaches enable the establishment of robust phenetic and phylogenetic relationships among bacterial species.

Current classification models are fundamentally based on the comparative analysis of molecular markers, selected for their evolutionary stability or low variability. These markers may be genomic such as DNA, RNA, or their derived proteins, or structural, including membrane components (e.g., lipoquinones, fatty acids) and cell wall constituents (e.g., peptidoglycan, teichoic acids).

6.2. Nomenclature

Nomenclature is the naming of microorganisms according to established rules and guidelines set forth in the International Code of Nomenclature of Bacteria (ICNB) or the Bacteriological Code (BC). In this binomial (two name) system of nomenclature, every organism is assigned a genus and a species of Latin or Greek derivation. Each organism has a scientific “label” consisting of two parts:

- * **The genus designation**, in which the first letter is always capitalized.

- * **The species designation**, in which the first letter is always lowercase.

The two components are used simultaneously and are printed in italics or underlined when written in the text. For example, the staphylococci include *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*, among others.

The name may be abbreviated by using the uppercase form of the first letter of the genus designation followed by a period (.), followed by the full species name, which is never abbreviated (e.g., *S. aureus*).

Frequently an informal designation (e.g., lactobacilli) may be used to label a particular group of organisms. These designations are not capitalized or italicized.

Scientific names can describe the organism, honor a researcher, or refer to the habitat of a species for example: the genus name of *Escherichia coli* was given in honor of the scientist Theodor Escherich, while the specific epithet coli refers to its habitat, the colon or large intestine.

6.3. Identification

Identification involves assigning an individual organism to a known taxon. An unknown strain is compared to previously described species (type strains), and the name of the most similar species is proposed. The bacterial identification process typically begins by obtaining a pure culture of the isolate, which is then subjected to a series of tests and compared to a broad range of known species until a match is found.

Advancements in molecular techniques have increasingly enabled the identification of bacteria without the need for culturing, providing valuable alternatives to conventional biochemical assays.

A wide range of identification methodologies is now available, relying on both phenotypic traits and, more recently, molecular characteristics. The identification strategy should take into account the specific criteria of each method and follow a funnel-shaped approach, starting with the simplest and most broadly informative techniques, and gradually moving toward more complex and precise analyses.

In practice, the determination of the genus and species of a newly discovered prokaryotic organism is based on polyphasic taxonomy, an integrative approach that considers phenotypic, phylogenetic, and genotypic characteristics.

Chapter 2. Different groups of bacteria and archaea

In the absence of an official classification system for bacteria, bacteriologists widely rely on the Bergey's classification, which was originally based exclusively on phenetic characteristics, the only data available at the time. These traits remain critically important today, as bacteria with distinct genomes can exhibit similar phenotypes, and conversely, organisms with similar genomes may display markedly different phenotypes. While the contributions of modern taxonomy, particularly in differentiating bacterial groups, are substantial, it is generally accepted that a robust classification should integrate all available approaches: genotypic, phenotypic, chemotaxonomic, immunological, and others. Taxonomy is a continually evolving science, and taxa at all hierarchical levels are frequently revised or redefined in terms of their composition and phylogenetic relationships.

Bacteria are grouped within the kingdom Procaryotae, which includes four divisions defined based on the presence or absence of a cell wall, and on its nature when it is present. This common characteristic among members of the same division brings together bacteria that are otherwise very heterogeneous in terms of their morphology, trophic types, modes of reproduction, and ecology.

The first three divisions consist of Eubacteria, of which the first two possess a cell wall, while the third lacks one. The fourth division includes all Archaeobacteria most of which have a cell wall that differs in structure and composition from that of the Eubacteria

The four divisions of bacteria are named and defined as follows:

1. Gracilicutes

Eubacteria possessing a Gram-negative type cell wall, i.e., composed of two elements: peptidoglycan and an outer membrane specific to them.

2. Firmicutes

Eubacteria with a Gram-positive type cell wall, i.e., composed of a single element: peptidoglycan.

3. Tenericutes

Eubacteria lacking a cell wall; their cytoplasmic membrane constitutes their outer envelope.

4. Mendosicutes

Group of Archaeobacteria that generally possess a cell wall, though some species lack one. When present, the wall has a chemical composition different from that of Eubacteria, but it has the same type of structure and serves the same function.

Table 5. Summary of the main bacterial groups as differentiated in the four divisions defined by the Bergey's Manual (1994, 9th edition), which was based primarily on phenotypic characteristics such as cell wall structure, Gram stain, morphology, and metabolism (Torche, 2016).

Division 1: Gram negative bacteria	1. The spirochetes 2. Aerobic/microaerophilic, motile helical/vibroid gram-negative bacteria 3. Nonmotile (or rarely motile) curved bacteria 4. Gram-negative aerobic/microaerophilic rods and cocci 5. Facultatively anaerobic gram-negative rods 6. Gram-negative, anaerobic, straight, curved, and helical rods 7. Dissimilatory sulfate- or sulfur-reducing bacteria 8. Anaerobic gram-negative cocci 9. The rickettsiae and chlamydiae 10. Anoxygenic phototrophic bacteria 11. Oxygenic phototrophic bacteria 12. Aerobic chemolithotrophic bacteria and assorted organisms
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	13. Budding or appendaged bacteria 14. Sheathed bacteria 15. Nonphotosynthetic, nonfruiting gliding bacteria 16. Fruiting gliding bacteria: the myxobacteria
Division 2 : Gram positive bacteria	17- Cocci 18- Endo-spore forming rods 19- Regular, nonsporing Gram positive rods 20- Irregular, nonsporing Gram positive rods 21- Mycobacteria 22- Actinomycetes
Division 3: Cell wall-less eubacteria: mycoplasmas	23- Mycoplasmas
Division 4: Archaea	24- The methanogens 25- Archaeal sulfate reducers 26- Extremely halophilic archaeobacteria 27- Cell wall-less archaeobacteria 28- Extremely thermophilic and hyperthermophilic sulfur metabolizers

Chapter 3. Principles of bacterial taxonomy

Bergey's Manual of Determinative Bacteriology has been the major provider of an outline of bacterial systematics since it was published by American bacteriologist David Hendricks Bergey in 1923 and has provided a resource ever since to workers at the bench who need to identify bacterial isolates and recognize new species. It originated in the Society of American Bacteriologists (SAB) but it has since become a truly international enterprise directed by an independent Trust which was founded in 1936.

It has gone through nine editions and has generated, as a more comprehensive resource, a unique compendium on bacterial systematics, Bergey's Manual of Systematic Bacteriology, which now enters its second edition.

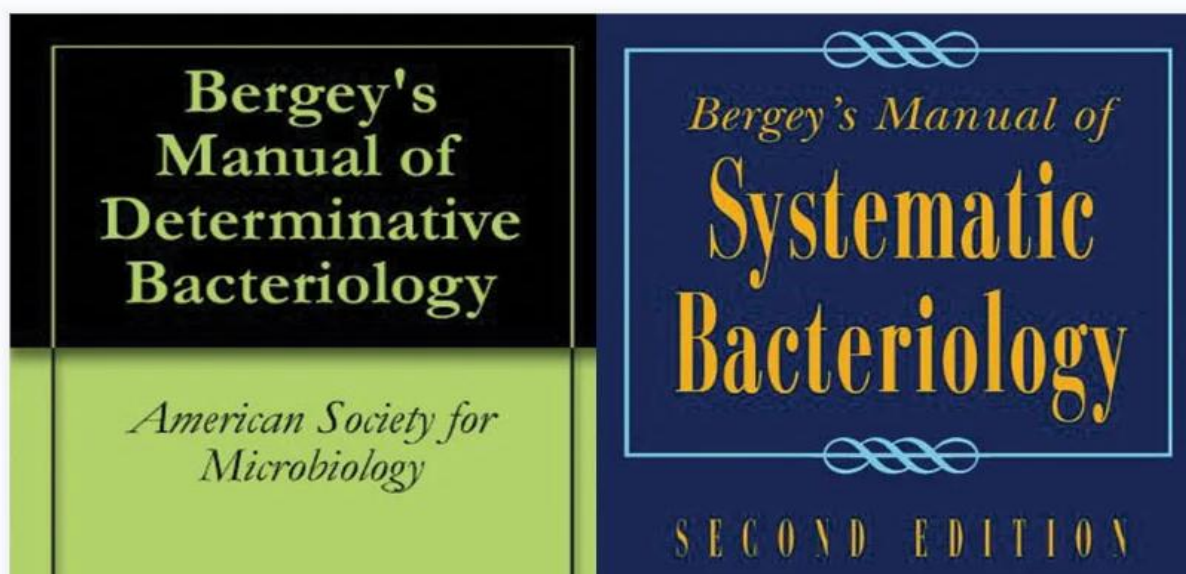


Figure 2. Covers of Bergey's Manual of Determinative Bacteriology and Bergey's Manual of Systematic Bacteriology

1. The first edition of the Bergey's Manual

The first edition of the Bergey's Manual of Systematic Bacteriology was published in 1984. It provided comprehensive descriptions of all known bacterial and archaeal species at the time. The system used in the first edition is primarily **phenetic**. Each of the 33 sections across the four volumes includes prokaryotes that share a number of easily determinable characteristics and bears a title that either describes these properties or provides the common names of the prokaryotes described. The characteristics used to define the sections are common traits such as cell shape and general morphology, Gram-staining properties, oxygen requirements, motility, presence of endospores, mode of energy production, and others.

Prokaryotic groups are distributed among the four volumes as follows:

Volume 1: Gram-negative bacteria of general, medical, or industrial importance;

Volume 2: Gram-positive bacteria other than actinomycetes;

Volume 3: Gram-negative bacteria with distinctive properties;

Volume 4: Actinomycetes (Gram-positive filamentous bacteria).

Responses to Gram staining play a particularly important role in this phenetic classification, even determining the volume in which a species is placed. Gram-staining responses are also correlated with many other bacterial properties. Typical Gram-negative bacteria, Gram-positive bacteria, and mycoplasmas (cell wall-deficient bacteria) ...

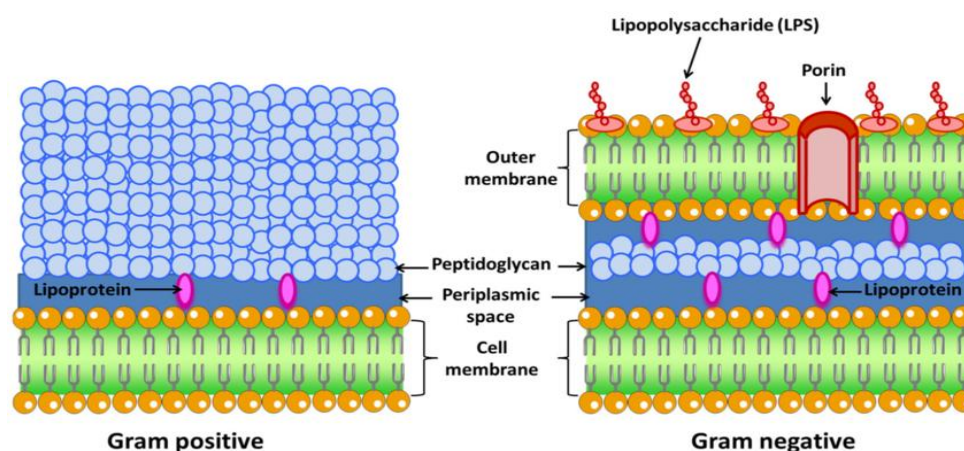


Figure 3. Gram-Positive and Gram-Negative Bacteria ((Jiménez-Jiménez et al., 2022).

Even though Gram stain reactions are usually seen as phenetic characteristics, they also contribute to the phylogenetic categorization of microorganisms.

2. The second edition of the Bergey's Manual

The more recent second edition, consisting of five volumes, was published in 2001. Each volume focuses on a specific group of microorganisms and is authored by leading experts in the respective fields. These volumes offer detailed information on microbial morphology, physiology, growth conditions, ecology, and other key characteristics, establishing the series as a foundational reference in microbiology. The second edition reflects significant advancements in microbial taxonomy since the original publication, particularly through the integration of molecular approaches to phylogenetic analysis.

The five volumes are as follows:

Volume 1: the Archaea and the deeply branching and phototrophic Bacteria.

Volume 2: the Proteobacteria

Volume 3: The firmicutes

Volume 4: The Bacteroidetes, Spirochaetes, Tenericutes, Mollicutes, Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes

Volume 5: Actinobacteria

Volume 5 also contains a section that updates the descriptions and phylogenetic classifications that have been revised since the publication of Volume 1.

Chapter 4. Main types of classification

Modern bacterial taxonomy adopts a **polyphasic approach**, integrating phenotypic, genotypic, and phylogenetic data to establish a more comprehensive and stable classification system.

1. Phenotypic taxonomy

The spectacular and classical approach of classifying microorganisms is exclusively based upon the phenotypic characteristic features found in them, which include morphology, staining properties, metabolic and biochemical activities, growth conditions, and ecological traits.

1.1. Morphological characteristics

Microbiologists typically start with microscopic examination (e.g., Gram staining) or colony morphology on specific media. These characteristics can offer rapid clues about an organism's identity, such as whether it is Gram-positive or Gram-negative, or whether it forms distinctive colony shapes or pigments. Moreover, the differences between structures such as endospores, capsules, or flagella provide valuable clues. The different morphological characteristics used in the classification and identification of microorganisms are summarized in the table 6.

Table 6. Morphological Characteristics Used in Bacterial Classification

Characteristic	Description / Categories	Examples
Cell Shape	Cocci (spherical), Bacilli (rod-shaped), Spirilla (spiral), Vibrio	<i>Staphylococcus</i> (cocci), <i>E. coli</i> (bacilli)
Cell Arrangement	Single, Pairs (diplo), Chains (strepto-), Clusters (staphylo-)	<i>Streptococcus</i> (chains), <i>Staphylococcus</i> (clusters)
Cell Size	Varies (e.g., 0.2–10 µm in length)	Small: <i>Mycoplasma</i> , Large: <i>Bacillus</i>
Gram Reaction	Gram-positive (purple), Gram-negative (pink/red)	<i>Bacillus</i> (G ⁺), <i>E. coli</i> (G [−])
Motility	Motile (flagella, gliding) or non-	<i>Proteus</i> (highly motile),

	motile	<i>Klebsiella</i> (non-motile)
Flagella Arrangement	Monotrichous, Lophotrichous, Amphitrichous, Peritrichous, None	<i>Vibrio cholerae</i> (monotrichous), <i>E. coli</i> (peritrichous)
Spore Formation	Presence or absence of endospores	<i>Bacillus</i> , <i>Clostridium</i> (spore-forming)
Capsule Presence	Encapsulated or non-encapsulated	<i>K. pneumoniae</i> (encapsulated)
Colony Morphology	Shape, margin, elevation, color, texture (on agar plates)	Round, irregular, smooth, rough colonies
Staining Characteristics	Acid-fast (e.g., Ziehl-Neelsen stain), Capsule stain, Endospore stain	<i>Mycobacterium</i> (acid-fast), <i>Bacillus</i> (spores)
Cell Wall Composition	Peptidoglycan (Gram+), outer membrane (Gram-), absent (e.g., mycoplasmas)	<i>Mycoplasma</i> (no cell wall)

1.2. Differential staining

Most bacteria are either Gram positive or Gram-negative. Other differential stains, such as acid-fast staining, can be used to identify microorganisms that belong to more specific groups. These stains are developed based on the chemical composition of the cell wall.

1.3. Biochemical characteristics

biochemical panels are used to assess an organism's ability to ferment sugars, produce certain enzymes (e.g., catalase, oxidase), or utilize specific substrates. Common examples include: Triple Sugar Iron (TSI) agar for enteric bacteria.

1.4. Physiological and metabolic characteristics

Physiological and metabolic characteristics are highly valuable, as they are directly related to the nature and activity of microbial enzymes and transport proteins. Table 7 lists some of these properties.

Table 7. Some Physiological and Metabolic Characteristics Used for Classification and Identification (Torche, 2016)

Carbon and nitrogen sources
Cell wall components
Fermentation products
General mode of nutrition
Optimum and range of growth temperature
Luminescence
Mechanisms of energy conversion
Motility
Osmotic tolerance
Photosynthetic pigments
Salt requirements and tolerance
Secondary metabolites produced
Sensitivity to metabolic inhibitors and antibiotics
Reserve inclusions (e.g., granules, storage materials)

1. 5. Serotyping

Serology is the science that studies blood serum and the immune responses detectable through serum analysis. Commercial kits containing antibody solutions are available on the market and are used for the identification of various medically important microorganisms.

Serological methods such as agglutination tests or ELISAs detect organism-specific antigens or antibodies. This approach is often used to differentiate closely related strains (e.g., various serotypes of *Salmonella* or *E. coli*).

1. 6. Ecological characteristics

The capacity of a microorganism to colonize a specific environment holds taxonomic relevance. Microorganisms that are phenotypically and genotypically similar may nevertheless occupy distinct ecological niches.

Ecological characteristics with taxonomic significance include life cycle patterns, the nature of symbiotic associations, host specificity in pathogenic interactions, and environmental preferences, such as optimal temperature, pH, oxygen availability, and osmotic conditions.

These ecological parameters contribute to the differentiation and classification of microbial taxa, especially when other distinguishing features are limited.

1.7. Chemotaxonomy

Chemotaxonomy or chemical taxonomy is the identification and classification of an organism based on the differences or similarities of the chemical features present in the cell structure such as peptidoglycan types/compositions, polar lipids, fatty acids, mycolic acids, respiratory quinones, and polyamine patterns. Considering their importance in their capacity to differentiate prokaryotes even at a higher taxonomic level, the characterization of chemical features was considered an integral part of the development of the polyphasic approach to prokaryotic systematics.

1.8. Numerical taxonomy

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

The selected characters are assigned equal weight and are quantified numerically to establish taxonomic distances that reflect both phenotypic similarity and evolutionary relationships (phylogenetic relatedness) among the organisms under comparison. Binary scoring (0 or 1, indicating absence or presence) of similarities and differences enables the characterization of taxa through a similarity coefficient, which is computed using various methods depending on

the selection of traits, the coding scheme, and the analytical treatment applied to the dataset.

Among the most commonly used indices is the Jaccard–Sneath index, given by the following

relation: $S_{AB} = n_s^+ / n_s^+ + nd$

where:

S_{AB} : similarity coefficient between strain A and strain B

n_s^+ : number of similar characters

nd : number of different characters

The results of numerical taxonomic analysis are often represented in the form of a branching diagram analogous to a tree. This is known as a dendrogram, in which organisms with the greatest similarity are grouped into clusters called phenons. However, the taxonomic significance of these phenons remains a subject of debate, particularly regarding the similarity threshold at which a pheno can be considered equivalent to a species or a genus, etc. Generally, it is assumed that phenons sharing more than 80% similarity may be regarded as belonging to the same species.

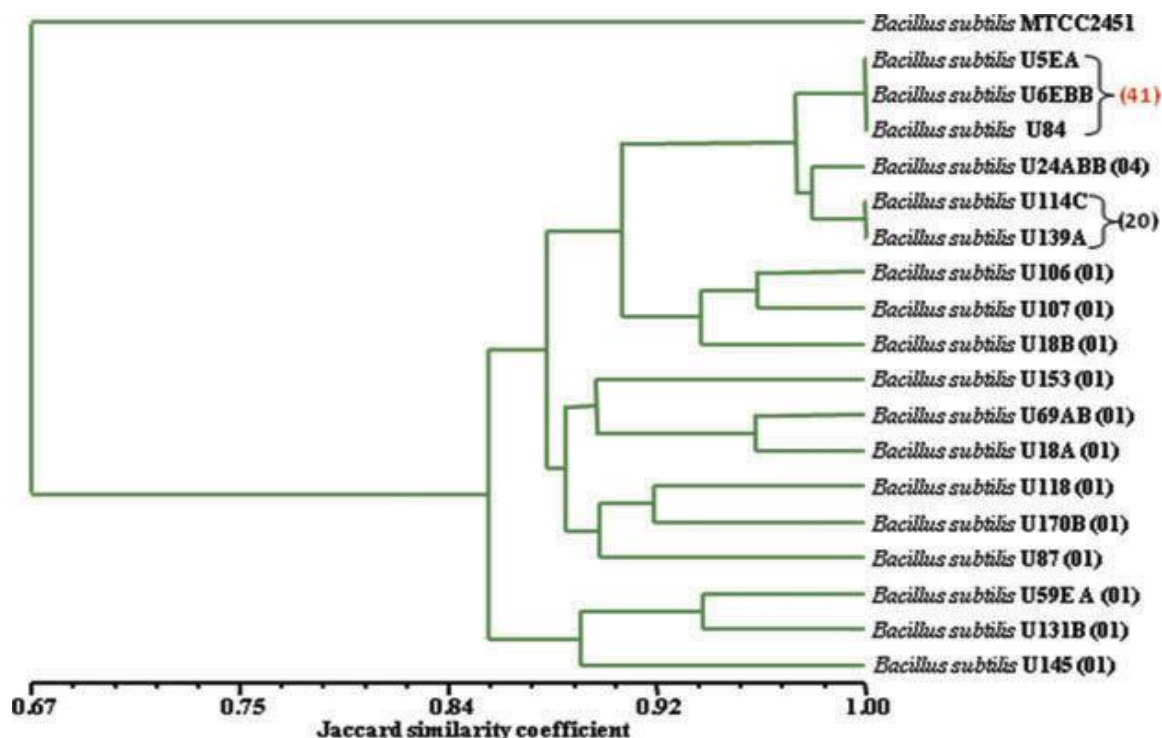


Figure 4. Dendrogram based on UPGMA clustering (unweighted pair group method with averages) of Jaccard similarity coefficient (S_j) of *B. subtilis* strains (Adewumi et al., 2014).

2. Phylogenetic taxonomy

The phylogenetic system of classification is a method of classifying organisms based on their evolutionary history and genetic relationships. It relies on common ancestors and genetic similarities rather than just physical characteristics.

Phylogenetic classification is based on hereditary relationships between bacteria, encoded by 16S or 23S rRNA sequence data. The phylogenetic concept of a bacterial species is considered as a group of isolates with a common origin. The generation of genetic diversity results in a clone with varying degrees of recombination, and is characterized by a certain level of phenotypic consistency, a significant degree of DNA-DNA hybridization, and more than 97% homology in 16S rRNA gene sequences.

The phylogenetic system of classification is also known as cladistic classification. Microbial taxa within the Bacteria and Archaea form distinct genealogically related groups that can be represented in the form of phylogenetic trees (cladograms) to represent evolutionary relationships between species.

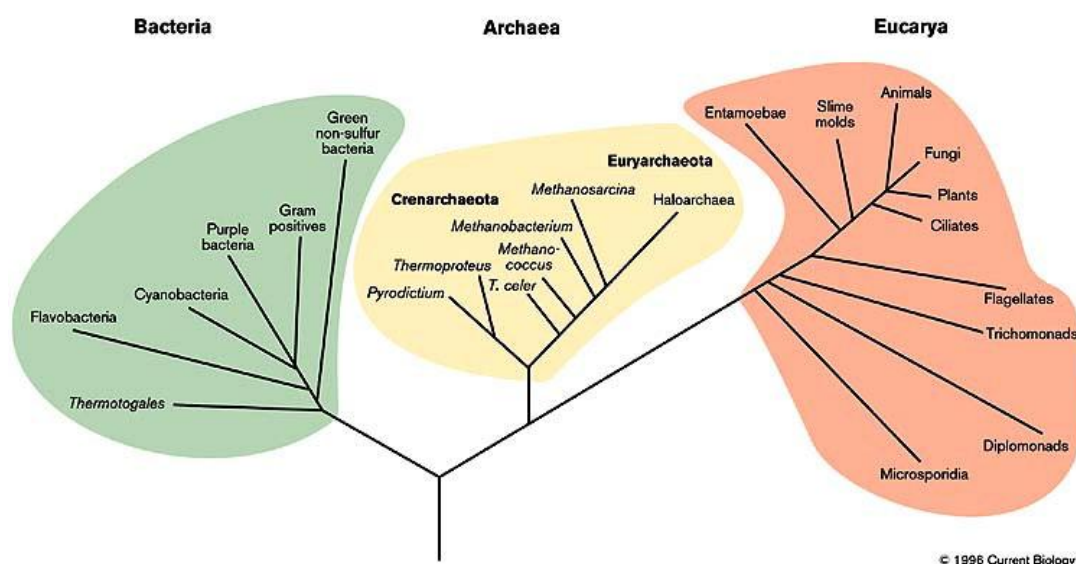


Figure 5. Universal phylogenetic tree (Woese, 1994)

3. Molecular taxonomy

Some of the most powerful approaches to taxonomy are through the study of proteins and nucleic acids. Because these are either direct gene products or the genes themselves, comparisons of proteins and nucleic acids yield considerable information about true relatedness. The more recent molecular approaches have become increasingly important in prokaryotic taxonomy are:

3.1. Comparison of Proteins

The amino acid sequences of proteins are direct reflections of *mRNA* sequences and therefore closely related to the structures of the genes coding for their synthesis. For this reason, comparisons of proteins from different microorganisms are very useful taxonomically.

The sequences of proteins with dissimilar functions often change at different rates; some sequences change quite rapidly, whereas others are very stable. If the sequences of proteins with the same function are similar, the organisms possessing them are probably closely related. The sequences of cytochromes and other electron transport proteins, histones, heat-shock proteins, transcription and translation proteins, and a variety of metabolic enzymes have been used in taxonomic studies.

3.2. Nucleic Acid Base Composition

The deoxyribonucleic acid (DNA) of most organisms contains the purine bases adenine (A) and guanine (G) and the pyrimidine bases thymine (T) and cytosine (C). Occasionally an organism may contain modified bases such as 5-methylcytosine or hydroxymethylcytosine. In most cases the modified base substitutes for only a small fraction of the parent base. The DNA in prokaryotic consists of double strands with specific base pairs (A + T) or (G + C) located at each position along the complementary strands. Since the recognition that the ratio of A + T to G + C base pairs may differ from one organism to the next and that the ratio in a given organism, usually expressed mole percentage G + C (mol% G + C), is relatively constant, the mol% G + C values have been used for the comparative characterization of organisms.

$$\text{Mol\% G+C} = [(G+C)/(G+C+A+T)] \times 100$$

It is generally accepted that bacteria whose G+C content differs by more than 5% cannot belong to the same species, and those with a G+C content difference greater than 10% cannot belong to the same genus.

A significant difference in G+C content typically indicates that two organisms belong to different species. However, the inverse is not necessarily true: distinct species may share similar or even identical G+C content, as this metric does not account for differences in the sequence and arrangement of bases within the genome.

Table 8. Representative G + C Contents of some bacteria (Lemos, 2013)

Species	Approximate genome size (Mb)	Approximate no. of genes	Approximate GC content	Lifestyle
<i>E. coli</i> *	4.7*	4500	50 %	Free-living and host-associated
<i>B. subtilis</i>	4.2	4100	43 %	Free-living
<i>S. mutans</i>	1.6	1950	37 %	Host-associated
<i>S. pyogenes</i>	1.8	1800	39 %	Host-associated
<i>S. agalactiae</i>	2.2	2200	35 %	Host-associated
<i>S. pneumoniae</i>	2.1	2100	40 %	Host-associated
<i>Staphylococcus aureus</i>	2.8	2850	33 %	Host-associated
<i>Listeria monocytogenes</i>	2.9	2800	38 %	Free-living and host-associated

*Sequenced genomes range in size from 4.6 to 6.2 Mb.

3.3. Nucleic Acid Hybridization

The similarity between genomes can be compared more directly by use of nucleic acid hybridization studies. If a mixture of single stranded DNA formed by heating dsDNA is cooled and held at a temperature about 25°C below the T_m , strands with complementary base sequences will reassociate to form stable dsDNA, whereas noncomplementary strands will remain single.

In one of the more widely used hybridization techniques, nitrocellulose filters with bound nonradioactive DNA strands are incubated at the appropriate temperature with single-stranded DNA fragments made radioactive with ^{32}P or ^{14}C . After radioactive fragments are allowed to hybridize with the membrane-bound ss- DNA, the membrane is washed to remove any nonhybridized ssDNA and its radioactivity is measured. The quantity of radioactivity bound to the filter reflects the amount of hybridization and thus the similarity of the DNA sequences.

If DNA molecules are very different in sequence, they will not form a stable, detectable hybrid. Therefore DNA-DNA hybridization is used to study only closely related microorganisms.

Two strains whose DNAs show at least 70% relatedness under optimal hybridization conditions are considered members of the same species. Moreover, DNA–DNA hybridization values exceeding 25% suggest that two isolates may be assigned to the same genus.

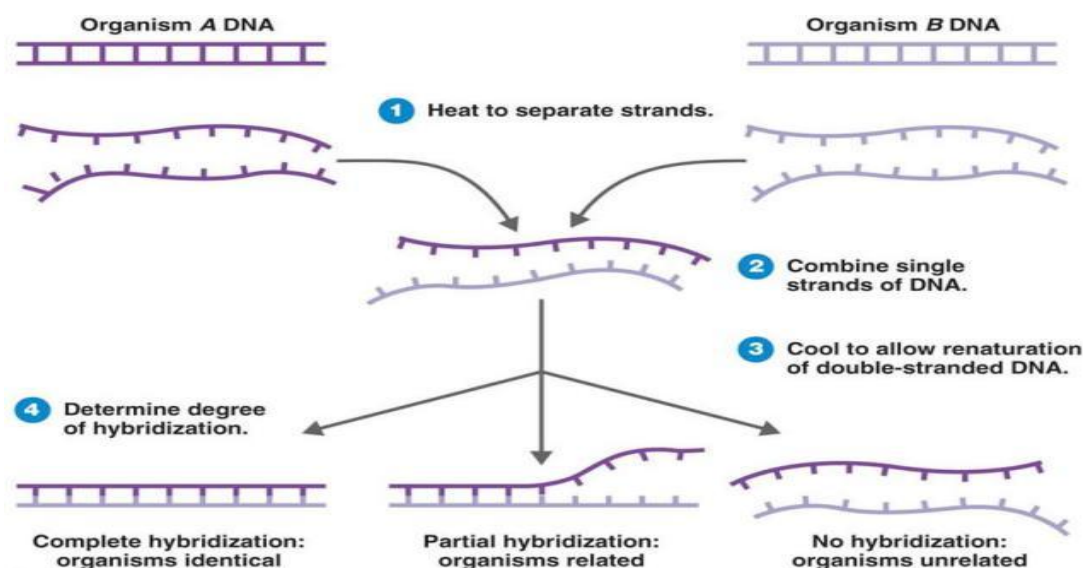


Figure 6. DNA-DNA Hybridization (Madigan et al., 2018)

4. Nucleic acid sequencing

Comparative rRNA gene sequence analysis currently represents the standard approach in microbial phylogeny, taxonomy and identification. Special database projects provide quality checked rRNA alignments as well as online facilities for data processing.

16S rRNA is the most commonly used in bacterial classification and identification since it is present in all bacteria. The nucleotide of 16S rRNA can be compared online with those of strains in international databases. The sequence of the gene encoding 16S rRNA is known for about 4,000 strains and can be accessed by querying databases (EMBL, GenBank).

The **FASTA** and **BLAST** programs enable comparative analysis of the nucleotide sequence from an unknown strain against sequence databases, retrieving the most homologous or closely related sequences.

The 16S rRNA gene sequence does not contain enough discriminating power to delineate species within certain groups, and additional DNA–DNA hybridizations are often required.

- When the 16S rRNA gene sequence similarity between two bacterial isolates is below 97%, they are considered to belong to distinct species. Consequently, DNA–DNA hybridization analyses are not warranted below this threshold.
- If the sequence similarity percentage is greater than 97%, the assignment of two strains to the same species or to different species depends on the results of DNA–DNA hybridization analyses

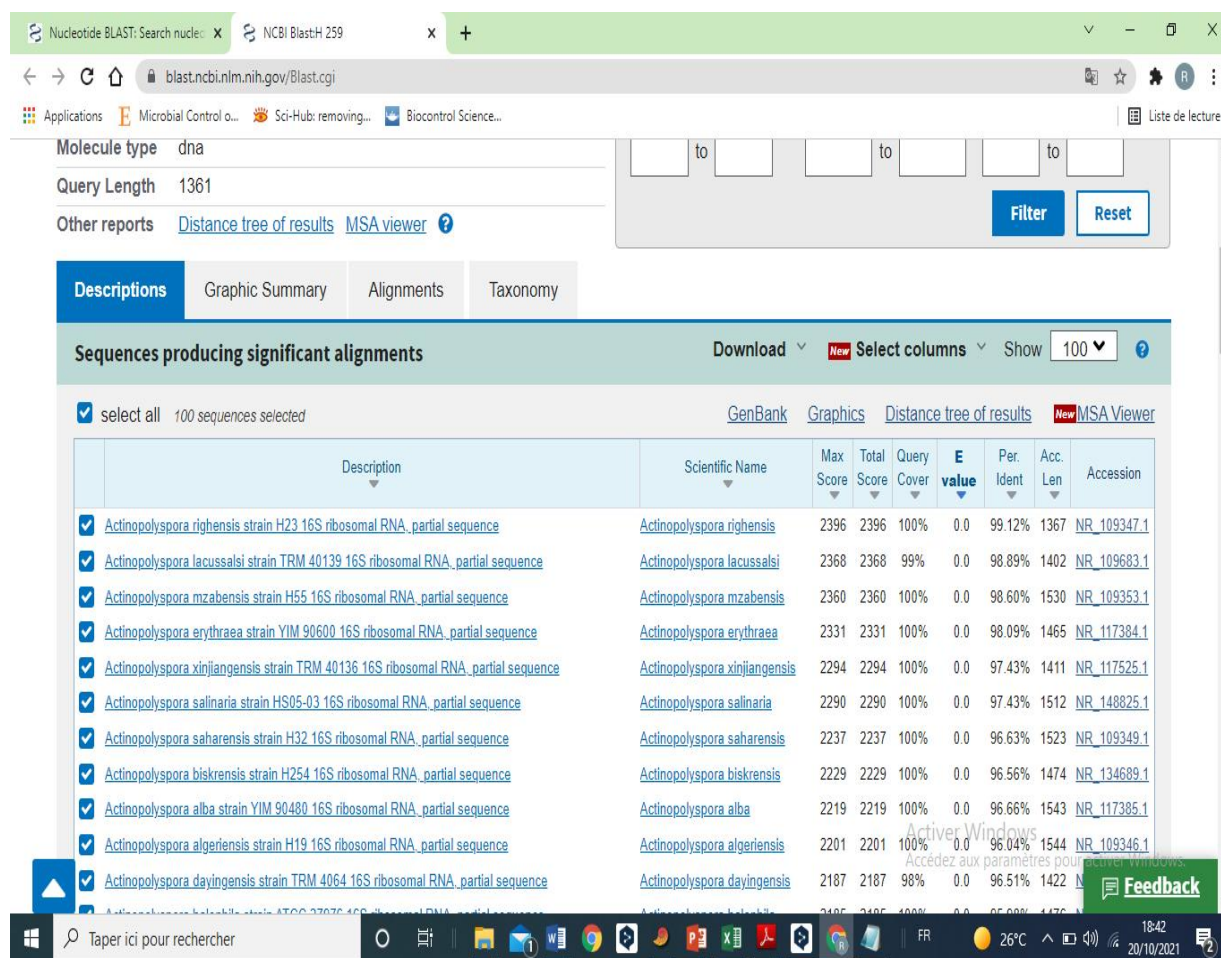


Figure 7. Example of the Blast results of 16S ribosomal RNA of *Actinopolyspora righensis* strain H23

Chapter 5. Major bacterial groups

1. Photosynthetic bacteria

- ✧ Photosynthetic bacteria are classified within five distinct bacterial phyla.
- ✧ Chlorobi (green sulfur bacteria), Phylum Chlorobi
- ✧ Chloroflexi (filamentous green bacteria), Phylum Chloroflexi
- ✧ Cyanobacteria, Phylum Cyanobacteria
- ✧ Photosynthetic Proteobacteria, Phylum Proteobacteria
- ✧ Photosynthetic Firmicutes (heliobacteria), Phylum Firmicutes

1.1. Phylum Chlorobi (Green Sulfur Bacteria)

The chlorobi phylum is composed of photosynthetic, obligately anaerobic, Gram-negative bacteria that are frequently known as green sulfur bacteria. It has only one class (Chlorobia), order (Chlorobiales), and family (Chlorobiaceae). In addition, these bacteria are very diverse morphologically, they can be vibrios, rods, or cocci; while some develop singly, others form chains and colonies.

Their name comes from the fact that most species are green due to their characteristic bacteriochlorophylls, mainly Bchl c, Bchl d, or Bchl e.

They form a small group of obligate anaerobic photolithotrophs that use hydrogen sulfide, elemental sulfur, and hydrogen as electron sources. These bacteria are most abundant in anoxic, sulfide-rich zones of lakes.

The photosynthetic pigments of these bacteria are located in ellipsoidal vesicles called chlorosomes, or chlorobium vesicles, which are attached to the cytoplasmic membrane but are not continuous with it.

They are non-motile and lack flagella; however, some species possess gas vesicles that allow them to position themselves at the optimal depth for light and hydrogen sulfide. They are pale green or chocolate brown in color. Representative genera include *Chlorobium*, *Prosthecochloris*, and *Pelodictyon*.

1.2. Phylum Chloroflexi (Green Non-Sulfur Bacteria)

The phylum Chloroflexi includes both photosynthetic and non-photosynthetic members. Within this phylum, the class *Chloroflexi* contains two main orders: *Chloroflexales* and *Herpetosiphonales*.

The order *Chloroflexales* includes the family *Chloroflexaceae*, which contains genera such as *Chloroflexus*, *Chloronema*, *Heliothrix*, and *Roseiflexus*. These bacteria are usually filamentous, often found in hot environments, and many are capable of photosynthesis without producing oxygen. Another family, *Oscillochloridaceae*, includes the genus *Oscillochloris*, which also performs photosynthesis.

Chloroflexus, the main representative of the green non-sulfur bacteria is a thermophilic, filamentous bacterium that exhibits gliding motility; it is often isolated from neutral to alkaline hot springs, where it forms reddish-orange mats, typically in association with cyanobacteria.

1.3. Phylum Cyanobacteria

The phylum Cyanobacteria indeed forms the largest and most diverse group of photosynthetic bacteria. Their photosynthetic system closely resembles that of eukaryotes because they possess chlorophyll a and thus perform oxygenic photosynthesis.

Cyanobacteria do not have internal cell membranes that delimit the cell nucleus from other organelles and are therefore classified as prokaryotic organisms, which microscopically distinguishes them from algae, microalgae, and plants. They can be found as unicellular, colonial, or multicellular organisms and inhabit the most diverse environments; they can be planktonic (suspended in water), benthic (attached to surfaces), or metaphytic (attached to macrophytes or other surfaces submerged in water).

They are photosynthetic organisms and therefore have a pigment called chlorophyll-a. However, due to the absence of internal cell membranes, cyanobacteria do not have

chloroplasts, and, therefore, chlorophyll is found inside simple thylakoids, where light-dependent photosynthetic reactions take place. Cyanobacteria also have carotenoids (whose main function is photoprotection) and accessory pigments, such as phycobilins (e.g., phycocyanin and phycoerythrin), which do more than provide the characteristic cyanobacterial color: **phycocyanins** and **phycoerythrins** absorb some wavelengths of active radiation and transfer the absorbed light energy to chlorophyll-a in photosystem II.

Cyanobacteria reproduce by asexual reproduction, the division of vegetative cells. In unicellular species, cells divide completely, and some can form colonies, an aggregate of single cells in a mucilaginous matrix. When their cell division occurs in a single plane, they can form pseudofilaments, linear colonies formed by unicellular cyanobacteria. In true filamentous cyanobacteria, the cells remain connected after division, forming structures called trichomes or filaments. The filaments divide through a single plane of division that can also project in multiple directions, forming false-branched filaments, or they can divide through multiple planes of cell division, forming true-branched filaments.

The cellular morphology of these organisms is quite diverse and can be used to identify different species and taxonomic groups. The cell shape of cyanobacteria can be spherical, ellipsoid, cylindrical, conical, or discoid. Their size also varies greatly, from the so-called picobacteria, spherical cyanobacteria with a very small cell diameter (0.2 μm), to filamentous forms that can reach up to a few millimeters (Figure 8).



Figure 8. Illustration of morphological diversity in cyanobacteria. Groups (orders) follow Rippka et al. (1979). I. Chroococcales: a *Chroococcus subnudus*, b Ch. limneticus, c *Cyanothece aeruginosa*, d *Snowella litoralis*, e *Microcystis aeruginosa*. II. Pleurocapsales: f *Pleurocapsa minor*. III. Oscillatoriales: g *Planktothrix agardhii*, h *Limnothrix redekei*, i *Arthrospira jenneri*, j *Johanseninema constrictum*, k *Phormidium* sp., l, m *Oscillatoria* sp., n *Schizothrix* sp., o *Tolypothrix* sp., p *Katagnymene accurata*., IV. Nostocales: q *Dolichospermum planctonicum*, r *Dolichospermum* sp., s *Nostoc* sp., t *Nodularia moravica*. V. Stigonematales: u, v *Stigonema* sp (Dvorak et al., 2015).

Despite not having flagella, many cyanobacteria have mobility mechanisms, although they are not very well elucidated. In fact, some filamentous species can develop hormogonia, reproductive and mobile units, which are formed by the fragmentation of filaments and then released from the parental filament. Hormogonia perform gliding movements until they develop into a new trichome.

Bergey's Manual of Systematic Bacteriology grouped the genera of Cyanobacteria into five subsections which are distinguished by the morphology of the cell or filament and the modes of reproduction.

Subsection I includes unicellular cyanobacteria that reproduce by binary fission (e.g., *Gloeobacter*, *Gloeocapsa*, *Gloeotheca*, *Synechococcus*, *Synechocystis*) or by budding (e.g., *Chamaesiphon*).

Subsection II consists of unicellular cyanobacteria that divide by multiple fission (formation of motile and non-motile baeocytes) or by both binary and multiple fission (e.g., *Dermocarpa*, *Xenococcus*, *Dermocarpella*, *Myxosarcina*, *Chroococidiopsis*).

Subsection III groups filamentous cyanobacteria lacking differentiated cells (e.g., *Spirulina*, *Oscillatoria*, *Pseudanabaena*).

Subsection IV includes filamentous cyanobacteria possessing differentiated cells such as heterocysts and akinetes (e.g., *Anabaena*, *Nodularia*, *Cylindrospermum*, *Scytonema*, *Calothrix*).

Subsection V contains heterocystous filamentous cyanobacteria exhibiting true branching (e.g., *Chlorogloeopsis*).

1.4. Photosynthetic Proteobacteria

The photosynthetic Proteobacteria are commonly called purple bacteria because of their reddish-violet coloration.

Photosynthetic Proteobacteria are divided into two subgroups. Purple sulfur bacteria belong to the γ -proteobacteria (class Gamma-proteobacteria), within the families Chromatiaceae and Ectothiorhodospiraceae. Purple non-sulfur bacteria are distributed among the α -proteobacteria (five different families) and two families of β -proteobacteria.

1.4.1. Purple Sulfur Bacteria

According to *Bergey's Manual*, purple sulfur bacteria are classified into two families within the order **Chromatiales**: **Chromatiaceae** and **Ectothiorhodospiraceae**.

Purple sulfur bacteria are relatively large unicellular microorganisms that can reach more than 6.0 μm in diameter. Many species are motile by means of polar flagella, and some possess gas vacuoles that help them maintain their position in the water column. All members of this group are capable of using hydrogen sulfide (H_2S) as an electron donor during photosynthesis, producing elemental sulfur granules. This sulfur is subsequently oxidized to sulfate (SO_4^{2-}).

These bacteria typically inhabit anoxic but illuminated (euphotic) zones of aquatic environments, such as lakes and other water bodies where hydrogen sulfide accumulates.

Members of the genus *Ectothiorhodospira* deposit sulfur globules extracellularly, whereas species of the **Chromatiaceae** family oxidize hydrogen sulfide to sulfur and store it inside the cell in the form of sulfur granules.

1.4.2. Purple Non-Sulfur Bacteria

Purple non-sulfur bacteria exhibit a wide diversity of cell morphologies. They may appear as spirals (*Rhodospirillum*), rods (*Rhodopseudomonas*), semicircular or circular cells (*Rhodocyclus*), and some species can even form prosthecae and reproduce by budding, as observed in *Rhodomicrobium* (Figure 9).

Historically, purple non-sulfur bacteria were considered incapable of using sulfide as an electron donor for CO_2 reduction. However, it is now known that many species in this group can utilize sulfide, although the sulfide concentrations that are optimal for purple sulfur bacteria (1–3 mM) are often toxic to most purple non-sulfur bacteria.

In the absence of light, most of these bacteria can grow aerobically as chemoorganotrophs. However, when growing anaerobically in the presence of light, they perform photosynthesis in a manner very similar to that of purple sulfur bacteria.

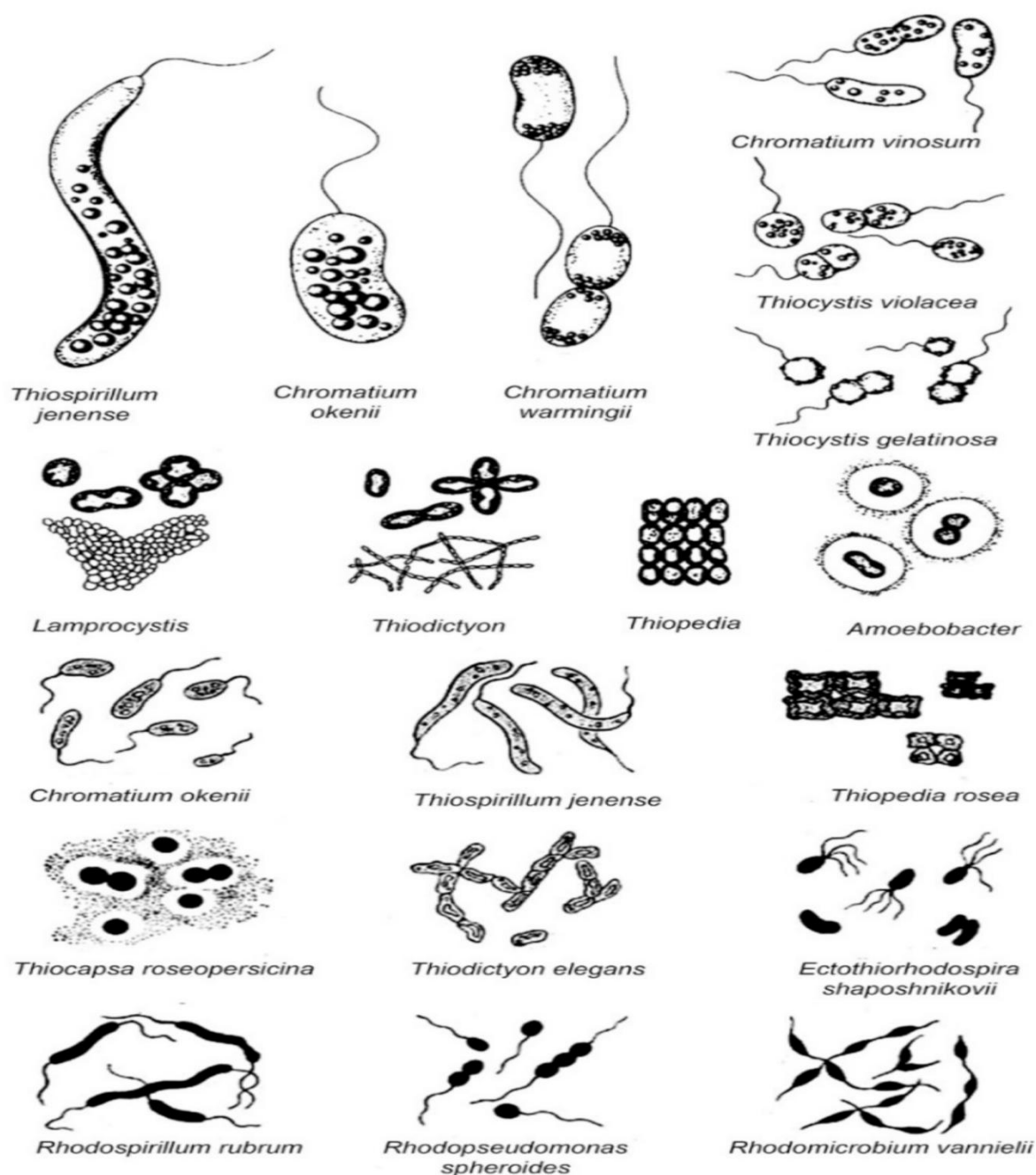


Figure 9. The morphological diversity of some phototrophic purple bacteria (Kushkevych et al., 2021).

1.5. Phylum Firmicutes (Heliobacteria)

Heliobacteria are Gram-positive photosynthetic bacteria that are strict anaerobes. They are distinguished by the presence of bacteriochlorophyll *g*, which makes them unique among photosynthetic microorganisms.

The family **Heliobacteriaceae** includes four genera: *Heliobacterium*, *Heliophilum*, *Heliorestis*, and *Heliobacillus*. All known heliobacteria exhibit rod-shaped or filamentous morphologies (Figure 10). However, species of *Heliophilum* display an unusual morphology, forming motile aggregates.

Heliobacteria are strictly anaerobic, anoxygenic phototrophic bacteria belonging to the phylum *Firmicutes*. They are distinct from other anaerobic anoxygenic phototrophs in that they produce unique photosynthetic pigments (bacteriochlorophyll *g* is the major pigment) located directly in the cytoplasmic membrane. They have a Gram-positive cell structure and are capable of producing heat-resistant endospores rich in calcium and dipicolinic acid. Heliobacteria are obligate photoheterotrophs, requiring organic carbon sources for growth,

Heliobacteria are typically found in environments such as alkaline soils. However, their ecological distribution and functional roles in terrestrial ecosystems are still not well understood.

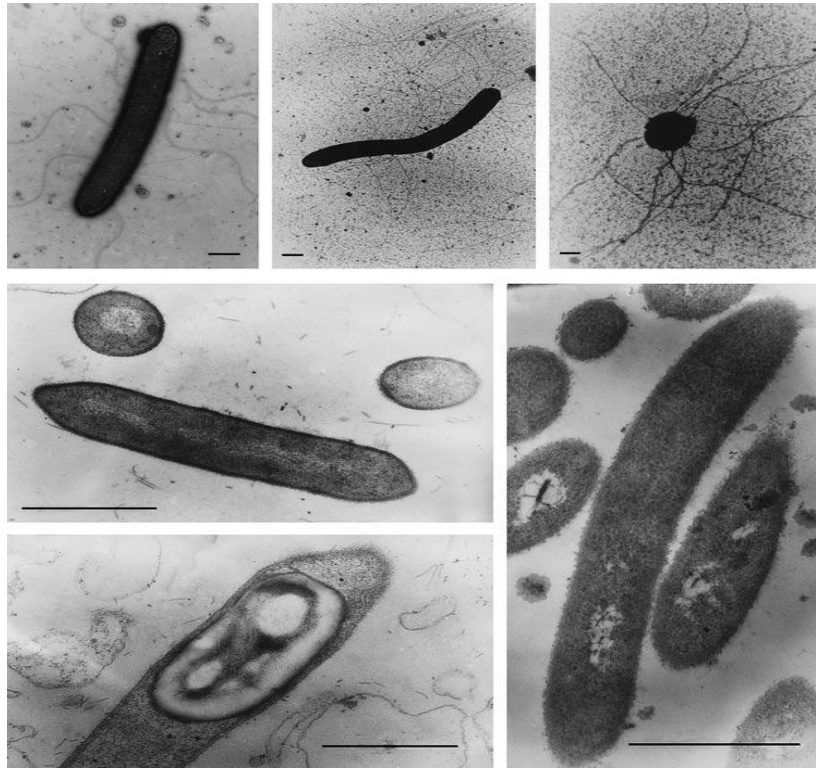


Figure 10. Morphology and fine structure of heliobacteria (Bryantseva., 2012)

2. Autotrophic bacteria

Autotrophic bacteria synthesize their own food. They derive energy from light or chemical reactions. They utilize simple inorganic compounds like carbon dioxide, water, hydrogen sulfide, etc. and convert them into organic compounds like carbohydrates, proteins, etc. to supplement their energy requirements.

Depending on the types of sources utilized, autotrophic bacteria are categorized into two types:

- Photoautotrophs or photosynthetic. They derive energy from sunlight.
- Chemoautotrophs or chemosynthetic. They use chemical energy to prepare their food.

Apart from energy requirements, both types of bacteria need a carbon source to synthesize their food, e.g. carbon dioxide and other compounds.

2.1. Photoautotrophic bacteria

Phototrophic bacteria are defined as microorganisms that utilize solar electromagnetic radiation for energy conservation, typically involving the reduction of CO₂ into organic matter through photosynthesis, using various pigments such as chlorophylls and bacteriochlorophylls. They can be categorized into oxygenic and anoxygenic types, with the latter relying on electron donors other than water and not producing oxygen.

2.1.1. Oxygenic Photosynthetic Bacteria

Cyanobacteria (blue-green algae) perform oxygenic photosynthesis. They use H₂O as an electron donor and oxygen is produced in the reaction. They do not possess chloroplasts but photosynthetic pigments like chlorophyll-a are present in the cytosol.



Cyanobacteria are filamentous or colonial, they may also perform nitrogen fixation and have specialized cells for that known as heterocyst. E.g. *Nostoc*, *Anabaena*, etc.

2.1.2. Anoxygenic Photosynthetic Bacteria

Most of the photosynthetic bacteria are anoxygenic, i.e. they do not utilize water as an electron donor, instead, they use H₂S, H₂ or thiosulphate as reducing agent and hydrogen sources. They contain a photosynthetic pigment known as bacteriochlorophyll (BChl), which is like chlorophylls in plants.

Examples include green sulphur bacteria, purple sulphur bacteria, purple non-sulphur bacteria, phototrophic acidobacteria and heliobacteria, FAPs (filamentous anoxygenic phototrophs).

$\text{CO}_2 + 2\text{H}_2\text{A} + \text{light energy} \rightarrow [\text{CH}_2\text{O}] + 2\text{A} + \text{H}_2\text{O}$; where H₂A can be any electron donor, e.g. H₂S, H₂ etc.

- **Purple sulphur bacteria (PSB)**

They are found in hot sulphur springs and stagnant water. These bacteria thrive in anaerobic or oxygen poor environments. They belong to the order Chromatiales of proteobacteria. They utilize hydrogen sulfide or thiosulphates as a reducing agent and release sulphur. The main pigments are bacteriochlorophyll 'a' and 'b' located in the plasma membrane. Their photosystem is similar to PSII of higher plants. They are two types:

- Chromatiaceae – contains intercellular sulphur granules
- Ectothiorhodospiraceae – contains extracellular sulphur granules

- **Purple non-sulphur bacteria (PNSB)**

They mainly use hydrogen as a reducing agent. They belong to the order Rhodospirillales. Purple photosynthetic bacteria are very important as they produce various beneficial substances such as polyphosphates, vitamins, pigments, hydrogen, extracellular nucleic acids and growth promoting substances for plants. They can increase the plants yield, resistance to environmental stress and improve biomass quality. They are also helpful in bioremediation of heavy metals and reducing emission of greenhouse gases.

- **Green sulphur bacteria (GSB)**

They are obligate anaerobe and generally non-motile. They belong to the Chlorobiaceae family. They are found deep in the ocean in extremely low light and anoxic environment and near thermal vents. The electron donor is sulphide, hydrogen or ferrous ion. Carbon fixation is done by reverse tricarboxylic acid or RTCA cycle. They contain bacteriochlorophyll 'c', 'd' and 'e' along with bacteriochlorophyll 'a'. Pigments are present in the plasma membrane and chlorosomes.

2. 2. Chemoautotrophic bacteria

Chemoautotrophic bacteria perform chemosynthesis, which utilizes chemical energy. They lack photosynthetic pigments. Here carbon sources can be carbon dioxide, hydrogen sulphide, methane, etc. The chemical energy is produced from oxidation of inorganic compounds such as hydrogen, H₂S, carbon monoxide, ammonia, methane, iron salts, nitrite, etc. The energy liberated from oxidation is trapped in ATP for the synthesis of organic compounds.

They can be aerobic or anaerobic. Depending on the source, where they derive energy from, they are categorized into various types such as sulphur bacteria, hydrogen bacteria, iron bacteria, nitrogen bacteria, methanotrophs, etc. They play an important role in nutrient recycling such as nitrogen, phosphorus, sulphur, iron, etc.

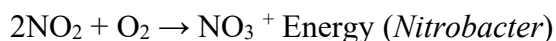
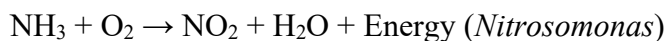
- **Sulphur bacteria**

They oxidize hydrogen sulphide or thiosulphates to molecular sulphur or sulphates. E.g. *Beggiatoa*, *Thiobacillus*, *Thiothrix*, *Sulfolobus*, etc.



- **Nitrogen bacteria**

Nitrifying bacteria convert ammonia to nitrite and then to nitrate. In this oxidation process, energy is released. Nitrate is utilized by plants. E.g. *Nitrosomonas*, *Nitrobacter*



- **Hydrogen bacteria**

As the name implies, they oxidize molecular hydrogen. Aerobic hydrogen-oxidizing bacteria use O₂ as an electron acceptor, whereas anaerobic hydrogen bacteria use nitrogen dioxide or

sulphate as an electron acceptor. E.g. *Helicobacter pylori*, *Hydrogenobacter thermophilus*, *Hydrogenovibrio marinus*, etc.

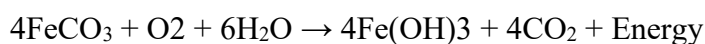


- **Methanotrophs**

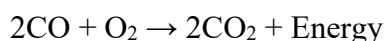
They use methane as a carbon source and to derive energy. They can be aerobic or anaerobic. Aerobic methanotrophs oxidize methane to formaldehyde, which is then utilized in various pathways to form organic compounds. Anaerobic methanotrophs utilize other compounds as electron acceptors. E.g. *Methylomonas*, *Methylococcus capsulatus*, etc. It assimilates formaldehyde by the RuMP pathway. It is used to produce animal feed. Some methanotrophs assimilate formaldehyde by serine pathway.

- **Iron bacteria**

They oxidize ferrous ions to ferric ions. They are present in iron-rich environments like hot lava bed, hydrothermal vents. E.g. *Thiobacillus ferrooxidans*, *Geobacter metallireducens*, *Zetaproteobacteria*, *Gallionella*, *Ferrobacillus*, etc.



Carbon bacteria: These bacteria use the energy released by the oxidation of carbon monoxide, e.g. *Bacillus oligocarbophilus*.



3. Gram negative heterotrophic bacteria

4. 3.1. Spirochaetes (Spirochetes)

The phylum Spirochaetes (from the Greek, spira meaning “coil” and chaite meaning “hair”) contains Gram-negative, chemoheterotrophic bacteria that are distinct in both their structure and their mechanism of motility.

The Bergey (Manual) divides the phylum Spirochaetes into one class, one order (Spirochaetales) and three families (Spirochaetaceae, Serpulinaceae, and Leptospiraceae). Currently, the phylum encompasses thirteen genera.

The Spirochetes are a heterogeneous group of bacteria that have a distinctive cell structure: their body is slender unicellular helical or spiral and is intertwined with an organelle termed the axial filament, a bundle of fibrils that winds around the cell body. The cytoplasm is surrounded by a cytoplasmic membrane and a peptidoglycan layer contributes to cell rigidity and shape. The individual fibrils that compose the axial filament originate near the ends of the cell and are normally more than half the length of the cell. Spirochetes have a typical gram-negative cell wall and a well-developed periplasmic space that encloses the flagella called endoflagella (axial filament). Endoflagella are constrained somewhat like limbs in a sleeping bag, their flexing propels the cell by rotation and even crawling motions.

The fibrils of the axial filament have a fine structure essentially the same as that of flagella; a basal body containing a series of disks; a hook; and the filament itself. The number of disks varies among the spirochetes: *Leptospira* typically has two pairs of disks, whereas most other spirochetes have a single pair of disks. The filaments are often enveloped in a proteinaceous sheath unlike the eubacterial flagellum which, if sheathed, is surrounded by an extension of the outer membrane. The close structural and chemical resemblance between the axial fibril and the flagellum, as well as their similar role in motility, suggests that they are homologous organelles. The term endoflagella and periplasmic flagella have accordingly been proposed to describe axial fibrils (Figure 11).

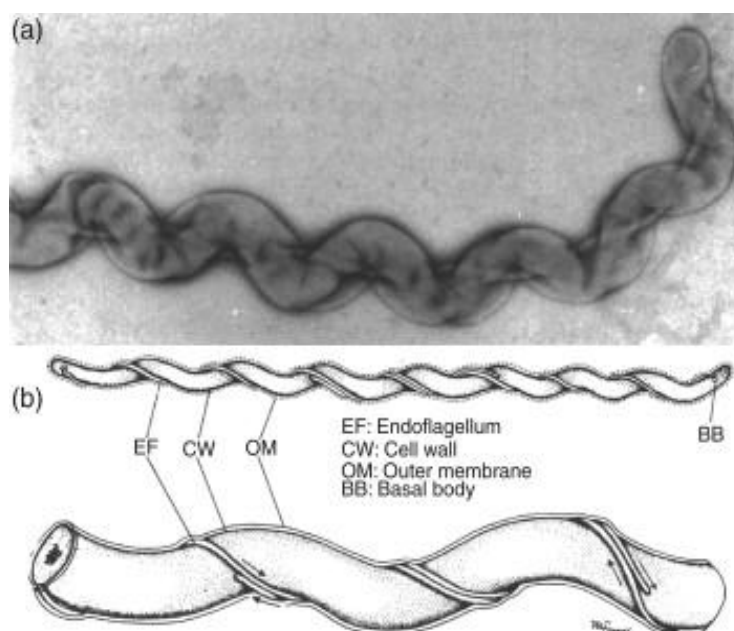


Figure 11. Spirochetal architecture. Spirochetes share a unique structure and motility strategy in which the endoflagella are inserted at opposite poles and wrap around the protoplasmic cylinder. (a) Electron micrograph of *Leptospira* showing a single endoflagellum at one end of the cell. (b) Schematic diagram showing endoflagellar location relative to the outer membrane and cell wall (Holt, 1978).

The majority of spirochetes are free living saprobes or commensals of animals and are not primary pathogens. But three genera that contain major human pathogens are *Treponema*, *Leptospira* and *Borrelia*.

3.2. Bacteroidetes

The Bacteroidetes phylum is highly diverse. It includes three classes (Bacteroides, Flavobacteria, and Sphingobacteria), 12 families, and 63 genera.

The class Bacteroides contains Gram-negative rods of various shapes, anaerobic, non-spore-forming, and either motile or non-motile. These bacteria are chemoheterotrophs and usually produce a mixture of organic acids as end products of fermentation. These bacteria grow in habitats such as the oral cavity, the intestinal tract of vertebrates, and the rumen of ruminants.

3.3. Verrucomicrobia

This phylum consists of a single class (Verrucomicrobiae) and genera such as *Verrucomicrobium*, *Prostheco bacter*, and *Xiphinematobacter*. Members are aerobic or anaerobic heterotrophs that grow in complex media. They form cytoplasmic appendages called prosthecae and divide symmetrically. Species occur in freshwater, marine, soil habitats and in animal gut microbiomes including those of humans.

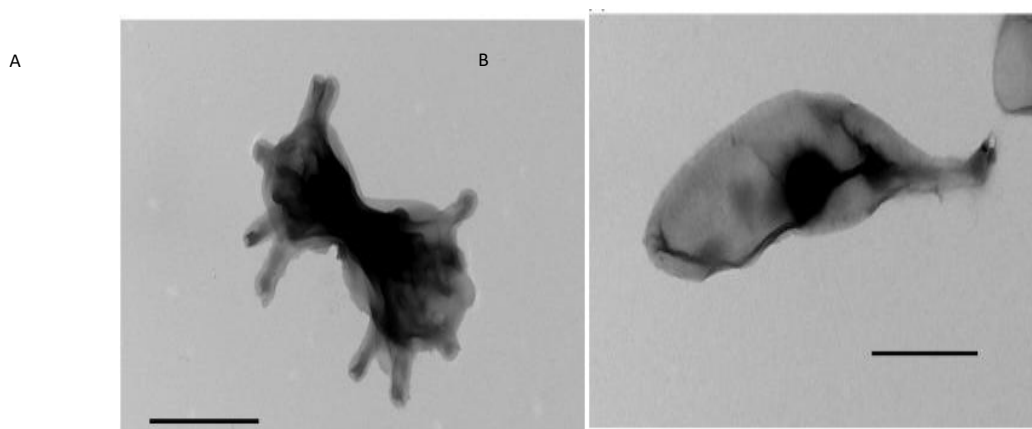


Figure 12. (A) Transmission electron micrograph of negatively-stained cell of *Verrucomicrobium spinosum* showing many tube-like prosthecae projections on all regions of the cell surface. (B) Transmission electron micrograph of negatively-stained cell of *Prostheco bacter dejongeii* showing single stalk-like prostheca projection on one pole of the cell only (Fuerst, 2019).

3.4. Fusobacteria

Fusiform bacteria constitute another phylum of anaerobes. These bacteria are often polymorphic but, as their name suggests, they generally have a spindle-like shape (fusus = spindle). *Fusobacterium* is the genus of immobile anaerobic bacteria, appearing as long, thin rods whose ends are more pointed than rounded

In humans, they are most commonly found in the gingival sulcus, where they are responsible for dental abscesses, and in the intestine. The species most frequently encountered is *F. nucleatum* (Figure 13).

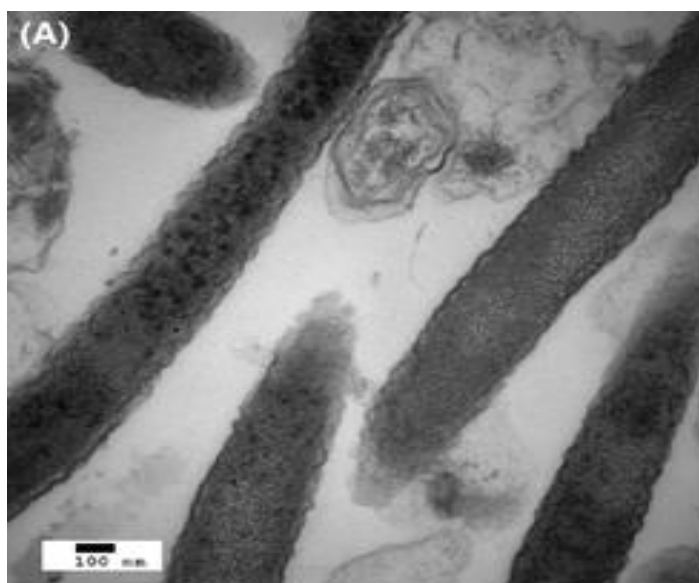


Figure 13. Electron micrographs of *Fusobacterium nucleatum* (Hendrickson et al., 2014)

4. Gram-positive heterotrophic bacteria

Gram-positive bacteria are divided into two major categories according to their guanine–cytosine (G+C) content, which can be high or low. They are therefore classified into distinct phyla:

- Firmicutes (low G+C content)
- Actinobacteria (high G+C content)

4.1. Gram-positive bacteria with low GC%: The phylum Firmicutes

4.1.1. Class Clostridia

Clostridium form a prominent phylum within the phylum Firmicutes. These bacteria are characterized by their anaerobic lifestyle and the ability to form spores, which allows them to survive in harsh environmental conditions. The phylum clostridia is divided into three orders and 11 families. Concerning metabolic capacities and ecological roles, clostridia exhibit a variety of metabolic capacities, reflecting their adaptation to different environments. They are obligate anaerobes, rely on fermentation or anaerobic respiration to produce energy, and can degrade a wide range of organic compound. Many *clostridium* species can form robust and inert endospores that allow them to survive adverse conditions, which is a major

survival strategy in the environment. Clostridia play important role in the cycling of organic matter and nutrients in various ecosystems, especially in anaerobic environments such as the rumen, sediment, and human intestine. In addition to non-pathogenic species, the Clostridia also comprise pathogens causing tetanus (*Clostridium tetani*), blackleg (*Clostridium chauvoei*) or gas gangrene (*Clostridium perfringens*). This pathogenicity is usually associated with the ability to produce toxins.

4.1.2. Class Bacilli

The second edition of Bergey's Manual groups a wide variety of Gram-positive bacteria into a single class, the Bacilli, which contains two orders: Bacillales and Lactobacillales. These orders include 17 families and more than 70 Gram-positive genera, comprising cocci, rods, spore-forming cocci, and non-spore-forming rods.

4.1.2.1. Order Bacillales

➤ Family Bacillaceae

Bacillus is the type genus of the family Bacillaceae, the ability to produce endospores is considered to be a defining character of the genus *Bacillus*, and the spores produced are highly resistant to heat, cold, dehydration and many disinfectants to maintain the strong survival of *Bacillus* in relatively extreme environments. It includes Gram-positive rods, chemoheterotrophic bacteria that are generally motile, with peritrichous flagella. The genus is aerobic, or sometimes facultatively anaerobic, and catalase positive.

Bacillaceae play an important ecological role and are of great biotechnological interest. Some *Bacillus* species contributing to soil fertility and plant protection. Certain species are exploited in the production of enzymes, biopesticides, probiotics, antibiotics and biofuels. Members of Bacillaceae are generally harmless, some species are pathogens of human and animals such as *Bacillus cereus* (food poisoning), *Bacillus anthracis* (anthrax), and *Bacillus thuringiensis* (pathogen of insects). The main genera and their characteristics are summarised in table 9.

Table 9. Characteristics of the main genera of Bacillaceae (Driche, 2024)

Genus	Characteristics
<i>Bacillus</i>	- Aerobic, endospore-forming Gram-positive rods - Diverse metabolic capabilities, including aerobic respiration, fermentation, and degradation of complex organic compound - Includes both beneficial and pathogenic species (e.g., <i>B. subtilis</i>, <i>B. cereus</i>,
<i>Paenibacillus</i>	- Closely related to <i>Bacillus</i>, but with some distinct phylogenetic and physiological differences - Able to form endospores - Known for nitrogen fixation and plant growth-promoting activities
<i>Lysinibacillus</i>	- Separated from the genus <i>Bacillus</i> based on the presence of diaminopimelic acid in the cell wall - Involved in the degradation of complex organic compounds
<i>Anoxybacillus</i>	- Aerobic, endospore-forming, thermophilic Gram-positive rods- Capable of growth at high temperatures and in the absence of oxygen- Potential sources of thermostable enzymes for industrial applications

➤ Family Staphylococcaceae

Staphylococcaceae is defined as a family of non-spore-forming, non-motile, spherical Gram-positive bacteria that divide in multiple planes to form irregular grape-like clusters, classified within the order Bacillales and characterized by facultative anaerobiosis and catalase positivity. They ferment glucose primarily to produce lactate.

The family Staphylococcaceae includes five genera, the most important of which is the genus *Staphylococcus*.

Staphylococcus species are widely distributed and can colonize skin, skin glands, anterior nares and mucous membranes of humans and warm-blooded animals. Some species like *Staphylococcus epidermidis* are known to form biofilms on medical devices. *Staphylococcus aureus* is an opportunistic human pathogen that can cause a wide variety of diseases ranging from local abscesses to life-threatening diseases like septicaemia, endocarditis and necrotizing pneumonia.

➤ Family Listeriaceae

The bacterial family of Listeriaceae includes two genera: *Listeria* and *Brochothrix*.

The genus *Listeria* currently includes more than 20 different species. The *Listeria* are Gram-positive rods (approximately 0.5 µm in diameter and 2.0 µm in length). The Listeriae are neither spore-forming nor encapsulated. They are catalase-positive and oxidase-negative. They are facultative anaerobes, being able to develop in aerobic and anaerobic conditions but growth is enhanced in the presence of CO₂. They cannot produce H₂S nor hydrolyze urea. *Listeria* bacteria are also indole-negative, methyl-red- and Voges-Proskauer positive. Glucose is fermented to lactate and other products, but no gas (CO₂) is produced. In aged cultures and after an osmotic shock, rough cultures and long filamentous cells appear. In their filamentous form the Listeriae can measure more than 100 µm in length. *Listeria* is motile due to its peritrichous flagella at 20–25 °C, but not at 37 °C. The loss of flagella is necessary for full virulence. *Listeria monocytogenes*, pathogenic for humans and other animals, is responsible for listeriosis, an important foodborne infection.

The genus *Brochothrix* is mainly found in meat and is non-pathogenic. *B. thermosphacta* is one of the main spoilage bacteria in raw meat stored aerobically or packaged in a modified atmosphere. It also grows on vacuum-packed meat stored chilled. Growth of *B. thermosphacta* is in fact most efficient when the level of O₂ is reduced and CO₂ is present. It causes discolouration and off-flavours in meat and meat products by breaking down fat and protein. Slime and souring are the result.

4.1.2.2. Order Lactobacillales

Lactic acid bacteria are usually immobile and non-spore-forming. For their energy, they normally depend on the fermentation of sugars. They are facultative anaerobes, but some are considered aerotolerant.

Several members of the order Lactobacillales produce lactic acid as the main or only fermentation product. The most important members of this group are given in table 10.

Table 10. Characteristics of some important genera of the order Lactobacillales (Driche, 2024).

Family	Genera	Morphology	Metabolism	Characteristics
Lactobacillaceae		Rod-shaped, non-spore-forming	Facultative anaerobic, lactic acid fermentation	Widely used in yogurt, cheese, and other fermented foods. Known for health benefits as probiotics.
<i>Lactobacillus</i>				
<i>Leuconostoc</i>		Coccoid to rod-shaped, non-motile	Heterofermentative, CO ₂ and lactic acid production	Involved in fermentation of cabbage to sauerkraut, sourdough, and kefir. Enhances flavor and texture.
<i>Pediococcus</i>		Coccus, non-spore-forming, tetrads	Homofermentative, lactic acid production	Used in sausage fermentation, cheese, and sour beer production.
Streptococcaceae			Facultative anaerobic, lactic acid fermentation	Includes <i>S. thermophilus</i> for yogurt and cheese. Some species are pathogenic.
<i>Streptococcus</i>		Coccus, chains		
<i>Lactococcus</i>		Coccus, pairs or chains	Homofermentative, lactic acid production	Used in cheese making (e.g., cheddar, gouda).
Enterococcaceae		Coccus, pairs or short chains	Facultative anaerobic, lactic acid fermentation	Used in cheeses, indicators of water quality, potential pathogens.
<i>Enterococcus</i>				
Leuconostocaceae		Coccoid to rod-shaped, non-motile	Heterofermentative, lactic acid, acetic acid, ethanol, CO ₂ production	Involved in various fermentation processes, researched for probiotic properties.
<i>Weissella</i>				
Carnobacteriaceae		Rod-shaped, non-spore-forming, motile	Facultative anaerobic, lactic acid fermentation	Found in meat, dairy products; used for biopreservation and probiotics
<i>Carnobacterium</i>				

4.2. Gram-positive bacteria with high GC content: the Actinobacteria

Actinobacteria is one of the largest phylum under Bacteria domain and can be found in a wide range of aquatic and terrestrial ecosystems. Actinobacteria are Gram-positive bacteria with > 50% of guanine and cytosine (*G + C*) content in their DNA.

4.2.1. Morphological characteristics of actinobacteria

The morphology of actinobacteria shows mycelia or linear or branched hyphae that give them a resemblance to fungi, but unlike fungi and like bacteria the presence of muramic acid residues in their cell wall structure classifies these microbes as Gram-positive bacteria.

However, the fungal morphology distinguishes this group of microbes from other bacteria. Some species such as *Micrococcus*, are unicellular.

The biological cycle of many actinomycetes includes the development of filamentous cells, called hyphae, and spores. When they grow on a solid substrate such as soil or agar, actinomycetes develop a branched network of hyphae. These hyphae grow both on the surface and within the substrate, forming a dense mat of hyphae known as the vegetative mycelium (Figure 14).

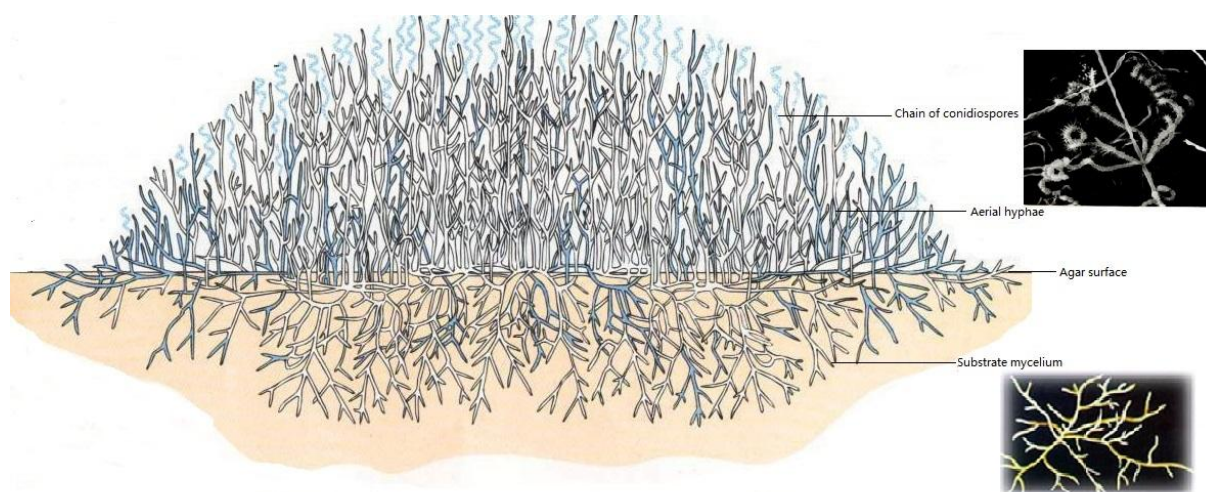


Figure 14. Actinomycetes colony growing on agar (common morphology of actinomycetes, the cross section of an actinomycete colony showing the substrate mycelium and aerial mycelium with chains of conidiospores) (Li et al., 2016).

Morphological characteristics are important for actinobacteria classification. Some factors such as the growth of substrate mycelium, the position and number of spores, the surface structure of spore, the shape of sporangia or conidia, and whether sporangiospore have flagella or not determine the classification (Figure 15).

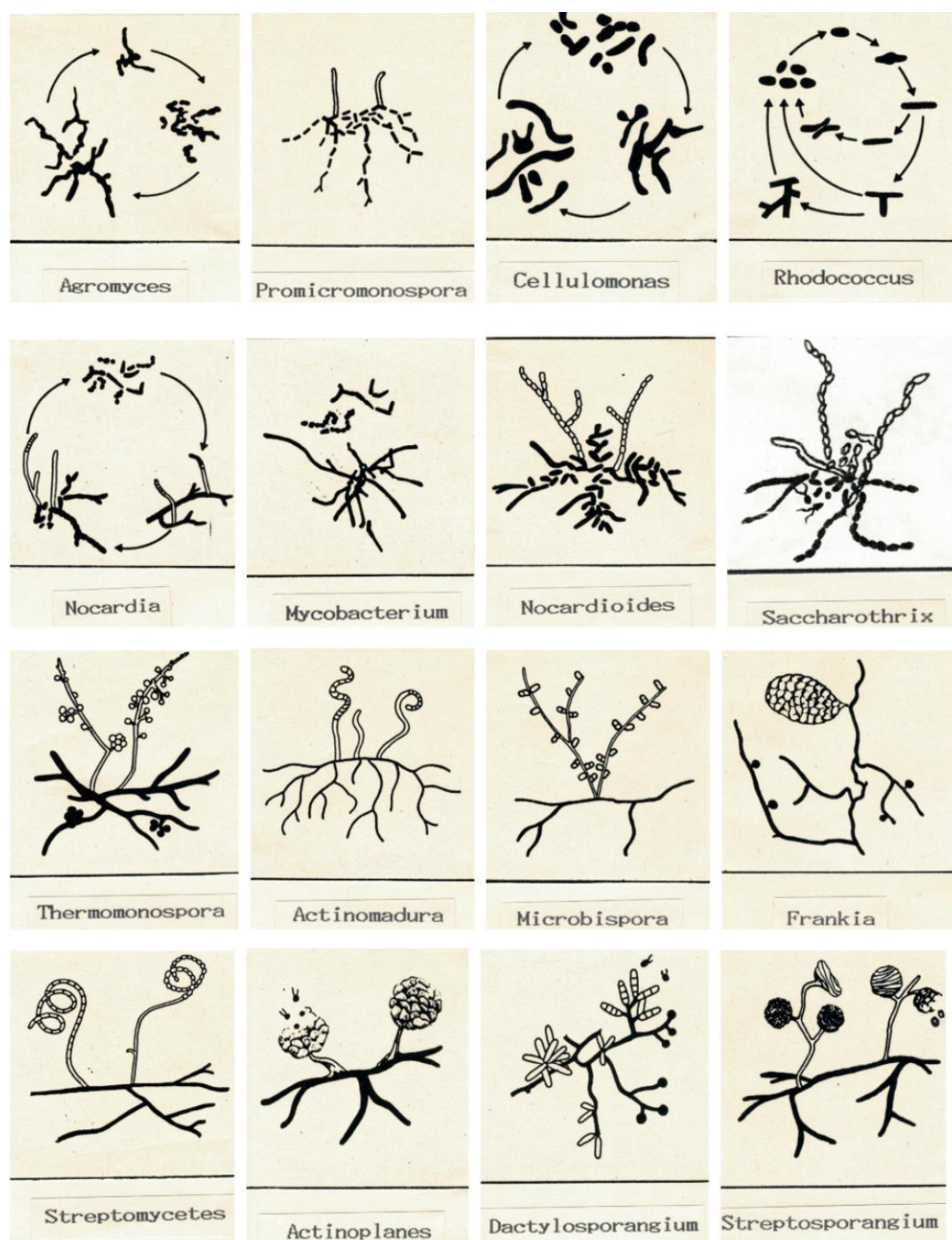


Figure 15. Diagram of some genera of Actinobacteria (Li et al., 2016).

4.2.2. The most common genera of Actinobacteria

➤ *Streptomyces*

These are the best-known actinomycetes and the most frequently isolated from soil. Species of the genus *Streptomyces* are strict aerobes. Most of them produce extracellular enzymes that enable them to utilize proteins, polysaccharides such as starch and cellulose, and many other

organic substances present in soil. These species are also highly valuable because they provide the majority of antibiotics produced on an industrial scale. In addition, *Streptomyces* species produce a characteristic gaseous compound called geosmin, which gives freshly turned soil its distinctive earthy, musty odor.

➤ ***Mycobacterium***

It belongs to the order Actinomycetales and constitutes the only genus of the family Mycobacteriaceae. They are slender, non-motile bacilli, aerobic, and do not produce endospores. The element myco refers to the fact that these bacteria sometimes exhibit filamentous outgrowths similar to those of fungi.

Their resistance to drugs and their pathogenicity are linked to the particular nature of their cell wall, which structurally resembles that of Gram-negative bacteria. The outer lipopolysaccharide layer is replaced by mycolic acids, which form a waxy, impermeable layer. As a result, these organisms become resistant to environmental stresses. They do not take up the Gram stain even though they are Gram-positive bacteria, but they are acid-alcohol-resistant (AARB / acid-fast bacilli).”

The most important pathogenic bacteria are *M. tuberculosis*, responsible for tuberculosis, and *M. leprae*, responsible for leprosy.

➤ ***Corynebacterium***

(Coryn = swollen, in the shape of a flask), they are generally pleomorphic. Their morphology varies depending on the age of the bacterium. The best-known species, *Corynebacterium diphtheriae*, is the causal agent of diphtheria. Non-pathogenic species of *Corynebacterium* are used for very important industrial applications. For example, *C. glutamicum*, whose name refers to its ability to produce glutamic acid under aerobic conditions, is used in the food industry as monosodium glutamate in the production of soy sauce and yogurt.

➤ ***Propionibacterium***

The genus contains pleomorphic rods that are non-motile and non-spore-forming, often shaped like clubs with one tapered end and one rounded end. Members of this genus are commonly found on the skin and in the intestine. They are important in cheese production and

in the development of acne.

➤ ***Frankia***

It induces the formation of nitrogen-fixing nodules in the roots of alder trees, similar to how *Rhizobium* causes nodule formation on the roots of legumes and fixes nitrogen.

➤ ***Nocardia***

Bacteria of the genus *Nocardia* have a morphology similar to that of Actinomyces bacteria, but they are aerobic. The cell wall has a structure similar to that of mycobacteria, so members of the genus *Nocardia* are often acid-alcohol-resistant. *Nocardia* species are commonly found in soil; some of them, including *Nocardia asteroides*, occasionally cause chronic pulmonary infections that are difficult to treat.

4.2.3. Applications of Actinobacteria

The phylum Actinobacteria represents the most recognized group of microorganisms with the ability to produce bioactive compounds. Thus, actinobacteria have received great interest in various applications in pharmaceuticals, biotechnology, food industries, agriculture and in the enzyme industry (Figure 16). Moreover, there is also interest to use Actinobacteria to assist in pollutant clean-up. Actinobacteria provides an economic and safe biological method for removal of contaminants as they can metabolize the contaminants for their growth.



Figure 16. Biotechnological applications of Actinobacteria (Ranjani et al., 2016).

4.2.4. Actinobacterial human and animal diseases

Actinobacteria have proved to be the causal agents of many human and animal infections, which include a number of common and intensively studied diseases, such as diphtheria, tuberculosis, and leprosy. There is also a wide range of infections that are less well known; some, like actinomycosis and nocardiosis, are proving to be more clinically significant than previously thought. In addition, it is becoming increasingly evident that Actinomyces play a role in the etiology of caries and periodontal disease.

5. Rickettsias

The Rickettsias are small rods with the fine structure typical of gram-negative eubacteria. The genus *Rickettsia* is an obligate intracellular parasite that enter the host cells by inducing phagocytosis, even by cells that are not normally phagocytic. Within host cells, the rickettsias reproduced by binary fission. They cling together in pairs after fission. Rickettsias are not motile as no flagella have been observed. They do not produce endospores. The organisms were considered to be the product of 'degenerative evolution' of bacteria as a result of

obligate intracellular parasitism. The DNA extracted from rickettsias is double stranded the organisms are capable of generating their own ATP as evidenced by oxidation of amino acid and glutamate.

The rickettsias cause several human diseases which are transmitted by arthropod vectors such as fleas, mites, lice and ticks. They are apparently not pathogenic to these arthropods but are pathogenic to man and other animals. Some diseases of man are fatal, the typhus fever which is epidemic caused by *R. prowazeki*, transmitted to man by body lice. The disease is characterized by abrupt severe ache, fever, and chills followed by rash on back and chest.

6. Chlamydiae

The phylum Chlamydiae contains one class, Chlamydiae, and a single order, Chlamydiales. It includes four families and only six genera. The genus *Chlamydia* is by far the most important and the best studied. The entire phylum consists of obligate intracellular parasites of eukaryotes (they must grow and reproduce inside their host cells).

Their pathogenic potential is well known, but many species can develop in protists and animal cells without causing harm. These hosts are considered a natural reservoir for Chlamydiae.

The growth cycle of Chlamydiae is shown in figure 17.

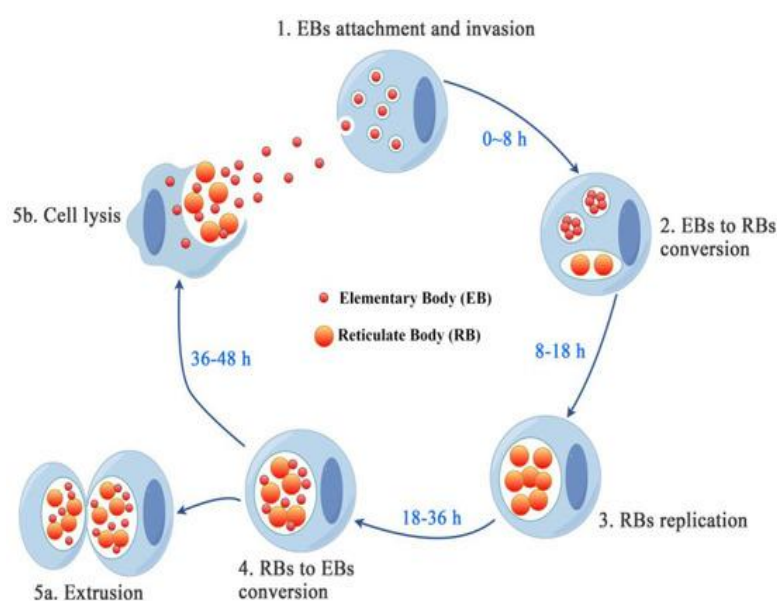


Figure 17. Chlamydia developmental cycle (Yang et al., 2024).

The cycle begins with the attachment of infectious elementary bodies (EBs) to host cells, followed by their invasion into the cells to form vacuoles. Within 8–18 h, EBs differentiate into non-infectious reticulate bodies (RBs) and utilize host cell nutrients for replication. Within 18–36 h, RBs re-differentiate into EBs. Within 36–48 h, EBs are released either through host cell lysis or extrusion, infecting adjacent cells and initiating a new round of development.

The chlamydial cell has a Gram-negative cell wall structure consisting of an outer membrane and an inner cytoplasmic membrane. Despite being sensitive to penicillin and containing penicillin-binding proteins, chlamydiae lack measurable muramic acid, a structure found exclusively in peptidoglycan.

Different kinds of pathogenic Chlamydia infect different hosts and cause different types of diseases. Three Chlamydia species are pathogenic to humans: *Chlamydia trachomatis* (*C. trachomatis*), *Chlamydia psittaci* (*C. psittaci*), and *Chlamydia pneumoniae* (*C. pneumoniae*). *C. trachomatis* is the leading bacterial cause of sexually-transmitted infections and trachoma worldwide. *C. psittaci* primarily affects psittacine birds and can be transmitted to humans causing psittacosis. *C. pneumoniae* is a major cause of respiratory tract diseases (community-acquired atypical pneumonia, bronchitis, pharyngitis, and sinusitis). Some distinguishing characteristics of these species are presented in table 11.

Table 11. Distinguishing characteristics of *Chlamydia* species (Rours et al., 2009)

	<i>C. trachomatis</i>	<i>C. pneumoniae</i>	<i>C. psittaci</i>
host	humans, mice	humans	rarely in humans, birds, mammals,
clinical disease	see page 34	pneumonia, asthma, endocarditis, arthritis	pneumonia
folate biosynthesis	yes	no	no
inclusion staining	iodine+	iodine-	iodine-
inclusion morphology	oval, granular, vacuolar	oval, dense	variable, dense, lucent
elementary body shape	coccoid	pear-shaped	coccoid
DNA homology	10	100	10

7. Mycoplasmas

Members of the class Mollicutes, commonly called mycoplasmas, are characterized by the complete absence of a cell wall and are incapable of synthesizing peptidoglycan or its precursors.

7.1. Taxonomy of mycoplasmas

The class Mollicutes is divided into 4 orders, 5 families, and 8 genera. The best studied genera are found in the orders: Mycoplasmatales (*Mycoplasma*, *Ureaplasma*), Entomoplasmatales (*Entomoplasma*, *Mesoplasma*, *Spiroplasma*), Acholeplasmatales (*Acholeplasma*), Anaeroplasmatales (*Anaeroplasma*, *Asteroleplasma*) (Table 12).

Table 12. Taxonomy and characteristics of genera of class *Mollicutes* (Mayla, 2010)

Order	Family	Genus	No. of taxa	Cholesterol requirement	Phenotypic characteristics	Habitat
1-Mycoplasmatales	Mycoplasmataceae	<i>Mycoplasma</i>	107	Yes	Opt.growth:37°C(Except some species isolated from cold-blooded vertebrates)	Human, animals
		<i>Ureaplasma</i>	7	Yes	Urease positive	Human, animals
2-Entomoplasmatales	Entomoplasmataceae	<i>Entomoplasma</i>	6	Yes	Opt.growth:30°C	Insects, plants
		<i>Mesoplasma</i>	12	No	Opt.growth:30°C	Insects, plants
	Spiroplasmataceae	<i>Spiroplasma</i>	34	Yes	Helical morphology	Insects, plants
3-Acholeplasmatales	Acholeplasmataceae	<i>Acholeplasma</i>	14	No	Opt. growth: 30-37°C	Animals, plant surfaces
		<i>Phytoplasma</i>	Uncultured <i>in vitro</i>			Insects, plants
4-Anaeroplasmatales	Aneroplasmataceae	<i>Anaeroplasma</i>	4	Yes	Obligate anaerobes	Bov. Ov. rumen
		<i>Asteroleplasma</i>	1	No	Obligate anaerobes	Bov. Ov. rumen

7.2. General characteristics of mycoplasma

- They are very small, non-motile (except *Spiroplasma*) and prokaryotic which may be parasitic or saprophytic.
- They lack cell wall and the outer boundary of the cells being the cytoplasmic membrane which is three layered unit membrane structure.

- Cells are non-motile but gliding motility has been observed in a few species.
- They do not show response towards Gram staining i.e. they are Gram negative in nature.
- Their cells possess plasticity (pleomorphic) and can assume various shapes ranging from spheres to branched filaments.
- The plasticity allows the cells to pass through bacteriological filters even though the smallest cells are about 0.3 μm in diameter.
- They require sterols for their growth. They are sensitive to supersonic vibrations desiccations and most of the physical environment factors.
- They are susceptible to lysis by osmotic shocks caused by sudden dilution of the medium with water.
- The colony of mycoplasma on solid agar medium appears as just like fried egg under stereo microscope. The colony shows spherical or hemispherical portion in the center, which is surrounded by surface growth towards periphery. The typical colony is biphasic with a fried egg appearance (characterized by opaque, granular central area with a translucent peripheral zone) (Figure 18).



Figure 18. Typical fried-egg colony morphology of the mollicute-like organism from *Scylla serrata* (Chen et al., 2011).

7.3. Parasitism of mycoplasma

Mycoplasmas are widespread in nature as parasites of humans, mammals, reptiles, fish, arthropods and plants. The list of hosts known to harbor mycoplasmas is continuously increasing, as does the number of established mollicutes species (Figure 19).

Taxonomy

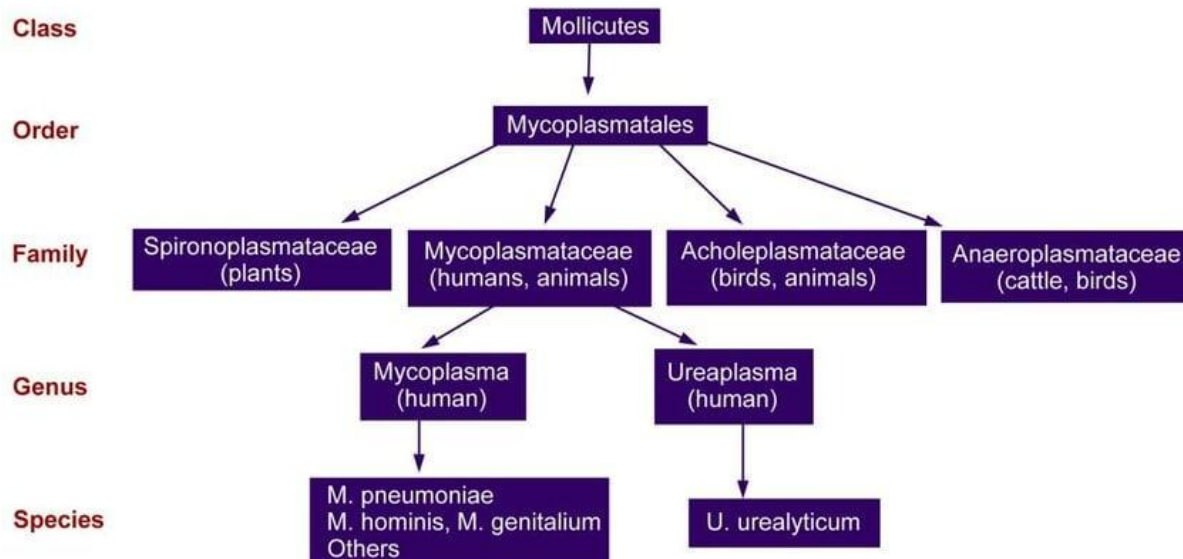


Figure 19. Show classification of *Mycoplasma* parasites (Mayla, 2010).

The study of *Mycoplasma* has become important in the understanding of chronic diseases. As both an extracellular and intracellular pathogen, a better understanding of the virulence mechanisms of *Mycoplasma* will provide fresh understanding of how to diagnose and combat this pathogen.

Due to their fastidious habit, usually there are no free-living mycoplasmas; most of them are strictly parasites. They parasitize a wide range of organism including humans, plants, animals, and insects. *Mycoplasma* grows very slowly, even under perfect conditions, with a generation time ranging up to nine hours in some species. They also have a very long lag phase, so it may take an entire week before colonies become visible on agar plates. Due to their degraded genome, and inability to perform basic functions, *Mycoplasma* relies on their host for much of their nutrition.

Many species of *Mycoplasma* are commensal, living innocuously with their host as part of the natural flora of the body, and having no detrimental effects. But when *Mycoplasma* acts as pathogens they cause chronic but mild infections that rarely, if ever, kill their host. They are usually surface parasites, although several species, including the aptly named *M. penetrans*, are intracellular pathogens.

Mycoplasma species are often found in research laboratories as contaminants in cell culture. Mycoplasmal cell culture contamination occurs due to contamination from individuals or contaminated cell culture medium ingredients.

Chapter 6. Phylum Proteobacteria

The phylum Proteobacteria represents a major group of Gram-negative bacteria. It is currently considered the largest and most diverse group of bacteria, comprising more than 500 genera and over 5,000 species. Analyses of 16S rRNA sequences demonstrate their phylogenetic relatedness.

The morphology of these Gram-negative bacteria is highly variable, ranging from simple forms such as rods and cocci to genera exhibiting prosthecae, budding, and even fruiting bodies. Their physiological diversity is equally remarkable, as they include photoautotrophic, chemolithotrophic, and chemoheterotrophic bacteria.

Phylogenetic relationships among proteobacteria have been established based on rRNA sequence analyses. Comparison of 16S rRNA sequences has led to the classification of the phylum Proteobacteria into five classes (Figure 20):

- Alphaproteobacteria
- Betaproteobacteria
- Gammaproteobacteria
- Deltaproteobacteria
- Epsilonproteobacteria.

A sixth class was identified in 2007, namely the Zetaproteobacteria. This class currently includes a single species, *Mariprofundus ferrooxydans*.

Phenotypic groups within the *Proteobacteria* include Gram-negative aerobic or microaerophilic rods and cocci; anaerobic straight, curved, and helical Gram-negative rods; anoxygenic phototrophic bacteria; non-photosynthetic, non-fruiting, gliding bacteria; aerobic chemolithotrophic bacteria and associated genera; facultatively anaerobic Gram-negative rods; budding and/or appendaged nonphototrophic bacteria; aerobic/microaerophilic, motile, helical/vibrioid Gram-negative bacteria; symbiotic and parasitic bacteria of vertebrate and

invertebrate species; fruiting, gliding bacteria; sheathed bacteria; and nonmotile or rarely motile curved Gram-negative bacteria.

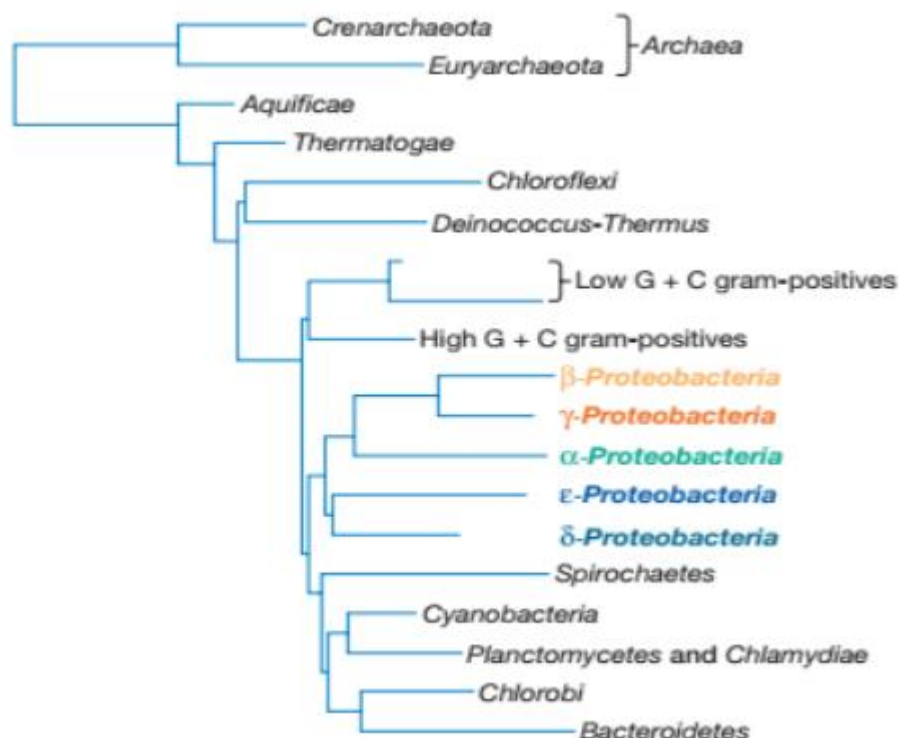


Figure 20. Phylogenetic relationships among the Prokaryotes (The Proteobacteria are highlighted).

1. Alphaproteobacteria

Alphaproteobacteria harbor a wide range of habitats, various kinds of metabolic processes, and cellular phenotypes. It includes mostly phototrophic genera (*Rhodobacter*), plant and animal pathogens (*Agrobacterium*, *Brucella* and *Rickettsia*), symbiotic relationship with plants (*Rhizobium*, *Azorhizobium*, *Mesorhizobium*, and *Sinorhizobium*). These groups are widely distributed in various environmental conditions such as volcanic eruptions, soil, symbiotic relationship with plant roots, and to survive on the surface of the ocean ecosystem. It is widely distributed in marine ecosystems which can contribute up to 10% of the microbial community. Members of genera such as *Rickettsia* and *Brucella* are important pathogens; in fact, *Rickettsia* is an obligate intracellular parasite. Many genera are characterized by distinctive morphology such as prosthecae.

1.1. Order Caulobacterales

The order *Caulobacterales* comprises predominantly aquatic bacteria characterized by a distinctive cellular morphology, notably the presence of a specialized appendage known as a **prostheca**, which contributes to surface attachment and nutrient uptake.

Caulobacters are aquatic, oligotrophic, prosthecate bacteria that occur widely in waters of low nutrient flux (clean freshwater lakes, open ocean) or content (drinking water, distilled water). Although their populations are typically sparse, they can often be detected in natural samples attached to algae and cyanobacteria. They are readily recognizable by the unique morphology of the dividing cell, which bears a flagellum at one pole and an outgrowth of the cell envelope – the stalk – at the opposite pole. Completion of fission separates the motile swarmer from its nonmotile sibling, which remains with the substratum to which the dividing cell is typically attached. The stalk provides several demonstrable properties (adhesion, nutrient uptake, and buoyancy) that enhance competitiveness of these bacteria as nutrient scavengers in low-nutrient environments, and dispersal as swimmers reduces competition between siblings. Investigation of development in the model species *Caulobacter crescentus* has revealed the elaborate sequence of protein synthesis, activation, localization, and destruction involved in accomplishing the alternation of swarmer and stalked stages by regulating the events at each caulobacter pole produced by cell fission. The genetic commitment of caulobacters to their unique cell cycle implies the importance of their dimorphic development to their competitiveness in oligotrophic habitats.

1.2. Order Rhizobiales

The order *Rhizobiales*, within the class Alphaproteobacteria, comprises 11 families exhibiting a wide diversity of phenotypes. One of the most important families within this order is Rhizobiaceae, which includes two well-known aerobic genera: *Rhizobium* and *Agrobacterium*.

Members of the genus *Rhizobium* are motile rod-shaped bacteria, typically measuring 0.5–0.9 × 1.2–3.0 µm. Under unfavorable environmental conditions, they may become pleomorphic.

Their cells often contain poly- β -hydroxybutyrate (PHB) inclusions, which serve as carbon storage compounds.

Rhizobium species are notable for their ability to establish symbiotic relationships with leguminous plants, where they inhabit the cells of root nodules in the form of specialized structures known as bacteroids. Within these nodules, the bacteria perform biological nitrogen fixation, converting atmospheric nitrogen (N_2) into ammonium (NH_4^+), a form readily assimilable by the plant.

Leguminous plants represent one of the most successful plant groups on Earth, with over 18,000 species. Their ecological success is largely attributed to their ability to form mutualistic associations with nitrogen-fixing bacteria, which enhance soil fertility and promote plant growth.

The genus *Agrobacterium*, a member of the family Rhizobiaceae, differs from *Rhizobium* by its phytopathogenic nature, as it infects the crown, roots, and stems of various plants and induces the transformation of normal plant cells into autonomously proliferating tumor cells. The most studied species, *Agrobacterium tumefaciens*, typically enters host plants through wounds, particularly in broad-leaved species, and causes crown gall disease.

In addition, the order *Rhizobiales* includes the family Brucellaceae, which comprises the genus *Brucella*, known for its role as a pathogen of humans and animals, especially livestock. *Brucella* species are responsible for brucellosis, also called undulant fever or Malta fever, a zoonotic disease transmitted from animals to humans.

2. Betaproteobacteria

Betaproteobacteria consists of various kinds of aerobic or facultative bacteria which includes 75 genera and 400 species of bacteria. It contains chemolithotrophic genera (e.g., ammonia-oxidizing *Nitrosomonas*) and some genera of phototrophs (*Rubrivivax* and *Rhodocyclus*). Most of the species of β -proteobacteria are heterotrophic and their energy is derived from organic compounds. It plays a major role in nitrogen (N) fixation in various

plants, oxidation of ammonium to nitrite and nitrate which is an important chemical reaction for plant growth and biogeochemical cycle. The class Betaproteobacteria currently comprises seven orders and 12 families. Two genera are important human pathogens: *Neisseria* and *Bordetella*.

2.1. Order Neisseriales

The order *Neisseriales* comprises a single family, Neisseriaceae, which includes approximately 15 genera. The most well-known and extensively studied genus is *Neisseria*. Members of this genus are Gram-negative cocci, strictly aerobic and non-motile, typically arranged in pairs (diplococci) with flattened adjacent sides. Some species may possess capsules and fimbriae, contributing to their virulence.

Neisseria species are chemoorganotrophic and characteristically oxidase-positive and catalase-positive. These bacteria commonly inhabit the mucous membranes of mammals, and several species are pathogenic to humans. Notably, *Neisseria gonorrhoeae* is the causative agent of gonorrhea, while *Neisseria meningitidis* is responsible for bacterial meningitis.

2.2. Order Burkholderiales

The order *Burkholderiales* includes **four families**, three of which contain well-known genera. The genus *Burkholderia*, belonging to the family **Burkholderiaceae**, is one of the most important representatives.

Members of the genus *Burkholderia* are Gram-negative, aerobic, non-spore-forming rods, typically mesophilic and non-fermentative. With the exception of one species, they are motile, possessing either a single polar flagellum or a tuft of polar flagella. These bacteria are catalase-positive and often oxidase-positive. Many species are capable of accumulating poly- β -hydroxybutyrate (PHB) as a carbon storage compound.

2.3. Order Nitrosomonadales

The order *Nitrosomonadales* includes numerous chemolithotrophic bacteria, which derive energy from the oxidation of inorganic compounds. Among them is the stalked bacterium *Gallionella*.

The family Nitrosomonadaceae contains important nitrifying bacteria, such as *Nitrosomonas* and *Nitrospira*, which play a key role in the nitrogen cycle by oxidizing ammonium (NH_4^+) to nitrite (NO_2^-). These organisms are essential for environmental nitrogen transformation processes.

2.4. Order Hydrogenophilales

The order *Hydrogenophilales* is relatively small but includes important genera such as *Thiobacillus* and *Acidithiobacillus*, which are among the most studied chemolithotrophic sulfur-oxidizing bacteria.

These bacteria are generally unicellular, with rod-shaped or spiral morphologies, and are capable of oxidizing reduced sulfur compounds. They may be non-motile or motile via flagella. Members of this group play a significant role in biogeochemical sulfur cycling and are also involved in industrial and environmental processes.

3. Gammaproteobacteria

Gammaproteobacteria is defined as a diverse class of bacteria that includes human pathogens such as *Pseudomonas aeruginosa*, *Salmonella*, and *Vibrio*, as well as genera involved in methane oxidation and symbiotic relationships with marine organisms. This group is notable for its applications in medical, industrial, and environmental fields. Members of Class Gamma Proteobacteria share a similarity in their ribosomal RNA, and, like all subclasses of Class Proteobacteria, they are Gram negative. Otherwise, the Gamma proteobacteria have widely divergent properties: some are aerobic, others not; some are rods, some are cocci; some have a short curve. Others are spiral-shaped, some cells are small, and others are large.

The exceptional diversity of Gammaproteobacteria is highlighted in *Bergey's Manual*, where they are classified into 14 orders and 28 families.

3.1. Order Thiotrichales

The order *Thiotrichales* comprises three families, the largest of which is the family Thiotrichaceae. Members of this family include several genera capable of oxidizing sulfur compounds. From a morphological perspective, they exhibit rod-shaped as well as filamentous forms.

Among the most extensively studied genera within this family are *Beggiatoa* and *Leucothrix*. *Beggiatoa* species are microaerophilic and typically inhabit sulfide-rich environments, such as sulfur springs, freshwater containing decomposing organic matter, rice paddies, salt marshes, and marine sediments.

In contrast, *Leucothrix* is an aerobic chemoorganotroph that forms long filaments (trichomes). It is predominantly found in marine environments and attaches to solid substrates by means of a holdfast structure.

3.2. Order Pseudomonadales

The order *Pseudomonadales*, defined based on 16S rRNA gene phylogeny, comprises two main families: *Pseudomonadaceae* and *Moraxellaceae*. Members of this order are generally Gram-negative, aerobic chemoorganotrophs with a predominantly respiratory metabolism. Many species are motile by means of flagella. These bacteria are widely distributed in soil, water, and host-associated environments, and they display remarkable metabolic diversity. The family *Pseudomonadaceae* includes the well-known genus *Pseudomonas*, while *Moraxellaceae* comprises genera such as *Moraxella*, *Acinetobacter*, and *Psychrobacter*, many of which are of medical and environmental importance.

3.3. Order Vibrionales

Gram-negative straight or curved rods. Motile by means of polar flagella. Additional lateral flagella may be produced when grown on solid media; these differ in wavelength and antigenicity from the polar flagellum and may number from a few to over 100 flagella/cell. Do not form endospores or microcysts. Chemoorganotrophs. Facultative anaerobes, having both a respiratory and a fermentative metabolism. Oxygen is a universal electron acceptor. Do not denitrify. Most strains: are oxidase positive, reduce nitrate to nitrite, ferment d-glucose and utilize it as a sole or principal source of carbon and energy, grow in minimal media with d-glucose or other compounds as the sole source of carbon and energy and use NH₄ as the sole nitrogen source. A few species require vitamins and amino acids. Ferment and utilize a wide variety of simple and complex carbohydrates and utilize a wide variety of other carbon sources. Most species require Na or a seawater base for growth and require 0.5–3% NaCl for optimum growth. Several species are bioluminescent (*Vibrio fischeri*, *V.harveyi* and *photobacterium phosphoreum*); other species include a few bioluminescent strains. Primarily aquatic. Found in fresh, brackish, and sea water, often in association with aquatic animals and plants. Several species are pathogenic for humans (*Vibrio cholerae* is the causative agent of **cholera**). Other species are pathogenic for fish, eels, and other aquatic animals (*Vibrio anguillarum*).

3.4. Order Enterobacterales

The Enterobacteriaceae are Gram-negative rod-shaped bacteria which are typically motile by virtue of peritrichous flagella, are non-sporing, facultative anaerobes which will grow in the presence of bile salts. They are oxidase negative, catalase positive, reduce nitrate and almost all ferment glucose often with the formation of acid and gas.

The Enterobacteriaceae include common human pathogens and are widely distributed in the environment, being found in the soil and water, on plants, and in the intestines of animals. They can be saprophytes, opportunist pathogens, zoonotic pathogens or primary human pathogen. Important genera and species of the Enterobacteriaceae are summarized in table 13.

Table 13. Selected important genera of the Enterobacteriaceae (Tortora et al., 1995).

Genera	Species	Significance
Escherichia	<i>E. coli</i>	One of the most common intestinal tract inhabitants; most familiar organism in microbiology; presence in food or water indicates faecal contamination; common cause of traveller's diarrhea through the production of enterotoxins
Salmonella		Common inhabitants of animal intestinal tracts, particularly poultry and cattle; cause of salmonellosis, one of the most common foodborne illnesses
Shigella	<i>S. typhi</i>	Cause of Typhoid fever
Klebsiella	<i>K. pneumoniae</i>	Found only in humans; causes bacillary dysentery or shigellosis; can cause traveller's diarrhoea
Serratia	<i>S. marcescens</i>	Major cause of septicemia in hospital pediatric wards; causes one form of pneumonia in chronic alcoholic and patients with compromised immune systems
Proteus		Important cause of nosocomial infections of the urinary and respiratory tracts.
Yersinia	<i>Y. pestis</i>	Implicated in urinary tract and wound infection and infant diarrhoea
Enterobacter	<i>E. cloacae</i>	Cause of plague, the Black Death; transmitted from urban rats and ground squirrels usually by fleas
Erwinia	<i>E. aerogenes</i>	Urinary tract and nosocomial infections; widely distributed in humans, animals, soils, water and sewage
		Plant pathogens

4. Deltaproteobacteria

The Deltaproteobacteria comprise a branch of predominantly aerobic genera. Deltaproteobacteria includes the fruiting-body-forming Myxobacteria which release myxospores under unfavorable environmental conditions. Deltaproteobacteria include a branch of strictly anaerobic genera, which contains most of the known sulfate- (*Desulfovibrio*, *Desulfobacter*, *Desulfococcus*, *Desulfonema*, etc.) and sulfur-reducing bacteria (e.g., *Desulfuromonas spp.*). The class Deltaproteobacteria is currently subdivided into eight orders comprising a total of 20 families.

4.1. Orders Desulfovibrionales, Desulfobacteriales, and Desulfuromonadales

The orders *Desulfovibrionales*, *Desulfobacteriales*, and *Desulfuromonadales* comprise a diverse group of sulfate- and sulfur-reducing bacteria (SRB). These bacteria are grouped together due to their strictly anaerobic metabolism and their ability to use elemental sulfur and

other oxidized sulfur compounds as electron acceptors during anaerobic respiration. The reduction of sulfate to sulfide involves a multi-step biochemical process, which is central to their energy metabolism and ecological function in sulfur and carbon cycles.

4.2. Order Bdellovibrionales

The order *Bdellovibrionales* consists of a single family, *Bdellovibrionaceae*, encompassing four genera. The genus *Bdellovibrio* is composed of Gram-negative, curved rods, which are aerobic and possess polar flagella for motility. Members of this genus are known for their predatory lifestyle, preying on other Gram-negative bacteria.

4.3. Order Myxococcales

Myxobacteria are unicellular, rod-shaped, Gram-negative eubacteria that exhibit gliding motility and are widely distributed in both terrestrial and aquatic environments. Phylogenetic studies suggest that they have evolved in parallel with purple bacteria. These bacteria are notable for their unique developmental cycle, which includes coordinated surface movement, aggregation of vegetative cells into slimy multicellular masses, and the formation of fruiting bodies that produce myxospores.

Myxobacteria can produce various bioactive compounds such as anti-viral, anti-bacterial, anti-fungal, anti-cancer and anti-parasitic compounds.

5. Epsilonproteobacteria

The Epsilonproteobacteria is the smallest class within the phylum Proteobacteria and has been primarily recognized as a group of pathogenic microorganisms. However, nonpathogenic Epsilonproteobacteria has been increasingly of great biological importance. They represent bacteria with the highest metabolic versatility. They all are slender gram-negative rods, which can be straight, curved, or helical.

This class comprises a single order, the *Campylobacterales*, and three families: *Campylobacteraceae*, *Helicobacteraceae*, and the more recently described *Nautiliaceae*.

However, recent phylogenetic analyses based on small-subunit rRNA (SSU rRNA) gene sequences from both cultured and uncultured organisms have proposed a revised classification into two orders: *Campylobacterales* (including the families *Campylobacteraceae*, *Helicobacteraceae*, and *Hydrogenimonaceae*) and *Nautiliales* (including the genera *Nautilia*, *Caminobacter*, and *Lebetimonas*).

5.1. Ordre Campylobacterales

The order *Campylobacterales* comprises Gram-negative, spiral or curved bacteria, typically motile by means of one or more polar flagella. These microorganisms are generally microaerophilic, requiring reduced oxygen levels for optimal growth, and some species exhibit thermophilic characteristics, growing best at elevated temperatures.

Members of this order are widely distributed in animal hosts, particularly in the gastrointestinal tract. Several species are of significant medical importance due to their pathogenic potential. For example, *Campylobacter jejuni* is one of the leading causes of bacterial gastroenteritis worldwide, while *Helicobacter pylori* is associated with gastric ulcers and stomach cancer.

These bacteria are therefore of major interest in both medical microbiology and public health, due to their role in foodborne and chronic infections.

Chapter 7. Archae

Archaea are unicellular prokaryotic microorganisms, which at a first glance resemble bacteria in that they have no nuclear compartment or complex endomembrane systems, and have circular genomes encoding genes lacking spliceosomal introns that are often co-located in operons and co-expressed. Surprisingly however, the machineries that archaea use to replicate and express genetic information, i.e., DNA replication, transcription, and translation, are evolutionarily more closely related to those found in eukaryotes than they are to those in bacteria. At the same time, archaea also share a set of common cellular hallmarks that separate them from both eukaryotes and bacteria. These features include distinct membranes made of lipids composed of isoprenoid chains ether-linked to a glycerol-1-phosphate backbone, a unique motility apparatus called the archaellum, and unique types of metabolism, like methanogenesis.

1. Archaeal Cell Structure

The basic cell morphology of archaea is comparable to that of bacteria and varies from rods, cocci, spirals to irregular shaped cells. The total cell volume ranges from 0.5 to 2.5 μm^3 , which is ideal for maximizing the surface area-to-volume ratio, optimizing the exchange of nutrients and reaction products via diffusion.

Coccoid cells are found within the Desulfurococcales, whereas lobed cocci are characteristic for members of the Sulfolobales as well as for *Nitrososphaera viennensis*. Members of the genera *Thermoproteus*, *Pyrobaculum* (Crenarchaeota) and *Methanothermus* (Euryarchaeota) depict short rods with a length in the range of usually 2–5 μm . In contrast, rod-shaped *Thermofilum* cells can exhibit a length of up to 100 μm with a diameter of just 150 nm. *Methanospirillum* (as the name already indicates) as well as *Methanosaeta* form long spiral chains. *Picrophilus*, *Hyperthermus* or the ultraflat cells typically found in *Pyrodictium* species are characterized by pleomorphic cell shapes. *Haloquadratum walsbyi*, is remarkable in that it forms an almost perfect quadrate flattened cell. Different archaeal cell structures and morphologies are given in Figure 21.

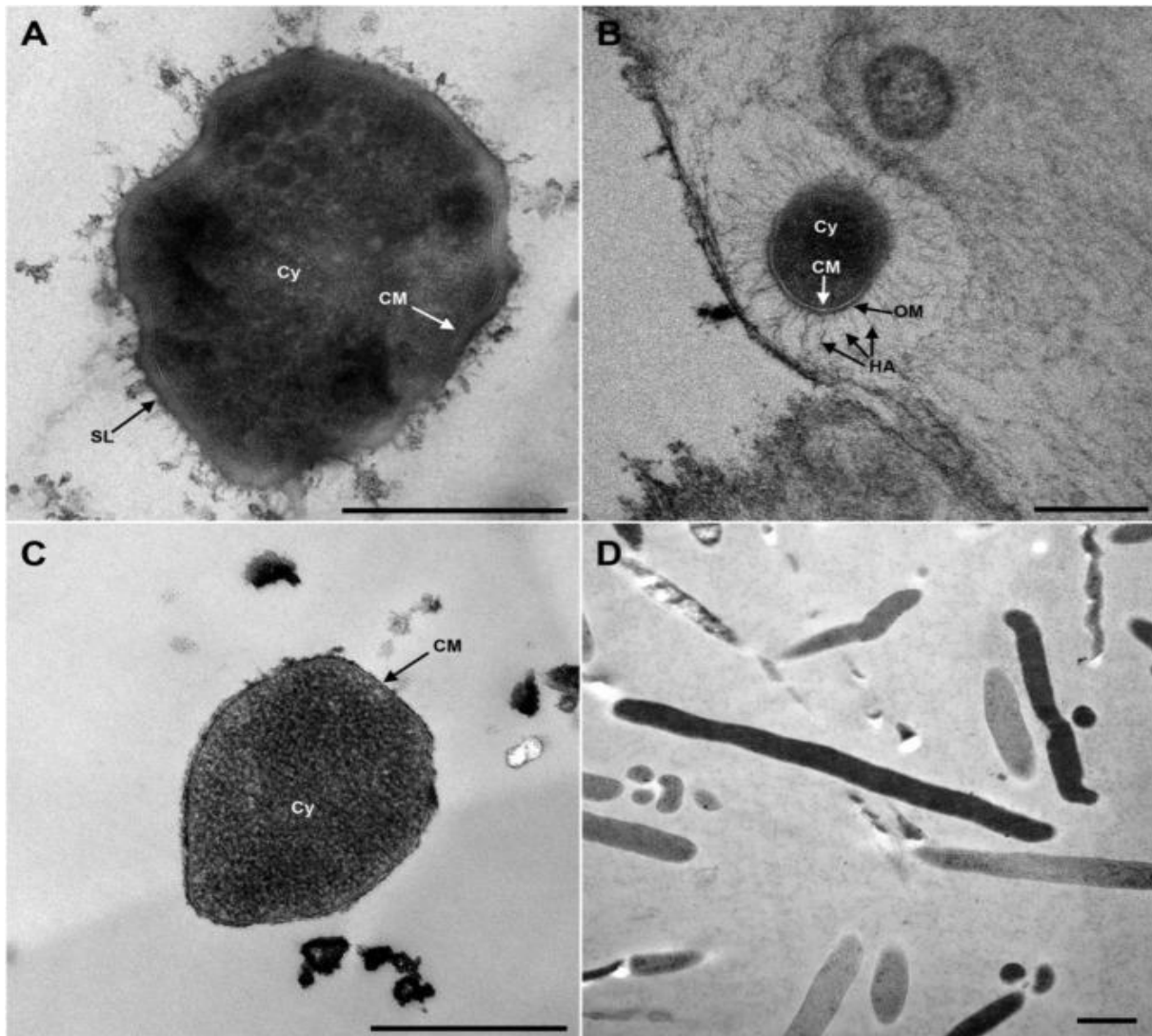


Figure 21. Transmission electron micrographs of ultrathin sections of different archaeal cells and structures. (A) Lobed, coccoid cell of thermoacidophilic *Sulfolobus metallicus*. The cell is surrounded by a cytoplasmic membrane and a S-layer. (B) A biofilm of *Cand. Altiarchaeum hamiconexum*. The cells are typically surrounded by a double membrane and reveal numerous appendages called hami. (C) Irregular, coccoid *Thermoplasma acidophilum* does not possess a cell wall outside the cytoplasmic membrane. (D) Rod-shaped cells of the methanogen *Methanothermobacter thermautotrophicus*. CM, cytoplasmic membrane; Cy, cytoplasm; HA, hami; OM, outer membrane; SL, S-layer. Scale bars: 500 nm (Blohs, 2019).

The cell wall is present in most archaea except *Thermoplasma* and *Ferroplasma*. The surface-layer proteins encoded constitute the cell wall or S-layer. The role of the S-layer or cell wall in archaea is for physical and chemical protection. While bacterial cell walls are made up of peptidoglycan, archaea cell walls lack it. They rather possess pseudo-peptidoglycan like in *Methanobacteriales*.

Pseudopeptidoglycan is similar to bacterial peptidoglycan (morphologically, functionally) but is chemically distinct (no D-amino acids & N-acetylmuramic acid). Rather, they have N-Acetylalosaminuronic acid (Figure 22).

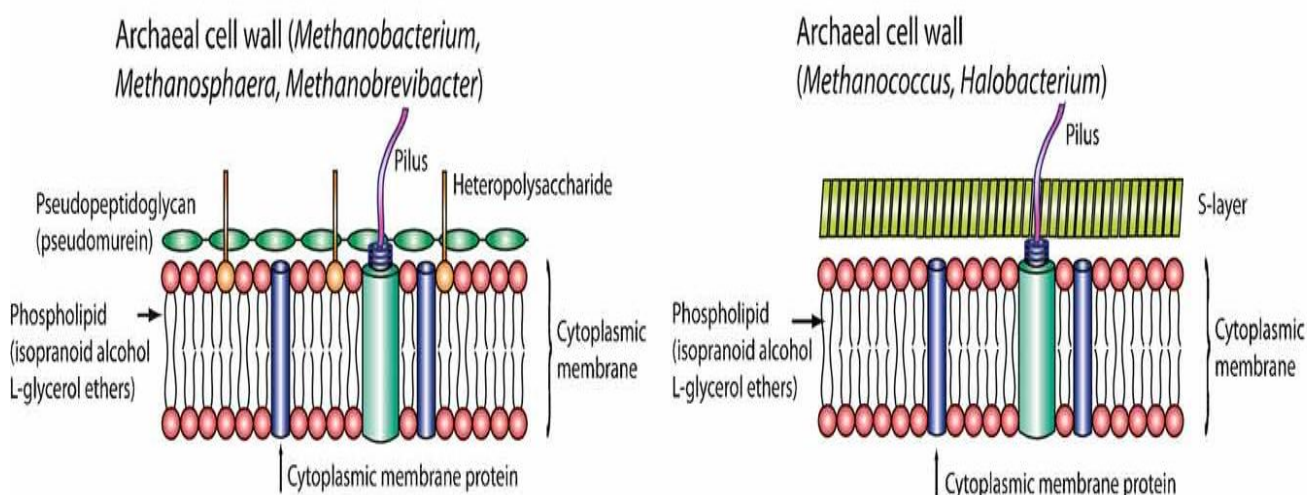


Figure 22. Archaeal cell wall (Gill and Brinkman, 2011)

Similar to bacteria, Archaea possess a variety of cell appendages. Although archaea do not encode flagella homologous to those of bacteria or eukaryotes, they have a functional equivalent known as the **archaeal flagellum** or **archaellum**. Archaeal flagella are 10–14 nm in diameter and share significant structural similarity with bacterial and archaeal type IV pili. Additional cell appendages include pili or fimbriae, as well as specialized structures such as hami (grappling hook-like appendages), cannulae, and extracellular fibers.

2. Physiological characteristics of Archaea

Archaea exhibit a wide range of physiological characteristics that reflect their remarkable adaptability to diverse environments. These microorganisms display significant variation in their metabolic pathways, energy sources, and environmental tolerances. The following table summarizes the main categories of Archaea and their physiological features (Table 14).

Table 14. Physiological characteristics of Archaea (Aparici-Carratalá et al., 2023).

Condition (dependence)	Category	Characteristic
Temperature	Thermophiles	High optimum growth temperature, between 45 and 80°C.
	Hyperthermophiles	Optimal growth at very high temperatures >80°C.
	Psychrophiles	Low optimum growth temperature ≤15°C.
pH	Acidophiles	Growth in places with low pH ≤5.
	Alkaliphiles	Very high optimum pH values ≥9.
Other features	Halophiles	Environments of high salinity (3–4M NaCl).
	Piezophiles	Places of high pressure (>1 atm) for their development.
	Radiophiles	Survival to large amounts of radiation.
	Methanogens	Methane production as a byproduct of their energy metabolism.
	Metallophiles	Environments with high concentrations of heavy metals.

3. Habitats of Archaea

Although the term *Archaea* is often associated with “extremophiles,” these microorganisms actually inhabit a wide range of environments and account for more than 20% of the total biomass. They can be found in oceans and their sediments, freshwater lakes, soils, plant roots, peat bogs, as well as in the oral cavity and gastrointestinal tract of mammals. In these diverse habitats, Archaea represent a significant fraction ($\approx 10\%$) of the microbial community.

Their fame, however, stems from their ability to thrive and sometimes dominate the microflora within ecological niches considered inhospitable to life, where certain physicochemical factors reach extreme values. These include temperature (thermophilic Archaea: $>50^{\circ}\text{C}$; psychrophilic: $<15^{\circ}\text{C}$), pressure (piezophilic Archaea: >1000 bar), pH (acidophilic Archaea: <7 ; alkaliphilic: >7), and salinity (halophilic Archaea: $[\text{NaCl}] > 2.5$ M).

For example, Archaea are present in the hot springs of Yellowstone National Park, hydrothermal vents, acidic waters, the Dead Sea, salt crystals, polar regions, and permafrost (Figure 23).

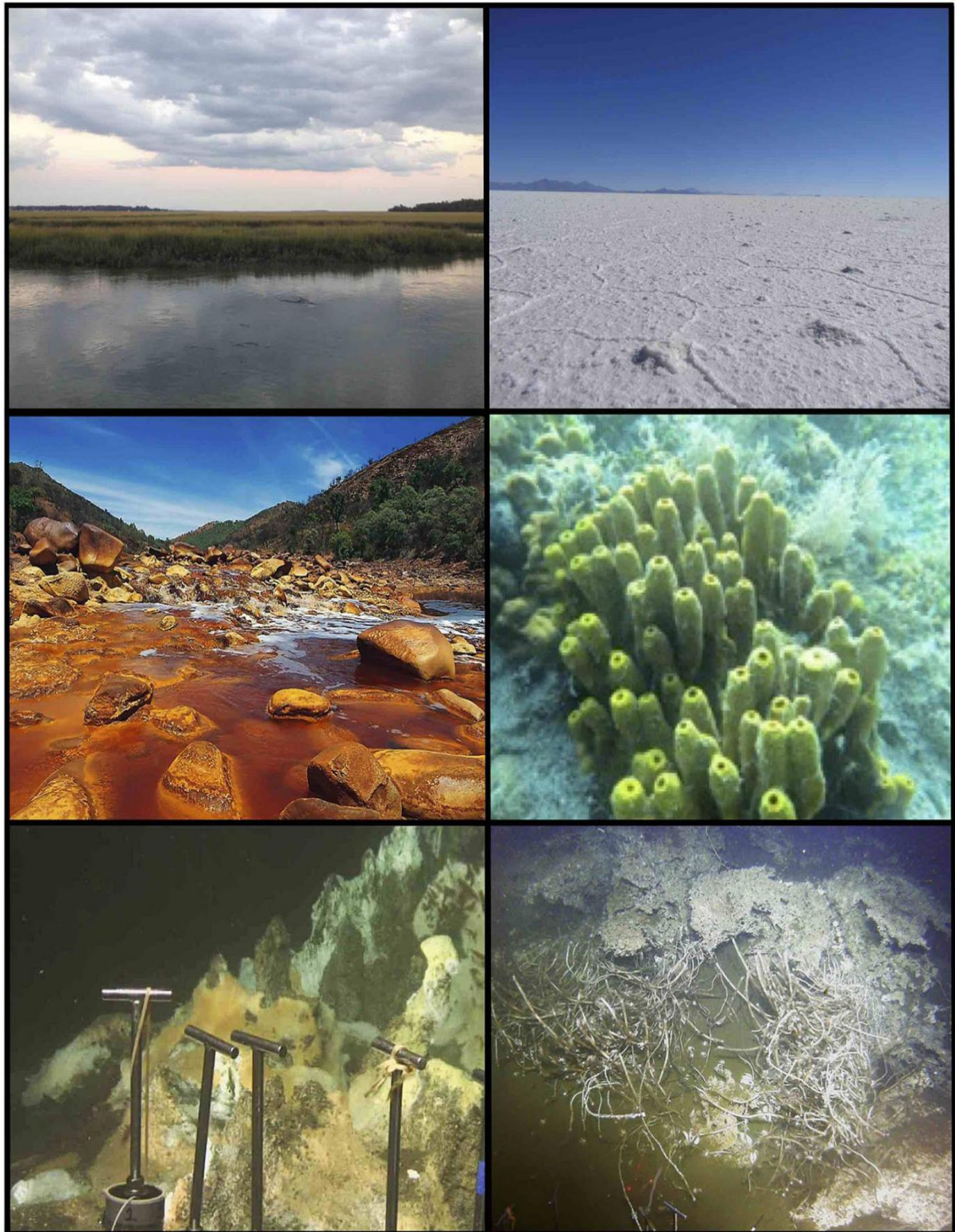


Figure 23. Diverse archaeal habitats (Teske et al., 2021).

4. Potential role in human diseases

Members of Archaea were viewed as extremophiles and thus foreign to the human habitat and microbial ecosystem. Current data suggest that archaea are able to colonize and survive in humans. However, no concerted efforts have been undertaken to implicate archaea in human disease.

5. Classification of Archaea

Archaea are divided into five phyla: Euryarchaeota, Nanoarchaeota, Korarchaeota, Crenarchaeota, Thaumarchaeota

The second edition of Bergey's Manual divides the Archaea into two phyla Euryarchaeota and Crenarchaeota, which contain the largest number of taxa. The three other phyla are poorly studied.

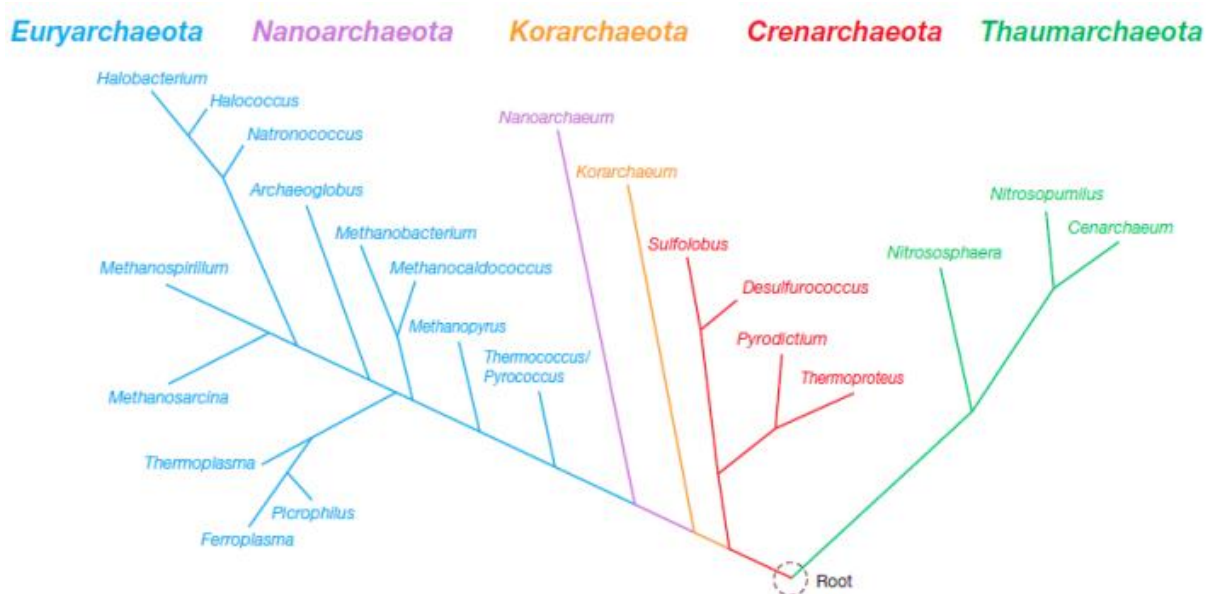


Figure 24. Detailed phylogenetic tree of the Archaea based on comparisons of ribosomal proteins from sequenced genomes (Eranga, 2015).

5.1. Euryarchaeota

The phylum Euryarchaeota includes five main groups: methanogens, halobacteria, thermoplasmas, extreme thermophiles that reduce sulfur, and sulfate-reducing Archaea.

5.1.1. Methanogenic Archaea

They are strict anaerobes that obtain energy by synthesizing methane. They possess several unusual cofactors involved in methanogenesis.

Representative genera include *Methanobacterium*, *Methanococcus*, *Methanomicrobium*, and *Methanosarcina*.

5.1.2. Extreme halophiles or halobacteria

They are aerobic chemoheterotrophs that require high concentrations of NaCl to grow. They are found in habitats such as salt flats, saline lakes, and salted fish.

5.1.3. Thermoplasma

This is a thermophilic archaeon that develops in hot, acidic coal heaps, where it survives despite lacking a cell wall.

5.1.4. Extreme thermophiles

The class Thermococci includes extreme thermophilic organisms that can reduce sulfur to sulfide.

5.1.5. Sulfate-reducing archaea

They belong to the class Archaeoglobi. The extreme thermophile Archaeoglobus differs from other archaea by using a wide variety of electron donors to reduce sulfate. The members of this phylum constitute a very large and highly diverse phylum, comprising numerous classes, orders, and families. Also, they constitute a very large and highly diverse phylum, comprising numerous classes, orders, and families.

5.2. Crenarchaeota

The phylum includes only a single class, Thermoprotei, which is divided into four orders.

5.2.1. Order Thermoproteales

This order contains two families: the family Thermoproteaceae includes genera of hyperthermophilic rods that are either anaerobic or facultatively anaerobic. The family Thermophilaceae has only one representative: *Thermophilum pendens*.

5.2.2. Order Sulfolobales

These are thermoacidophiles with a coccoid (spherical) shape.

5.2.3. Order Desulfurococcales

The two families of this order contain hyperthermophilic coccoid or disc-shaped organisms.

- The family **Desulfurococcaceae** includes the genus *Ignicoccus*.
- The family **Pyrodictiaceae** includes the genus *Pyrodictium*. The genus *Pyrolobus* also belongs to this family. *P. fumarii* is one of the most thermophilic microbes isolated to date. Its optimal growth temperature is 106°C, and its maximum temperature is 113°C.

5.2.4. Order Caldisphaerales

This order contains only one genus, *Caldisphaera*, whose members are thermoacidophilic, aerobic, and heterotrophic cocci.

5.3. Other phyla

Thaumarchaeota, Nanoarchaeota, and Korarchaeota, remain relatively poorly characterized due to limited cultivation and study. Despite this, available data reveal notable diversity in their physiological traits, metabolic strategies, and ecological distributions. The following table summarizes the main characteristics of these archaeal phyla based on the limited information currently available (Table 15).

Table 15. Characteristics and comparison of Thaumarchaeota, Nanoarchaeota and Koraarchaeota (Driche, 2024)

Phylum Characteristic	<i>Thaumarchaeota</i>	<i>Nanoarchaeota</i>	<i>Korarchaeota</i>
Morphology	Coccoid or rod-shaped, 0.5-2 μm in diameter	Extremely small, 0.4-0.5 μm , often found in close with other archaea	Small, 0.5-1 μm in diameter
Metabolism	Aerobic, chemolitho-autotrophic, ammonia-oxidizing	Obligate symbiotic or parasitic, limited metabolic capabilities	Poorly understood, presumably anaerobic and heterotrophic
Temperature Range ($^{\circ}\text{C}$)	10 - 55, with optimum "30-40 "	80 - 100,	80 - 105,
Habitat	Marine and terrestrial ecosystems, soil, sediments, and	High-temperature, anaerobic environments, such as deep-sea hydrothermal vents	High-temperature, anaerobic environments,
Ecological Role	players in nitrification and carbon cycling	potentially parasitic or symbiotic with other archaea	play a role in high-temperature ecosystems
Cultivated Representatives	Several species have been successfully cultivated	Few species have been successfully cultivated	Very few species have been successfully cultivated

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