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University M'hamed Bougara of Boumerdes

Faculty of Sciences

Department of Biology

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Course Handout

Bacterial Taxonomy

Carried out by:

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This handout is intended for students enrolled in Bachelor L3

Specialty BTM Students

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وزارة التعليم العالي والبحث العلمي

Ministry of Higher Education and Scientific Research

جامعة احمد بوقرة_ بومرداس

M'Hamed Bougara University of Boumerdès
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EDUCATIONAL HANDOUT

Bacterial Taxonomy

Course for Third-Year BTM Students

Presented by Dr. HEZIL DJAMILA

Academic Year **2024/2025**

Foreword

This **handwork** is intended for third-year students in Microbial Biotechnology (L3 BTM) as part of the **Bacterial Taxonomy** module. It aims to provide an in-depth understanding of the fundamental principles of microorganism classification, focusing on systematics, bacterial and archaeal diversity, and the classification criteria used in microbiology.

The objective of this document is to help students develop a structured understanding of microbial diversity, based on modern taxonomic approaches and the **Bergey's Manual** classification, a key reference in the field.

This course material is divided into **four parts**:

- **The first part**: Introduction to microbial systematics, covering the fundamental principles of microorganism classification, taxonomic approaches, and differentiation criteria.
- **The second part**: Overview of bacterial and archaeal groups, focusing on their main physiological, morphological, and ecological characteristics.
- **The third part**: Classification of major bacterial phyla according to Bergey's Manual, with special emphasis on **Proteobacteria** and their classes (Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Epsilonproteobacteria).
- **The fourth part**: Diversity and classification of archaeal phyla, presenting the five major **Archaea** phyla (Euryarchaeota, Crenarchaeota, Korarchaeota, Nanoarchaeota, Taumarchaeota) and their ecological significance.

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Chapter I: Introduction to Systematics and Taxonomic Approaches

1.1 Generalities

1.1.1 The Discovery of the Microbial World

Microorganisms, also commonly called microbes, form a group of living organisms of microscopic size. They are invisible to the naked eye and can only be observed using a microscope. This microscopic nature is their common characteristic; however, they are extremely diverse and differ in morphology, physiology, reproduction methods, and ecology.

1.1.2 Historical Background

In the 18th century, Carl Linnaeus (1735), a Swedish botanist, developed the first classification system of living organisms into two kingdoms: Vegetal and Animal. In 1857, Karl van Nägeli proposed classifying bacteria and fungi within the plant kingdom. In 1866, E. Haeckel divided the living world into three kingdoms: the Animal Kingdom, the Plant Kingdom, and the Protist Kingdom (microorganisms), which included algae, protozoa, fungi, and bacteria. In 1937, thanks to the invention of the electron microscope, Edward Chatton distinguished two types of cells: eukaryotic and prokaryotic cells. In 1968, R.G.E. Murray, continuing Chatton's work, divided the living world into two kingdoms: "Eucaryotae" and "Procaryotae" (or "Monera").

However, the five-kingdom classification proposed in 1969 by Robert H. Whittaker introduced a new classification system comprising:

- **The Animal Kingdom (Animalia):** A group of eukaryotic, heterotrophic organisms lacking a cell wall.
- **The Plant Kingdom (Plantae):** A group of eukaryotic, autotrophic organisms possessing a cell wall.
- **The Fungi Kingdom (Mycetes):** A group of eukaryotic, heterotrophic organisms with a cell wall.
- **The Protist Kingdom (Protista):** A group of unicellular eukaryotic organisms.
- **The Monera Kingdom (Monera or Procaryotae):** A group of unicellular prokaryotic organisms.

With advancements in molecular biology techniques and phylogenetic data, Carl Woese et al. (1977) proposed a new classification of living organisms into three domains, a taxonomic rank superior to kingdoms:

1. **Eucarya Domain:** Includes animals, plants, fungi, and protists.
2. **Bacteria or Eubacteria Domain:** Composed of bacteria, forming a distinct evolutionary group.
3. **Archaea Domain:** Includes archaebacteria (prokaryotic protists), which constitute a homogeneous yet highly distinct evolutionary group from bacteria.

1.2 Definitions

1.2.1 Taxonomy

- Derived from the Greek *taxis* (order or arrangement) and *nomie* (laws).
- The science of classification rules and laws for living beings (identification and nomenclature).
- It allows organisms to be classified into affinity groups or taxonomic units.

1.2.2 Systematics

- The scientific discipline of classifying living beings.
- Studies relationships between taxa (hierarchy and kinship).
- Organizes and classifies taxa in a logical order.
- Based on three disciplines :
    1. Classification
    2. Nomenclature
    3. Identification

1.2.3 Classification

The arrangement of living organisms into taxa based on similarity.

1.2.4 Nomenclature

Assigning a conventional name to each distinct taxon following a set of rules (International Code of Nomenclature of Bacteria).

1.2.5 Identification (Determination)

Placing a particular individual into a known taxon, previously defined based on the comparison of their specific characteristics.

1.2.6 Taxon

Groups of living organisms related by sufficiently specific criteria to make them both recognizable and distinct from other groups "classification groups of different but phylogenetically related organisms."

1.2.7 Phylogeny

A science that studies the genetic evolution of bacteria over time.

- It allows organisms to be grouped according to their kinship (seeking evolutionary relationships between groups of organisms, e.g., species, populations).

1.3 Importance of Taxonomy

Taxonomy is crucial for several reasons:

1. Essential for identifying new isolates.
2. Provides access to phylogeny (i.e., kinship relationships between different organisms).
3. Serves as a "database" on microorganisms.
4. Enables the description of distinct groups of organisms, facilitating:

    - The identification and utilization of beneficial microorganisms.
    - The control and protection against pathogenic microorganisms.

1.4 Main Principles of Taxonomy

1.4.1 Classification Units

1.4.1.1 Classification Ranks

The basic unit of classification is **the species.**

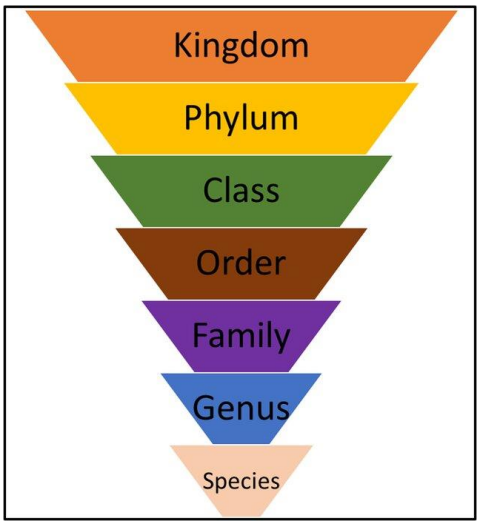
- Living organisms are classified into a **seven-rank hierarchical system**: **Species – Genus – Family – Order – Class – Phylum – Domain (Table 1)**
- Unlike in the **Eucarya domain**, where multiple phyla form a kingdom, in the **Archaea and Bacteria domains**, there are no defined kingdoms.
- Intermediate levels are sometimes used: **subphylum, subfamily (tribe), subspecies.**

1.4.2 Taxonomic Hierarchy

- The process of classifying microorganisms consists of placing them in hierarchical taxonomic levels.
- **At each level or rank, microorganisms share a set of specific characteristics.**
- The ranks are arranged in a **non-overlapping hierarchy**, ensuring that each level defines both the attributes of that rank and a new set of more restrictive traits.
- **The different classification groups at any rank are called taxa "From species to phylum, shared characteristics become progressively fewer."**

Each Category = Taxon

Scientists have divided each kingdom into a series **of narrower categories to clearly distinguish the different levels of similarity**.



Note: In the domains of Archaea and Bacteria, there are no clearly defined kingdoms, unlike in the domain of Eucarya, where multiple phyla combine to form a kingdom.

Table 1: The main taxa in descending order (Taxonomic Hierarchy):

Taxon	Example
Kingdom	***Bacteria***
Regnum	***Undefined***
Phylum	***Proteobacteria***
Class	***Gammaprotebacteria***
Order	***Enterobacteriales***
Family	***Enterobacteriaceae***
Genus	***Escherichia*(Set of species)**
Species	***Escherichia coli E.coli* (Set of strains)**
Serovar	***E.coli O157 :H7***

1.4.2.1 The Bacterial Species

- A collection of strains that share many stable properties (morphological, biochemical, and genetic) and differ from other strain groups.
- The bacterial species includes all strains considered sufficiently close to the type strain of the species to be classified within it.
- The type strain of the species is first clearly defined by its genomic characteristics and deposited as a reference strain.

1.4.2.2 The Bacterial Strain

- A strain = a clone = the exclusive progeny of a single mother bacterium (pure culture isolate).
- Strains of the same species may differ from each other by minor but identifiable differences.
- Strains of the same species are identified by adding a number to the species name (*E. coli* K12).
- A species thus includes multiple strains that differ by secondary characteristics.
- Subdivisions of the species are defined based on various specific characteristics:
  - **Biotypes (Biovars):** Biochemical characteristics (biovars are strains that differ biochemically from the type strain).
  - **Serotypes (Serovars):** Antigenic characteristics.
  - **Pathotypes (Pathovars):** Pathogenicity factors.
  - **Lysotypes (Lysovars):** Sensitivity to phages.
  - **Zymotypes (Zymovars):** Enzymatic capabilities.
  - **Antibiotypes (Antibiovars):** Sensitivity to antibiotics.

1.5 Bacterial Nomenclature

1.5.1 Nomenclature Code

There are rules governing bacterial nomenclature, compiled in the *International Code of Nomenclature of Bacteria*. This code is established by the *International Committee of Systematic of Prokaryotes (ICSP)*, particularly by its *Judicial Commission*.

1.5.2 Writing Bacterial Nomenclature

Two categories of names are distinguished:

- **Informal names** (vernacular names).
- **Specialized names** (scientific names of taxa).

Vernacular Name	Scientific Name
Colibacillus	*E.coli O157 :H7*
Koch's Bacillus	*Myobacterieum tuberculosis*
Golden Staphylococcus	*Staphylococcus aureus*

- All nomenclatures are Latin or Latinized words, and such words are traditionally written in italics or underlined in a manuscript.
- No diacritical marks (á, à, â, ä, ã, é, è, ê, ë, í, î, ï, ñ, ó, ò, ô, ö, õ, ú, ù, û, ü, æ...) are allowed, and words must not contain hyphens.

1.5.2.1 Rules for Naming:

The binomial system of the botanist Carl Von Linné is used.

- Species names consist of a binary combination: the first term is the genus name, and the second term is an "epithet."
- **Genus**: Written in italics, with its first letter capitalized. After its first mention in the text, the genus name is abbreviated to its first letter followed by a period.
- **Species**: Written in italics (or underlined in books and manuscripts), with the first letter in lowercase. (Example: *Staphylococcus aureus*). After the first mention, the use of the first letter of the genus name followed by a period and the epithet is allowed (Example: *S. aureus*).
- **Family**: Feminine, plural, and ends with **-aceae**.
- **Order**: Ends with **-ales**.
- **Subspecies** names consist of a three-part combination: the species name followed by the abbreviation "subsp." (or "ssp.") and a third term specific to the subspecies. (Example: *Staphylococcus aureus subsp. aureus*).

1.6 The Classification System

- **Before 1960**: Classical taxonomy was based on the study of morphological and structural characteristics of bacteria as well as their metabolic profiles.
- **Since the 1970s**: Modern analysis techniques based on different molecular genomic components (DNA, RNA) or genome-derived components (proteins) have made it possible to establish genetic relationships.
  The computational analysis of molecular markers has revolutionized bacterial taxonomy.
- There are two main types of classification: **Artificial Classification and Natural Classification**

1.6.1 Artificial Classification

Based on highlighting a set of significant phenotypic (observable) characteristics.

1.6.2 Natural Classification

Groups organisms into categories where members share numerous characteristics (a maximum of criteria).

- It reflects the biological nature of organisms as much as possible.

There are two different approaches:

- **Phenetic (Phenotypic Classification)**: Groups organisms based on the similarity of their phenotypic characteristics.
- **Phylogenetic (Phylogenetic Classification)**: Groups organisms according to evolutionary relationships. It is based on genetic characteristics and relies on molecular markers due to their high evolutionary stability and low variability.

"The current classification of bacteria and archaebacteria is natural, based on the phylogenetic data of bacteria and archaebacteria through the comparative analysis of their 16S rRNA."

1.7 Different Approaches to Taxonomy

1.7.1 Phenotypic Approach (Phenotypic Taxonomy)

Species identification is based on comparing many phenotypic characteristics of the studied strain with those of a reference strain. A small number of characteristics considered significant are used in phenetic or phenotypic classification, such as morphology, physiology, biochemistry, immunology, and physicochemical properties (chemotaxonomy). However, this

approach reflects only limited information, as the considered characteristics are subjective and depend on environmental conditions.

1.7.1.1 Morphological Characteristics

- Morphological characteristics are important in microbiome taxonomy for several reasons.
- Morphology is simple to study and analyze.
- Morphological comparisons are significant because the structure of characteristics depends on the expression of numerous genes, which are generally genetically stable (at least in eukaryotes).
- These characteristics include: colony morphology (shape, size, color, presence of diffusible or non-diffusible pigments, fluorescence, luminescence, and odor), cell morphology (bacillus, coccus, spiraled), and their arrangements. Staining techniques (Gram stain, methylene blue stain, Ziehl stain) are also considered, as well as motility (polar or peritrichous flagella, gliding motion) and the presence of endospores.

1.7.1.2 Physiological and Metabolic Characteristics

Physiological and metabolic characteristics are highly useful, as they are directly related to the nature and activity of microbial enzymes and transport proteins. These include:

- Trophic type (carbon and nitrogen sources)
- Type of energy metabolism (chemotrophic, phototrophic)
- Fermentation products
- General mode of nutrition
- Optimal growth temperature range
- Osmotic tolerance
- Photosynthetic pigments
- Salt tolerance and pH requirements

1.7.1.3 Biochemical Characteristics

Enzymatic activity is often used to differentiate bacteria. It is generally possible to distinguish closely related bacteria and group them into distinct species using biochemical tests, such as (Figure 1):

- Presence of catalase, oxidase, and other enzymes
- Glucose metabolism (oxidative or fermentative)
- Ability to degrade complex molecules (starch, tributyrin, cellulose)
- Acid production from sugars
- Indole and H2S production
- Methyl red test
- Nitrate reduction and Voges-Proskauer reaction

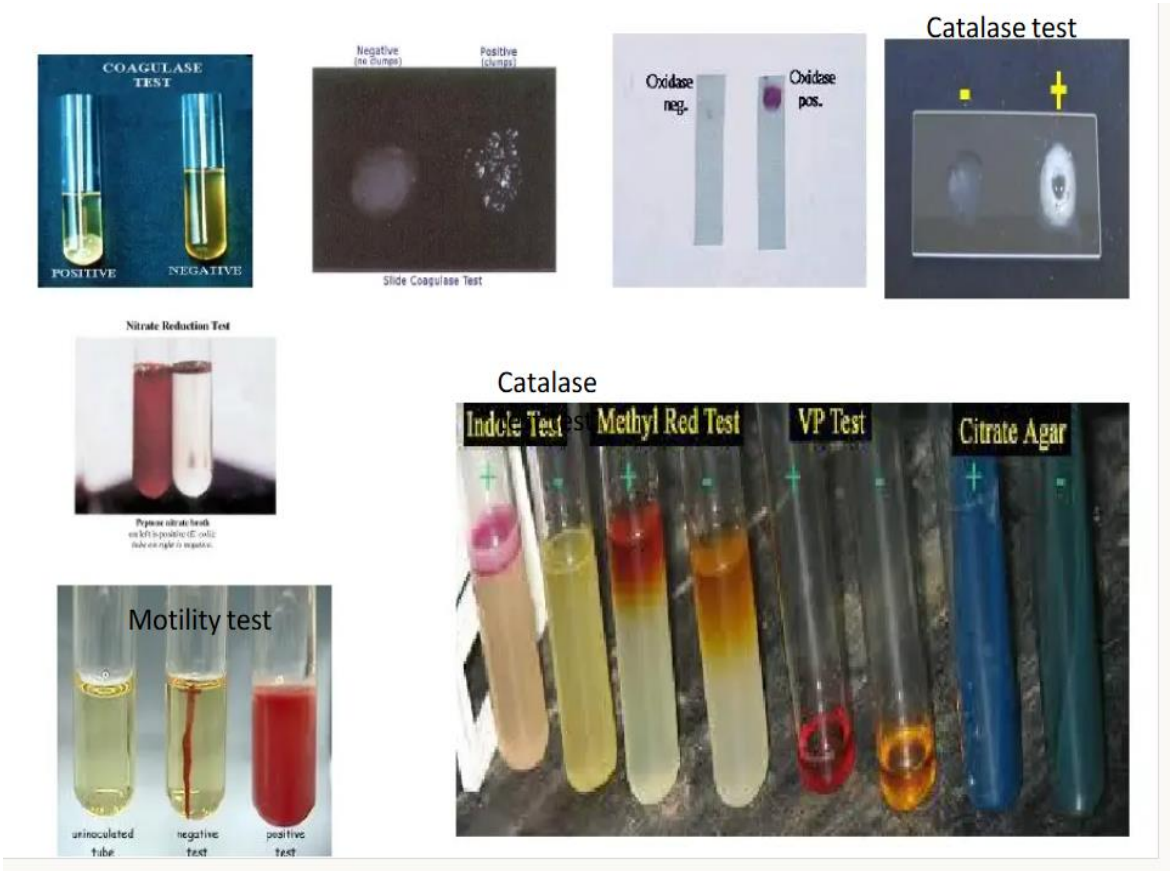


Figure 1 : Biochemical tests

1.7.1.4 Serotyping

Serotyping is based on the specific antigen-antibody reaction. This method allows for differentiation at the species level and even among strains within the same species. The targeted antigens include (Figure 2):

- **O antigen** (in Gram-negative bacteria)
- **H antigen** (flagellar)
- **K antigen** (capsular)

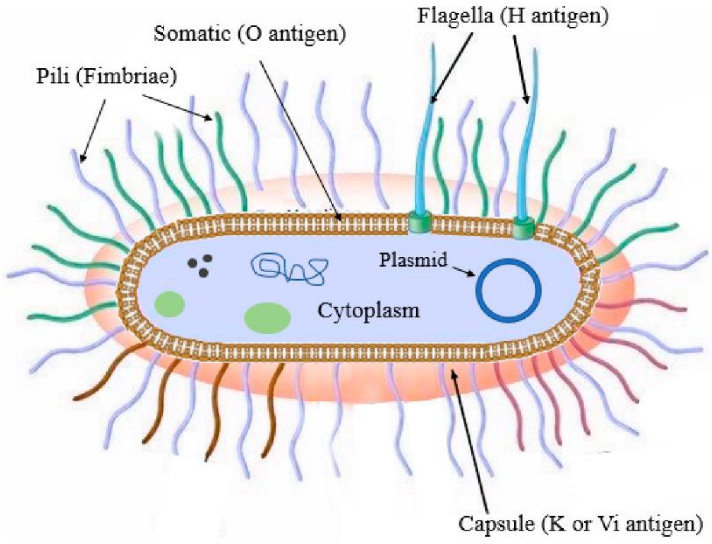


Figure 2: Schematic illustration of the structure of *Salmonella (***Teklemariam et al., 2023**)

1.7.1.5 Inhibition Tests

Microorganism growth is assessed in selective media and in the presence of antibiotics (antibiogram).

1.7.1.6 Chemotaxonomy

Chemotaxonomy determines the fatty acid profile of cell walls, total protein profiles, and acids using SDS-PAGE electrophoresis.

1.7.1.7 Lysotyping

Lysotyping identifies a specific typing profile using bacteriophages.

1.7.1.8 Automated Identification Methods – Identification Galleries (API System)

- These systems use the same principle as conventional biochemical techniques.
- They offer a **miniaturized and standardized** version.
- Ready-to-use wells containing the necessary lyophilized substrates for various biochemical tests (Figure3).

Advantages

- **Standardization** of biochemical characteristics improves inter-laboratory reproducibility by eliminating subjective choices of "important" tests for characterization.
- **Minimizes technical variability**.
- Easy to use

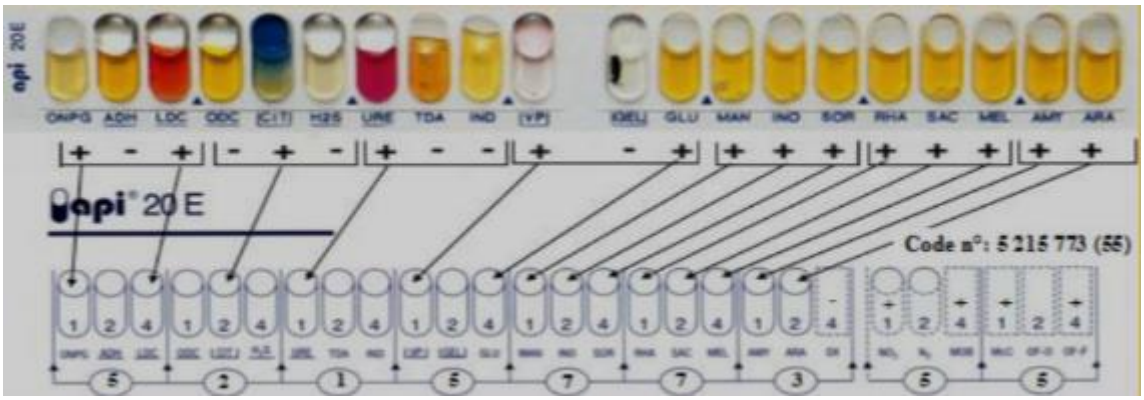


Figure 3: Api 20 E gallery reader.

1.7.1.9 Bacterial Identification

Dichotomous Approach

- Characteristics are hierarchized to construct an identification scheme followed step by step based on the results.
- However, this method has a high risk of error. Misreading or misinterpreting a single characteristic can lead to misidentification (Figure 4and figure 5).

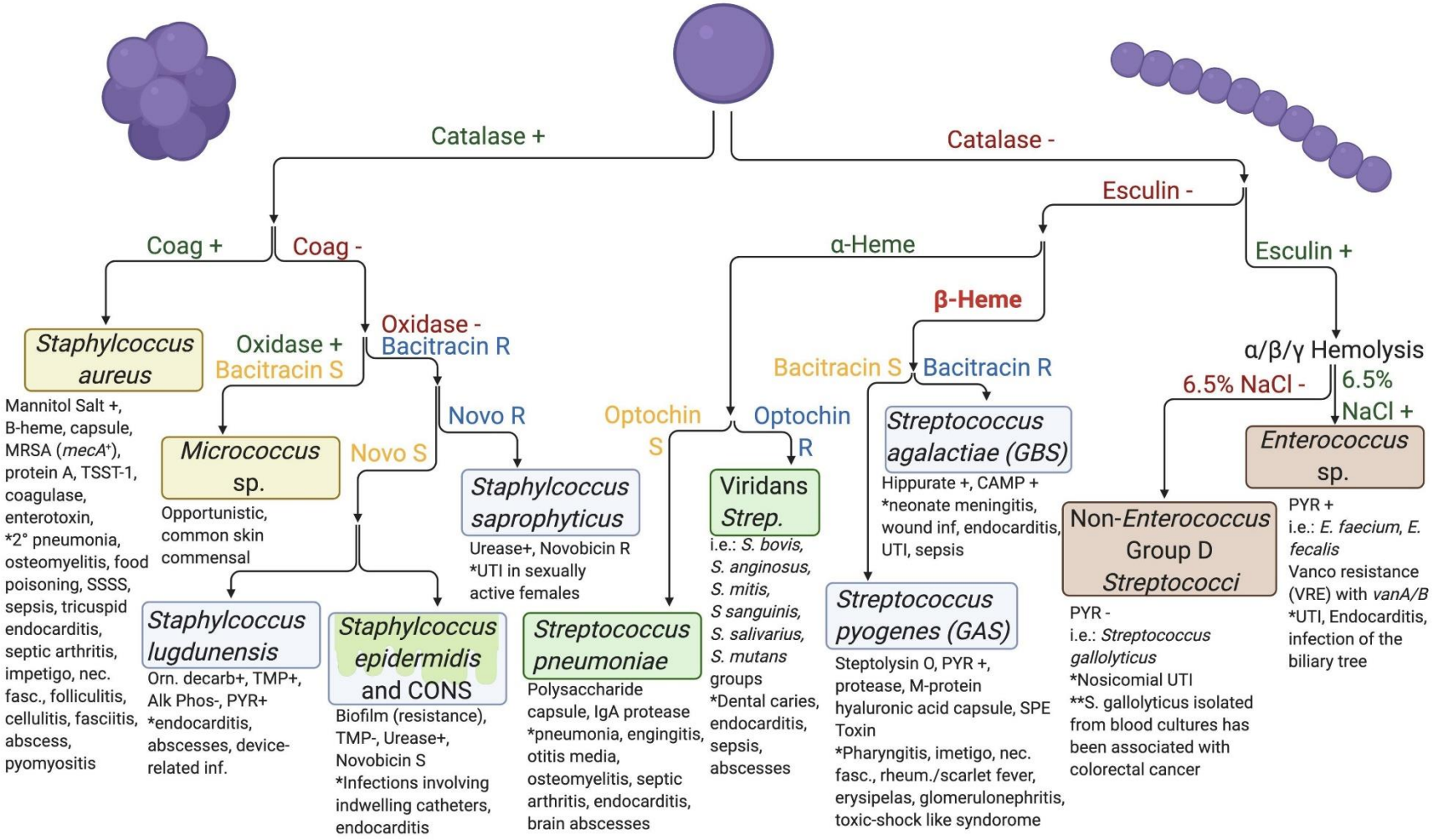


Figure 4: Phenetic identification use of dichotomous keys for Gram-positive bacteria

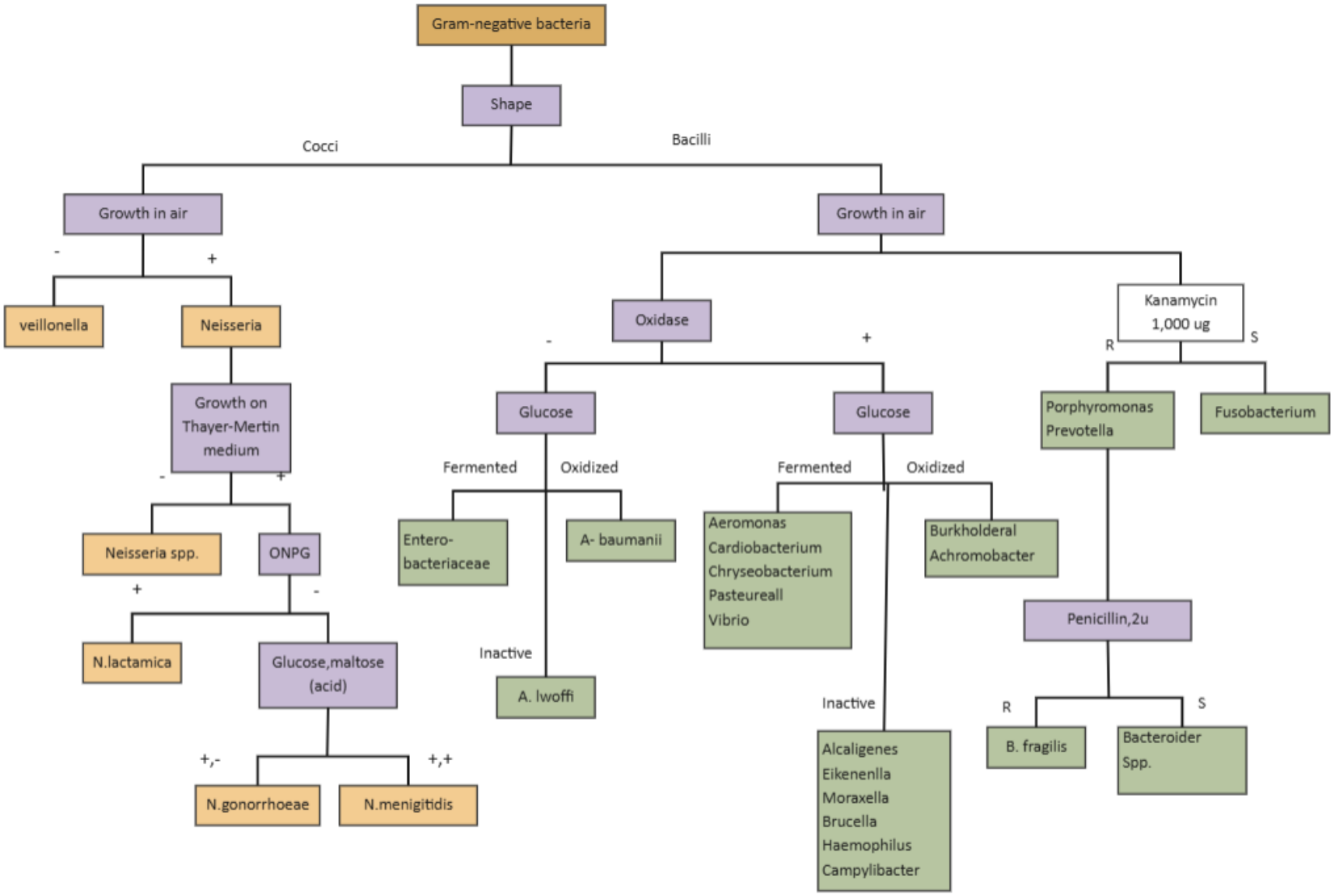


Figure 5 :Phenetic identification use of dichotomous keys for Gram-negative bacteria.

Probabilistic Approach Using Computer Software

- Result interpretation is based on the principle of numerical identification (each characteristic has the same weight, unlike the dichotomous approach).
- A database (table, online database, or included in an Excel spreadsheet) indicates, for each taxon and each characteristic, its probability of being positive.
- Use of software (e.g., UPBM online identification software).
- The bacterial name is obtained through probability calculations: the software ranks all results to provide the most probable taxa.

Use of API Coding (Numerical Profile)

- Final result = Code referenced in an analytical catalog/software.
- Identification of the most probable taxon (species or genus) based on probability calculations from the tested characteristics.

1.7.2 Limitations of Phenotypic Classification

- Slow or difficult culture (e.g., *Chlamydia*, *Rickettsia*).
- This classification relies on a limited number of phenotypic characteristics (a maximum of around 100) compared to the typical bacterial genome (approximately 5,000 genes). Even with 400 tests, only 5-20% of a bacterium's genetic potential is assessed (e.g., *E. coli* possesses 3,000 genes).
- Phenotypic techniques are unsuitable for diagnosing viable but non-culturable bacteria.
- Studied characteristics may be absent, particularly in mutant bacteria. Example: existence of *E. coli* lactose-negative strains.

1.7.3 Numerical Approach

- Numerical or Adansonian taxonomy evaluates similarity among various strains by comparing numerous morphological, biochemical, and physiological characteristics, assigning equal weight to each.
- The computer calculates similarities between individuals and groups those with high resemblance into phenons.
- The number of studied characteristics ranges between 50 and 200. Each test result is coded in binary (0 for absent, 1 for present).
- The number of analyzed characteristics for unknown isolates and reference strains should ideally be between 50 and 100. The results are coded in binary format for each test (0 for absent, 1 for present).
- Selected tests should be consistent (always positive or always negative), and redundant tests (e.g., motility and flagella presence) should be eliminated.

- Taxonomic data are presented as a data matrix: first, strains are grouped in a table based on their characteristics. Then, individuals are grouped based on similarity indices (similarity or distance). This results in polythetic taxa (constructed with multiple characteristics). These groupings are hierarchized by levels of resemblance:
  - **First level:** Organisms with high similarity.
  - **Subsequent levels:** Organisms with decreasing resemblance.

- **Simplified example of digital taxonomy: Data matrix (: results of phenotypic tests for 8 strains from A to H. 10 characters ((+ or 1), (- or 0)).**

	Strain A	**Strain B**	**Strain C**	**Strain D**	**Strain E**	**Strain F**	**Strain G**	**Strain H**
Test 1	1	0	1	1	1	1	0	1
Test 2	0	1	1	0	1	0	1	1
Test 3	1	1	0	1	0	1	1	1
Test 4	1	0	0	0	1	1	0	1
Test 5	1	0	0	1	1	1	0	0
Test 6	1	1	0	1	0	0	1	0
Test 7	0	1	1	1	1	0	0	1
Test 8	1	0	0	1	0	1	1	0
Test 9	1	0	1	1	1	1	0	1
Test 10	0	1	1	0	1	0	1	1

1.7.3.1 Calculation of a Numerical Index (Similarity Index = Jaccard Index)

- The results are analyzed using a computer program to determine the Jaccard Index (Jaccard-Sneath Coefficient).
- The Jaccard Coefficient is calculated as:

S (A, B) = (Ns+ / (Ns+ + Nd)) × 100

Where:

- **S (A, B)** = Similarity coefficient between A and B (ranges from 0 to 1)
- **Ns+** = Number of positive characteristics shared by A and B
- **Nd** = Number of differing characteristics between A and B

Example: **S (A, D) = 6 / (6+2) = 6 / 8 = 0.75**

- The distance index **d** can also be calculated as **d = 1 - S**. This index decreases as similarity increases:

- **d = 0** for two identical strains (**S (A, B) = 1**).
- **d = 1** for two strains with no common characteristics (**S (A, B) = 0**).

- The similarity index is calculated by comparing each strain with all others.

Strains	A	B	C	D	E	F	G	H
A	**100**							
B	**20**	**100**						
C	**20**	**43**	**100**					
D	**75**	**33**	**33**	**100**				
E	**40**	**38**	**71**	**40**	**100**			
F	**86**	**10**	**22**	**63**	**44**	**100**		
G	**33**	**67**	**25**	**33**	**20**	**22**	**100**	
H	**40**	**50**	**71**	**40**	**75**	**44**	**33**	**100**

1.7.3.2 Interpretation of Similarity Results

- Strains with **90% similarity** belong to the same species.
- Strains with **70% similarity** belong to the same genus.
- Strains **A and F** appear to form a single genus but require additional tests to confirm their classification within the same species.

1.7.3.3 Dendrogram Representation

- Results are represented as a **dendrogram** or **cladogram** (*clado* = branch). Each intersection point of two branches corresponds to shared characteristics among species located beyond that node.
- The dendrogram is visualized as a tree with multiple branches and twigs representing different strains.
- The **similarity percentages** allow the tree to be mathematically segmented into different levels, leading to the identification of taxonomic groups.

1.7.4 Molecular Approach (Genotypic or Phylogenetic Taxonomy)

The molecular approach relies on methods based on the analysis of DNA or RNA molecules, either at the whole genome level or by targeting specific fragments of the bacterial chromosome or plasmid.

The criteria considered are:

- Genome size.
- Determination of DNA base composition in the form of G+C% (GC% coefficient).
- DNA hybridization.
- 16S ribosomal RNA (rRNA) sequencing.

1.7.4.1 Genome Size

- Genome size varies depending on the species (Table 2). It corresponds to the amount of DNA contained in a single copy of a genome.
- It is measured either by genome mass in picograms (pg) or by the number of nucleotide base pairs in millions (Mb). One pg corresponds to 978 Mb. Number of base pairs = Mass in pg × 978 × 10^6.

Table 2: The genome sizes by certain bacteria

S.No.	Microorganisms (Bacteria)	Genome size (Daltons × 10^9)
1	*Bacillus subtilis*	2.500
2	*Escherichia coli*	25.000 (± 0.5)
3	*Micrococcus salivarius*	3.300
4	*Mycoplasma pneumoniae*	0.480
5	*Peptococcus aerogenes*	0.816
6	*Peptococcus saccharolydicus*	1.250
7	*Staphylococcus aureus*	1.458

1.7.4.2 DNA Base Composition as G+C% (GC% Coefficient, Chargaff's Rule)

- Chargaff demonstrated that the purine (guanine = G and adenine = A) and pyrimidine (cytosine = C and thymine = T) base content varies between genomes but remains relatively constant within the same species.
- GC% can be calculated from sequencing using the following formula: GC% (mol) = (G + C) / (A + T + G + C) × 100
- It is one of the most classical genotypic methods and is an integral part of the standard description of a bacterial taxon.
- Different techniques determine GC%, including melting temperature (Tm) measurement and high-performance liquid chromatography (HPLC):
    - Simple physical methods measure GC content through melting temperature (Tm), the temperature at which DNA is half denatured, detected spectrometrically at 260 nm (Figure 6).
    - Double-stranded DNA absorbs weakly at 260 nm due to base stacking in the helix. Heat-induced denaturation breaks hydrogen bonds, separating strands and significantly increasing absorbance at 260 nm (hyperchromic effect).
    - The Tm increases with GC content since G-C pairs have three hydrogen bonds compared to two for A-T pairs. Example: E. coli Tm = 72°C (GC% = 50), P. aeruginosa Tm = 79°C (GC% = 66).
- GC% is an exclusion criterion: it indicates genetic divergence but does not confirm phylogenetic proximity.
- Bacteria differing in GC% by more than 5% should not belong to the same species, and differences of more than 10% suggest different genera.

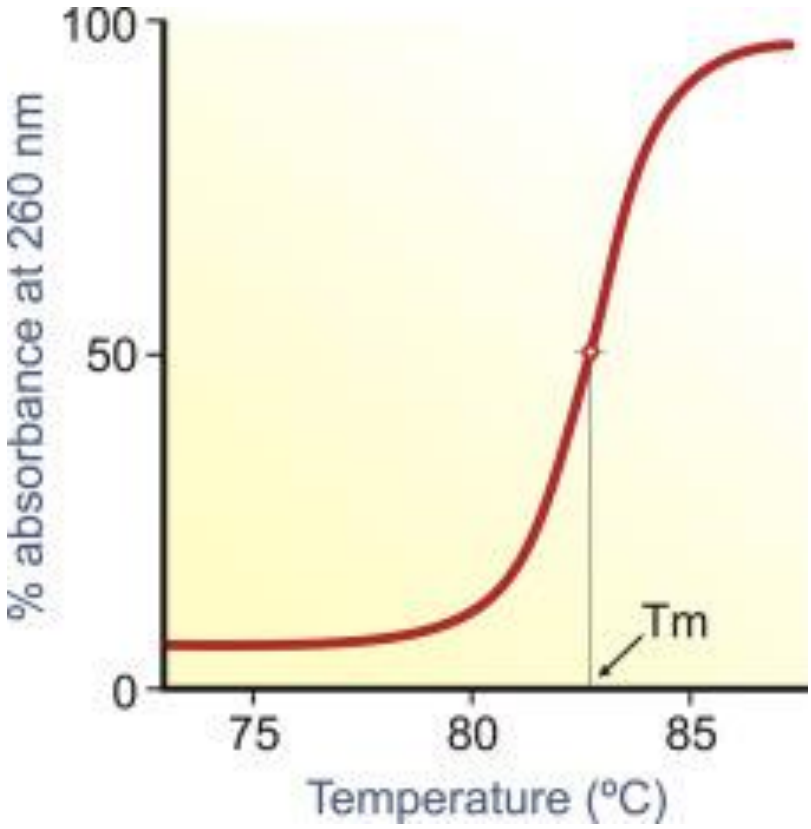


Figure 6: DNA denaturation.

The change in absorbency at 260 nm with increasing temperature (hyperchromic effect) is shown. When both DNA helices are completely separated, absorbance does not further increase with temperature. Tm (Melting temperature) corresponding to the temperature at which 50% of DNA is denatured **(Antonio Blanco et Gustavo Blanco, 2022).**

1.7.4.3 DNA Hybridization

- DNA hybridization is based on the ability of single-stranded DNA to reanneal into double-stranded DNA(Figure 7).
- When GC% values are identical, a common ancestor is confirmed only if nucleotide sequences are identical or highly similar. This is determined by measuring DNA renaturation rates.
- DNA from bacterial strains is purified, fragmented, denatured by heat, then renatured in the presence of labeled DNA. The most common technique involves radioactive labeling .
- Denaturation occurs at high temperatures (80°C), while renaturation typically occurs 15-20°C lower.
- Homology levels: 70-100% for the same species, 0-60% for different species.
- DNA-DNA hybridization remains a key criterion for species delineation.

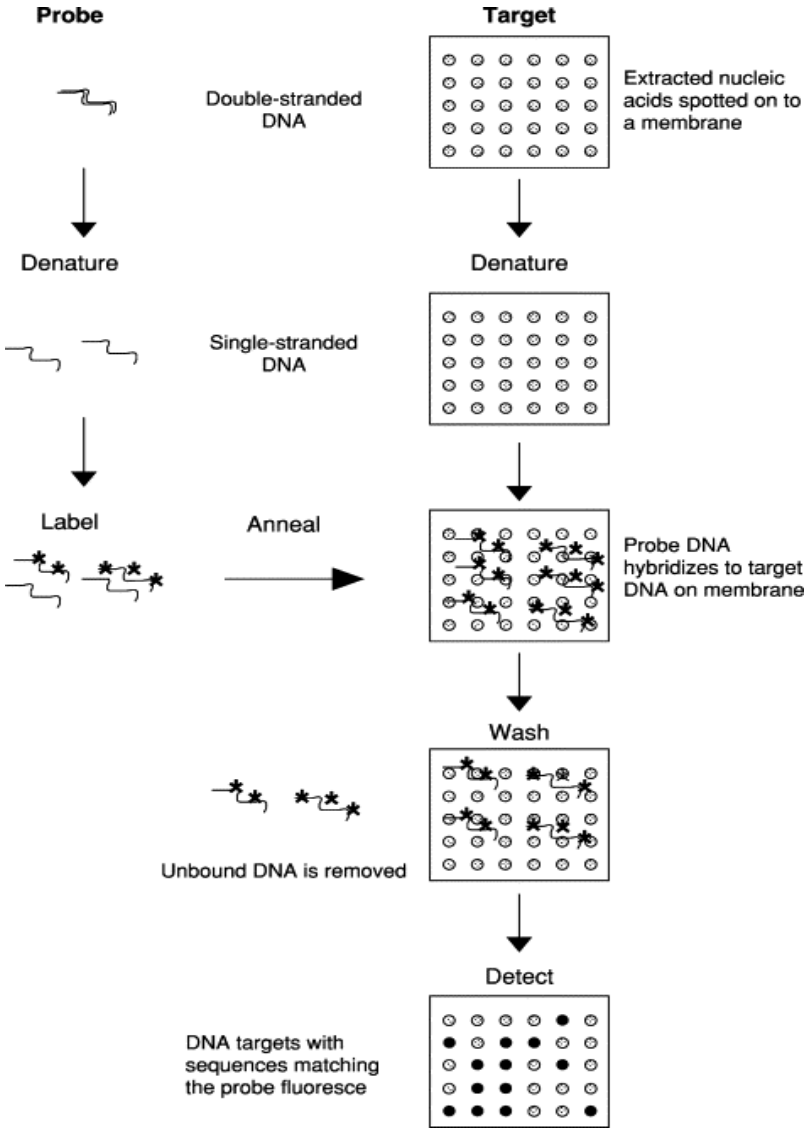


Figure 7: DNA/DNA hybridization (**Nakatsu, 2005)**.

1.7.4.4 16S Ribosomal RNA (rRNA) Sequencing

Ribosomal RNA has been selected for taxonomic and phylogenetic markers for several reasons:

- It is abundant, easily purified, and present in all living cells.
- Its structure is highly conserved due to its essential role in protein synthesis.
- rRNA sequences are identical across all living organisms.
- Among the three rRNA types (23S, 16S, and 5S), 16S rRNA (1500 nucleotides) is the most frequently analyzed.
- It contains conserved sequences common to higher taxonomic units and variable sequences specific to species.
- The 16S rRNA gene sequence is known for approximately 4000 strains.
- rRNA 16S sequences can be compared online with databases of deposited strains.

- The FASTA and BLAST programs compare unknown bacterial sequences with reference databases, identifying the closest matches.
- If homology is below 97%, two bacteria cannot belong to the same species. If homology is above 97%, species determination depends on DNA-DNA hybridization results.

Note: Two species may have highly similar 16S rRNA sequences but differ significantly in DNA-DNA hybridization. Example: Aeromonas trota and A. caviae (99.9% similarity in 16S rRNA but only 30% in DNA-DNA hybridization).

1.7.5 Polyphasic Approach

Many taxonomists believe that only a combination of genotypic and phenotypic data can establish phylogeny; this is the polyphasic approach. The first step groups strains based on phenotypic characteristics. The homogeneity of these phenons is then confirmed by genotypic methods. The final step ensures that genotypic groups share common, easily identifiable biochemical traits for classification.(Figure 8)

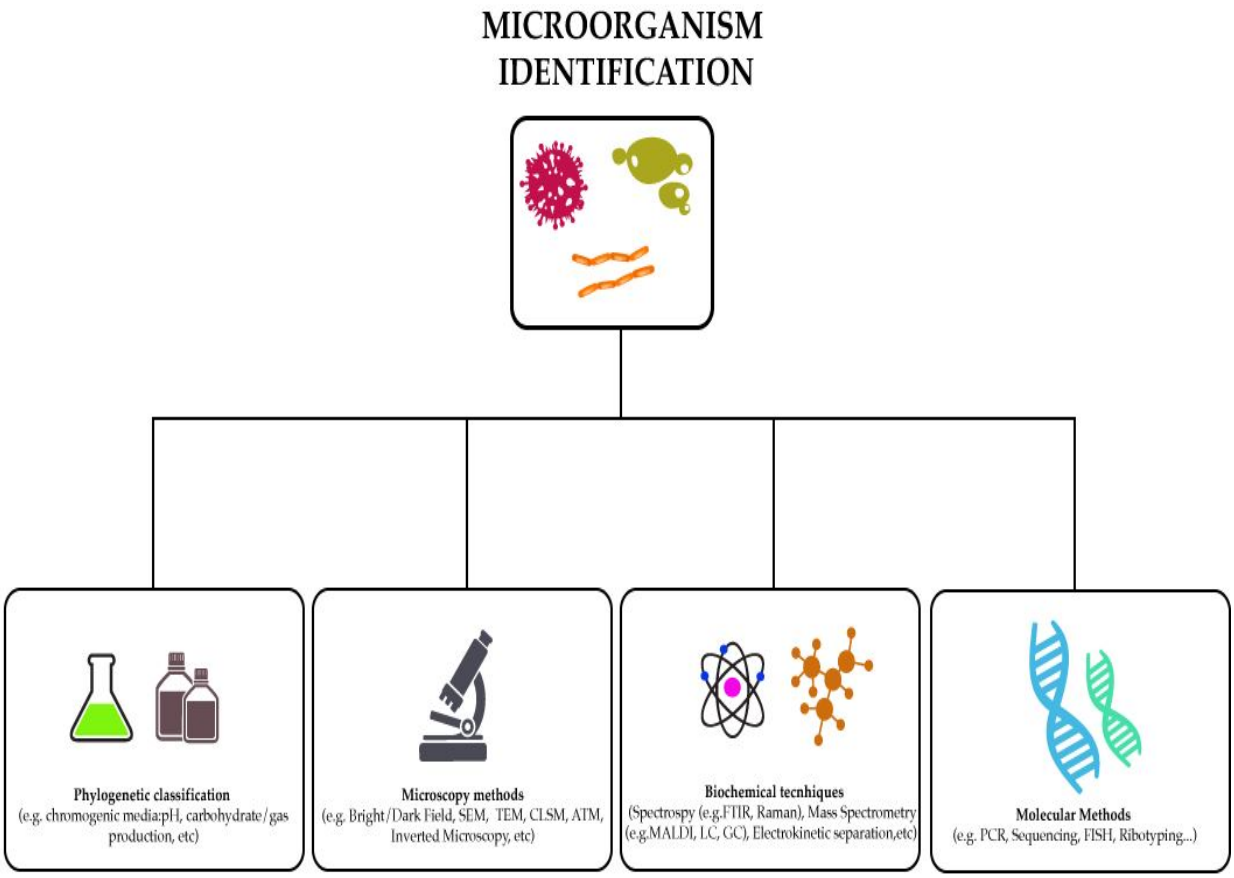


Figure 8: Polyphasic Approach (Franco-Duart et al., 2019).

Chapter II: The Different Groups of Bacteria and Archaea

2.1 Bergey's Classification

There is no official classification of bacteria; however, the classification outlined in Bergey's Manual is the most widely accepted among microbiologists. Until the early 1960s, bacterial taxonomy was based entirely on a phenotypic classification.In its early editions, dating back to 1936, Bergey's classification was based on the study of several criteria: microscopic and macroscopic morphology, motility, spore presence, Gram reaction, growth temperature, respiratory type, nutritional requirements, and the ability to utilize specific carbon or nitrogen sources. At that time, prokaryotes were divided into four recognized divisions based on the presence or absence of a cell wall: **Gracilicutes, Firmicutes, Tenericutes, and Mendosicutes** (**Table 3**).

This is the classification that is consensually used today.
It is, of course, evolving. It may be useful to consult Wikipedia, for example: https://en.wikipedia.org/wiki/List_of_bacterial_orders.

1. Gracilicutes: Thin skin (Gram-negative Eubacteria)

- **Anoxyphotobacteriae**: Photosynthetic bacteria that do not produce oxygen
- **Photobacteriae**: Cyanobacteria
- **Scotobacteriae**: Non-photosynthetic bacteria

2. Firmicutes: Thick skin (Gram-positive Eubacteria)

- **Firmibacteriae**: Low G+C content
- **Thallobacteriae**: High G+C content, branched bacteria

3. Tenericutes: Soft skin (Wall-less Eubacteria, Gram-negative)

- **Mollicutes**

4. Mendosicutes: Defective skin

- **Archaebacteriae**: Archaeobacteria (Gram-positive/negative)

In the modern edition, with the introduction of **polyphasic classification**, prokaryotes are now divided into two distinct domains: **Eubacteria** and **Archaeobacteria**,(**(Table 4)** comprising **35 phyla** in total: **30 for Eubacteria and 5 for Archaeobacteria**.

Table 3: The 35 Phyla of Prokaryotes and Some Classes and Subclasses

Domaine Archaea	Domaine Eubacteria (I-XIII)	Domaine Eubacteria (XIV-XXII)	Domaine Eubacteria (XXIII-XXX)
AI. Crenarchaeota	BI. Aquificae	BXIV. Firmicutes	BXXIII. Fusobacteria
AII. Euryarchaeota	BII. Thermotogae	CI. Clostridia	BXXIV. Verrucomicrobia
AIII. Korarchaeota	BIII. Thermodesulfo-bacteria	CII.Negativicutes	BXXV. Gemmatimonadetes
AIV. Nanoarchaeota	BIV. Deinococcus-Thermus	CIII. Bacilli	BXXVI. Lentisphaerae
AV. Thaumarchaeota	BV. Chrysiogenetes	CIV.Thermolithobacteria	BXXVII. Dictyoglomi
	BVI. Chloroflexi	CV. Erysipelotrichi	BXXVIII. Caldiserica
	BVII. Thermomicrobia	BXV. Tenericutes	BXXIX. Elusimicrobia
	BVIII. Nitrospirae	BXVI. Actinobacteria	BXXX. Armatimonadetes
	BIX. Deferribacteres	SCI. Acidimicrobidae	
	BX. Synergistetes	SCII. Rubrobacteridae	
	BXI. Cyanobacteria	SCIII. Coriobacteridae	
	BXII. Chlorobi	SCIV.Actinobacteridae	
	BXIII. Proteobacteria	SCV. Nitriliruptoridae	
	CI. Alpha-Proteobacteria	BXVII. Planctomycetes	
	CII.Beta-Proteobacteria	BXVIII. Chlamydiae	
	CIII. Gamma-Proteobacteria	BXIX. Spirochaetes	
	CIV. Delta-Proteobacteria	BXX. Fibrobacteres	
	CV. Epsilon-Proteobacteria	BXXI. Acidobacteria	
	CVI. Zeta-Proteobacteria	BXXII. Bacteroidetes	
		CI. Bacteroidetes	
		CII. Flavobacteria	
		CIII. Sphingobacteriia	
		CIV. Cytophagia	

Table 4: Differences between Archaea and Bacteria.

Basis of Comparison	Archaea	Bacteria
Definition	A group of primitive prokaryotes forming a distinct domain from bacteria and eukaryotes.	Unicellular organisms that vary in shape, size, structure, and habitat.
Phylogeny	More closely related to eukaryotes at the molecular and genetic levels (share similarities in transcription and translation).	More distantly related to eukaryotes and phylogenetically distinct from archaea.
Habitat	Mostly extremophiles, found in extreme environments such as deep-sea vents, hot springs, mountains, brine, etc.	Found in various habitats, including soil, water, and inside living and non-living organisms.
Cell Wall	Composed of pseudopeptidoglycan, lacking D-amino acids and N-acetylmuramic acid.	Composed of peptidoglycan with N-acetylmuramic acid and D-amino acids.
Membrane Lipids	Fatty acids in membrane lipids are linked to glycerol via ether bonds.	Fatty acids in membrane lipids are linked to glycerol via ester bonds.
Glucose Oxidation	Do not use glycolysis or the Krebs cycle but follow similar metabolic pathways.	Glycolysis and the Krebs cycle are key metabolic pathways for glucose oxidation.
Photosynthesis	Do not perform oxygen-generating photosynthesis but are phototrophic, using sunlight as an energy source.	Many bacteria with photosynthetic pigments can perform photosynthesis to produce their own food.
Types	Divided into methanogens, thermophiles, and halophiles based on characteristics.	Divided into Gram-negative and Gram-positive bacteria based on Gram staining.
Flagella	Archaeal flagella (archaella) are synthesized by adding subunits at the base.	Bacterial flagella are hollow and assembled by adding subunits from the central pore to the tip.
Reproduction	Reproduce by fission, budding, and fragmentation. Do not undergo sporulation.	Some bacteria can form spores to survive extreme conditions for extended periods.
tRNA	Thymine is absent in archaeal tRNA.	Thymine is present in bacterial tRNA.
tmRNA	Transfer-messenger RNA (tmRNA) is present in archaea.	tmRNA is present in bacteria.
Chromosomes	Introns are present in archaeal chromosomes.	Introns are absent in bacterial chromosomes.
RNA Polymerase	Complex, with more than eight polypeptides, and some archaea may have multiple RNA polymerases.	Simple, with four polypeptides.
Pathogenicity	Not pathogenic.	Can be pathogenic or non-pathogenic.
Examples	*Thermosphaera aggregans, Staphylothermus marinus, Sulfolobus tokodaii*.	*Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Salmonella Typhi*.

2.2 Study of photosynthetic bacteria

2.2.1 Definition:

Photosynthetic bacteria are organisms that convert light into chemical energy, often producing ATP or organic molecules.

- **Purpose**: To understand the differences between photosynthesis in bacteria and in plants/algae.
  - **Oxygenic photosynthesis** (producing oxygen, as in plants).
  - **Anoxygenic photosynthesis** (no oxygen production, but other compounds like sulfur can be used).

2.2.2 Types of Photosynthetic Bacteria

A. Oxygenic Photosynthesis

- **Organisms**: Cyanobacteria (e.g., *Synechococcus*, *Anabaena*).
- **Main pigment**: **Chlorophyll a** (similar to that of plants).
- **Process**:
  - Degradation of water (H_2O) to produce oxygen (O_2).
  - Use of light energy to produce ATP and NADPH.

B. Anoxygenic Photosynthesis

- **Organisms**: Purple bacteria, green bacteria (e.g., *Chromatium*, *Chlorobium*).
- **Main pigment**: **Bacteriochlorophyll** (different from chlorophyll).
- **Process**:
  - Use of electron donors like **sulfur (H_2S)** or other organic compounds.
  - No oxygen production.

2.2.3 Mechanisms of Photosynthetic Bacteria

- **Photosynthetic Pigments**: These pigments capture light energy and convert it into chemical energy.
  - **Bacteriorhodopsin**: Used by some archaea and bacteria to capture light and generate a proton gradient.
  - **Bacteriochlorophylls**: Found in purple and green bacteria, these pigments capture light at specific wavelengths.
- **Electron Transport System**:
  - Excited electrons from light pass through an electron transport chain.
  - This generates a proton gradient (H^+) that is used to produce ATP via **phosphorylation**.

- **Chemical Process**:
    1. **Excitation of electrons** by light.
    2. **Electron transport** through the electron transport chain.
    3. **ATP production** through a proton gradient.
    4. **Formation of NADPH** or **sulfur reduction** depending on the type of photosynthesis.

2.2.4 Examples of Photosynthetic Bacteria

- **Cyanobacteria**:
    - Examples: *Synechococcus*, *Anabaena*.
    - Importance: These bacteria perform **oxygenic photosynthesis**, contributing to the production of oxygen on Earth.
- **Purple Bacteria**:
    - Examples: *Chromatium*.
    - Importance: These bacteria perform **anoxygenic photosynthesis**, using sulfur as an electron donor.
- **Green Bacteria**:
    - Examples: *Chlorobium*.
    - Importance: Like purple bacteria, they perform photosynthesis without oxygen production, using sulfur as an electron donor.

Chapter III : Major Bacterial Phyla According to Bergey's Manual Classification(Phylum Proteobacteria)

3.1 Introduction

Proteobacteria is a vast and diverse bacterial phylum comprising five major classes: **α (Alpha), β (Beta), γ (Gamma), δ (Delta), ε (Epsilon)**. It includes 45 orders and over 500 genera(Figure 9). Some bacteria in this group are also referred to as **purple bacteria**. **Proteobacteria** are **Gram-negative** bacteria exhibiting a wide range of morphologies, including rods, cocci, prosthecate bacteria, budding bacteria, and bacteria forming fruiting bodies. This phylum includes many **important pathogens** (*Escherichia coli*, *Yersinia*, *Helicobacter*), as well as **nitrogen-fixing bacteria**. Most are **motile** via flagella, while some are **non-motile** or move by **gliding** (*e.g.*, *Myxobacteria*). Metabolically, most are **heterotrophic**, but some, such as **purple bacteria**, are **autotrophic** and **photosynthetic**.

3.2 The Class α-Proteobacteria

This class includes two major functional groups:

- **Non-sulfur purple bacteria**
- **Nitrifying bacteria**

α-Proteobacteria are **oligotrophic**, capable of growing in nutrient-poor environments. Some possess unique metabolic pathways such as **methylotrophy** and **nitrogen fixation**. They include agriculturally important bacteria (**Rhizobium**, nitrogen-fixing symbionts) and pathogens such as **Rickettsia typhi** (murine typhus) and **Agrobacterium tumefaciens** (causes crown gall disease in plants).

3.2.1 Non-Sulfur Purple Bacteria

With the exception of *Rhodocyclus* (**β-Proteobacteria**), these bacteria are classified under **α-Proteobacteria**. They exhibit diverse morphologies and can grow **photoorganotrophically heterotrophically** under anaerobic conditions. In the absence of light, most can grow **aerobically** as **chemoorganotrophic heterotrophs**, while some use **fermentation** under anaerobic conditions. They are primarily found in **lake sediments, ponds, and organic-rich environments** with low sulfide concentrations.

3.2.2 Nitrifying Bacteria

Nitrifying bacteria are **aerobic and non-spore-forming**. They are classified into several taxa:

- **Alphaproteobacteria**: *Nitrobacter* (**Bradyrhizobiaceae** family) is **motile, aerobic, and chemolithotrophic**.
- **Betaproteobacteria**: *Nitrosomonas* and *Nitrosospira* (**Nitrosomonadaceae** family).
- **Gammaproteobacteria**: *Nitrococcus* (**Ectothiorhodospiraceae**) and *Nitrosococcus* (**Chromatiaceae**).
- **Nitrification process**:
    1. *Nitrosomonas* oxidizes ammonia (**NH_3**) to nitrite (**NO_2^-**).
    2. *Nitrobacter* oxidizes nitrite to nitrate (**NO_3^-**), a form readily assimilated by plants.

3.3 The Class β-Proteobacteria

This class shares some characteristics with **α-Proteobacteria** but primarily utilizes nutrients from **anaerobic decomposition zones** (hydrogen, ammonia, methane). It exhibits significant metabolic diversity and includes several human pathogens.

a. **Neisseriales**: Non-motile, aerobic cocci, sometimes encapsulated (*Neisseria gonorrhoeae, Neisseria meningitidis*).
b. **Burkholderiales**: Aerobic bacilli with respiratory metabolism (*Bordetella pertussis*, causes whooping cough).
c. **Hydrogenophilales**: Sulfur-oxidizing bacteria contributing to soil fertility (*Thiobacillus*).

3.4 The Class γ-Proteobacteria

This class represents the most diverse group within **Proteobacteria**. It includes nitrifying bacteria (*Nitrosomonas, Nitrobacter*), sulfur bacteria (*Chromatiaceae*), and major bacterial orders such as **Enterobacterales, Pseudomonadales, Vibrionales**.

a. Purple Sulfur Bacteria

These bacteria belong to the order **Chromatiales** and are divided into two families: **Chromatiaceae** and **Ectothiorhodospiraceae**. They are **strict anaerobes,**

photolithoautotrophic, and oxidize **hydrogen sulfide**, storing sulfur intracellularly as granules.

b. Colorless Sulfur Bacteria

Thiobacillus (order **Thiotrichales**) oxidizes various sulfur compounds and thrives in sulfur-rich environments such as hot springs, salt marshes, and marine sediments.

c. The Order Vibrionales

Facultatively anaerobic, motile **Gram-negative** rods, oxidase- and catalase-positive (*Vibrio cholerae*, cholera-causing pathogen).

d. The Order Enterobacterales

Straight rods, motile or non-motile, **facultatively anaerobic**, oxidase-negative, catalase-positive (*Escherichia coli, Klebsiella, Salmonella*).

e. The Order Pseudomonadales

Includes *Pseudomonas spp.*, opportunistic bacteria with diverse metabolic capabilities (*Pseudomonas aeruginosa*).

3.5 The Class δ-Proteobacteria

This class consists of **chemoheterotrophic** and **sulfur-reducing** bacteria. It includes **Myxobacteria**, known for their predatory lifestyle, *Bdellovibrio* (parasitic on Gram-negative bacteria), and *Desulfovibrio* (sulfate reducers).

3.6 The Class ε-Proteobacteria

The smallest class within the phylum, **ε-Proteobacteria** includes several slender, curved, or helical rod-shaped bacteria. This group consists mainly of microaerophilic and pathogenic species, particularly within the genera *Campylobacter* and *Helicobacter*.

- *Helicobacter pylori* is a major cause of **peptic ulcers** and a significant risk factor for **gastric cancer**.
- *Campylobacter jejuni* is a leading cause of **bacterial gastroenteritis** in humans, often linked to **contaminated poultry**.

- Other *Campylobacter* species have been associated with **zoonotic infections** in domestic and wild animals.

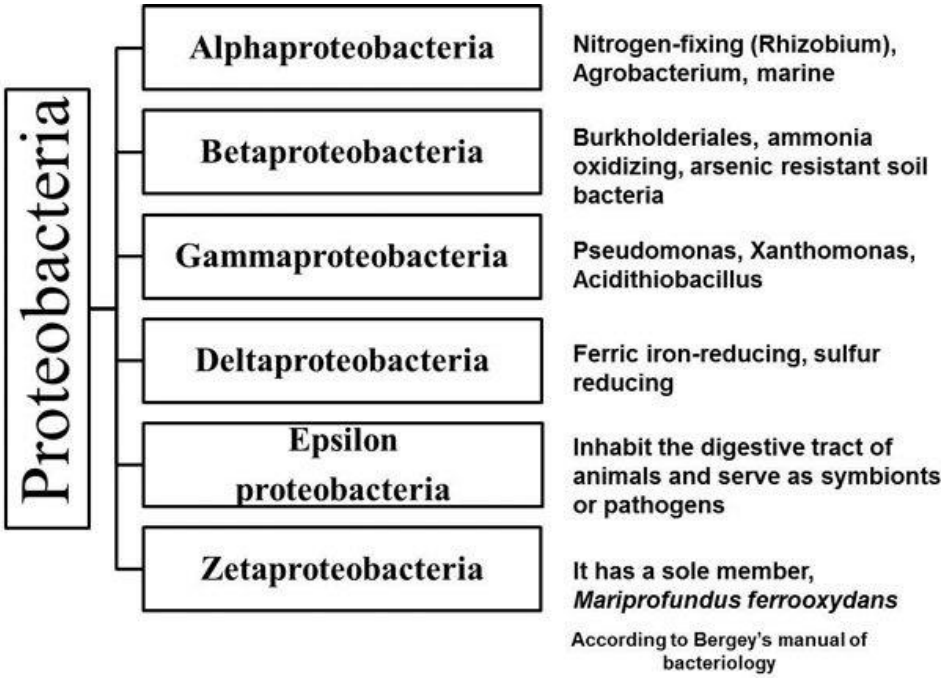


Figure 9 : The subgroups of proteobacteria and the main members of each subgroup

(Verma et Melcher , 2012).

3.7 Conclusion

Proteobacteria play an essential role in diverse ecosystems due to their vast metabolic capabilities. Their involvement in **nutrient cycling, bioremediation, agriculture, and human health** highlights their ecological and biomedical importance. The pathogenic members of this phylum underscore the need for ongoing research to better understand their interactions with hosts and develop new strategies for disease control and prevention. Further studies on their **genetics, evolution, and potential biotechnological applications** will continue to expand our knowledge of this critical bacterial phylum.

Chapter IV: The Phyla of Archaea

4.1 Introduction

Archaea are microorganisms adapted to extreme environments such as high salinity, elevated temperatures, low pH, and anoxic conditions. These extreme conditions resemble those of early Earth when life first emerged. While archaea are frequently found in extreme aquatic and terrestrial habitats (e.g., hypersaline environments, extreme pH, and high temperatures), many also thrive in more moderate environments, including soil, lakes, oceans, and even within the human body (e.g., methanogens in the intestines). Their growth temperatures range from psychrophilic to hyperthermophilic. Archaea exhibit diverse morphologies, including spherical, rod-shaped, spiral, lobed, cuboid, triangular, flattened, irregular, or pleomorphic forms. They may exist as isolated cells or form aggregates and can be aerobic, facultatively anaerobic, or strictly anaerobic. Their trophic modes range from chemolithoautotrophy to organotrophy, with some species displaying phototrophic capabilities.

4.2 Major Groups of Archaea

4.2.1 Extreme Halophiles:

These archaea require at least 9% salt for survival and can tolerate alkaline pH levels up to 11.5. They inhabit saline lakes, salt flats, marine sand, salt marshes, and brine pools. Some have been isolated from salt-preserved foods such as fish. Notably, extreme halophiles are the only archaea capable of photosynthesis, Their purple color is due to the presence of an archaeal pigment: **archaeorhodopsin**. Phototrophic archaea contain either **archaeorhodopsin** or carotenoids. Representatives of the Euryarchaeota class, order Halobacteria: *Halobacterium*, *Halococcus*, *Haloarcula*, *Haloferax.* These halophilic archaea are found in salt lakes (e.g., Salt Lake) and seas (e.g., the Dead Sea).

4.2.2 Extreme Thermophiles:

These archaea thrive at temperatures of 80°C or higher. They are typically anaerobic and acid-resistant. Examples include *Pyrodictium occultum* (optimal growth at 105°C) and *Sulfolobus sp.* (87–90°C, pH ~1). These archaea are often thermoacidophiles, living in geothermally heated water and sulfur-rich soils.

4.2.3 Methanogens:

These strictly anaerobic archaea, such as *Methanobacterium*, produce methane (CH4) from carbon dioxide (CO2). Found in the digestive tracts of ruminants, methanogens release 1–2

billion metric tons of methane annually, contributing to the greenhouse effect and global warming. They are also present in sediments, thermal springs, and wastewater treatment plants.

4.3 Classification of Archaea

Based on genetic comparisons (such as 16S rRNA gene analysis), archaea are classified into five phyla (Table 5):

4.3.1 Crenarchaeota

These include thermophilic and hyperthermophilic species, which can be autotrophic or heterotrophic, strictly anaerobic, or microaerophilic. Many are acidophilic and sulfur-dependent, using sulfur either as an electron acceptor in anaerobic respiration or as an electron source in lithotrophy.

4.3.1.1 Thermoproteus

- Rod-shaped archaea, sometimes curved or branched.
- Strictly anaerobic.
- Thermoacidophilic: optimal growth at 70–97°C and pH 2.5–6.5.
- Found in hot, sulfide-rich aquatic environments.
- Metabolism: chemo-organotrophic or chemolithotrophic.

4.3.1.2 Sulfolobus

- Spherical, lobed archaea (**Figure 10**).
- Aerobic (exceptional among archaea).
- Thermoacidophilic: optimal growth at 70–80°C and pH 2–3.
- Found in acidic hot springs and soils.
- Metabolism: chemo-organotrophic or chemolithotrophic.
- Uses oxygen as the primary electron acceptor but can also utilize ferric iron.

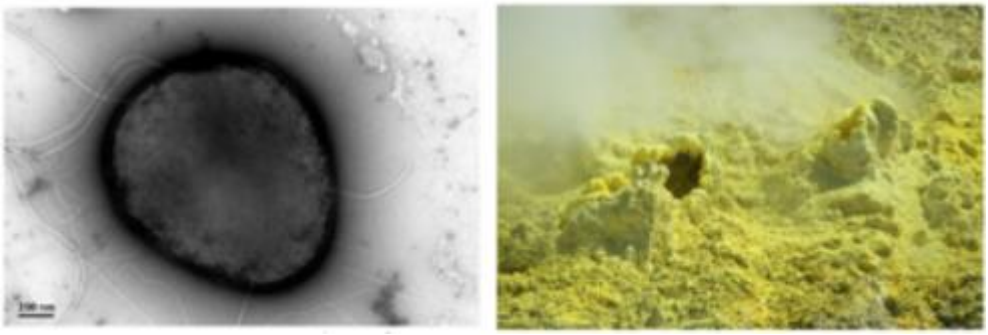


Figure 10 : *Sulfolobales* (65-90 °C, pH 1-3,5)

4.3.2 Euryarchaeota

Euryarchaeota are ecologically diverse and include methanogens, extreme halophiles, and extreme thermophiles. With numerous sequenced genomes, this phylum is the most well-characterized. *Haloferax volcanii* is a notable laboratory model.

4.3.2.1 Methanogens

- Rod-shaped or coccoid archaea.
- Strictly anaerobic (**Figure 11**).
- The largest group of archaea, comprising five orders (*Methanobacteriales, Methanococcales, Methanomicrobiales, Methanosarcinales,* and *Methanopyrales*).
- Found in anaerobic, organic-rich environments.
- Produce large quantities of methane, a greenhouse gas.
- Some oxidize iron, contributing to metal corrosion.

Some are found in the rumen of cattle .

Others in marine sediments, rice fields, marshes, or wastewater

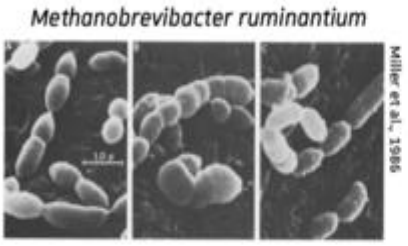


Figure 11 : Methanogens.

4.3.2.2 Extreme Halophiles

- *Halobacteria* class, *Halobacteriales* order, *Halobacteriaceae* family (Figure **12**).
- Require at least 1.5 M NaCl for survival; optimal growth at 3–4 M NaCl.
- Aerobic, chemo-organotrophic, and typically mesophilic.
- Found in salt marshes and hypersaline aquatic environments.
- May cause spoilage of salt-preserved foods.

Figure 12: **1**. Salt Lake, Californie 2. Halobacterium salibarum (Alain et al., 2024)

4.3.2.3 Thermoplasmata

- *Thermoplasmata* class, *Thermoplasmatales* order (*Thermoplasmataceae, Picrophilaceae, Ferroplasmaceae* families).
- Thermoacidophilic: optimal growth at 55–59°C, pH 1–2.
- Facultative anaerobes.

- Chemo-organotrophic.
- Found in coal mine waste.
- Morphology varies with temperature (filamentous at 59°C, spherical at lower temperatures).
- Lack a cell wall; membrane stabilized by diglycerol tetraethers, lipopolysaccharides, and glycoproteins.

4.3.2.4 Thermococci

- Class: Thermococci, Order: Thermococcales, Family: Thermococcaceae.
- Obligate thermophiles (70–110°C) (Figure 13).
- Acidophilic or neutrophilic.
- Strict anaerobes.
- Reduce sulfur to sulfide.

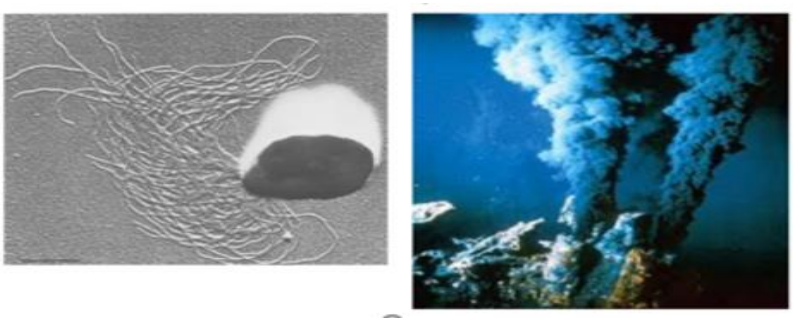


Figure 13: *Pyrococcus furiosus (Thermococci)* (100 °C, pH 7)(Alain et al.,2024).

4.3.2.5 Archaeoglobi

- Class: Archaeoglobi, Order: Archaeoglobales, Family: Archaeoglobaceae.
- **Sulfate-Reducing Archaea**
- Irregular spherical cells.
- Extreme thermophiles.
- Anaerobes.
- Can be autotrophic or heterotrophic.
- Reduce sulfate and thiosulfate to sulfide.

4.3.3 Nanoarchaeota and Korarchaeota

- *Nanoarchaeum equitans* is the only known representative of Nanoarchaeota.

- The smallest known living cell (~490 kb genome, ~400 genes).
- Obligate symbiont/parasite of hyperthermophilic *Ignicoccus hospitalis*.
- Evolved more rapidly than other archaea, a trait common among symbiotic and parasitic organisms.
- Korarchaeota, an ancient divergent lineage of Crenarchaeota, are primarily found in hot springs.
- No pure culture isolates of Korarchaeota exist.

4.3.4 Thaumarchaeota

- The most ancient archaeal lineage.
- Contains *Nitrosopumilales* and *Cenarchaeales*.
- Unique among archaea for possessing a class IB DNA topoisomerase, closely related to eukaryotic enzymes.
- This similarity suggests a potential evolutionary link between archaea and eukaryotes.

4.4 Conclusion

Archaea are fundamental to many ecological and biogeochemical processes. Their ability to thrive in extreme environments provides insights into the adaptability of life and the potential for extraterrestrial organisms. Methanogens contribute significantly to methane emissions, impacting climate change. Extreme halophiles influence salt cycling, while hyperthermophiles offer valuable enzymes for industrial and medical applications. Continued research on archaea will not only enhance our understanding of evolutionary biology but also open new avenues in biotechnology, medicine, and environmental sustainability.

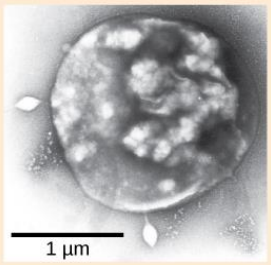
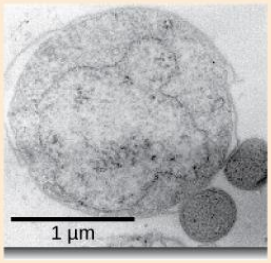
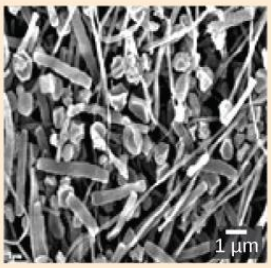
4.5 Perspectives and recent Approaches

In addition to the classical approaches described in this handout, recent advances in microbiology have introduced new methods for the classification of microorganisms. Among them, **phylogenomics**, which is based on the comparison of whole genomes, provides more robust and reliable phylogenetic trees than those built on a single or few genes (e.g., 16S rRNA).

Another innovative approach is **metagenomics**, which involves the direct analysis of DNA extracted from environmental samples (soil, water, gut microbiota, etc.) without the need for isolation and cultivation of microorganisms. This method reveals a large part of microbial diversity that remains inaccessible through traditional methods.

These modern tools complement classical phenotypic and molecular approaches and open new perspectives for a better understanding of microbial systematics and ecology.

Table 5: Archaea are separated into four phyla: the Korarchaeota, Euryarchaeota, Crenarchaeota, and Nanoarchaeota. (credit "Halobacterium": modification of work by NASA; credit "Nanoarchaeotum equitans": modification of work by Karl O. Stetter; credit "korarchaeota": modification of work by Office of Science of the U.S. Dept. of Energy; scale-bar data from Matt Russell).

Archaea		
Phylum	**Representative organisms**	**Representative micrograph**
Euryarchaeota This phylum includes methanogens, which produce methane as a metabolic waste product, and halobacteria, which live in an extreme saline environment.	*Methanogens* Methane production causes flatulence in humans and other animals. *Halobacteria* Large blooms of this salt-loving archaea appear reddish due to the presence of bacterirhodopsin in the membrane. Bacteriorhodopsin is related to the retinal pigment rhodopsin.	 *Halobacterium* strain NRC-1
Crenarchaeota Members of the ubiquitous phylum play an important role in the fixation of carbon. Many members of this group are sulfur-dependent extremophiles. Some are thermophilic or hyperthermophilic.	*Sulfolobus* Members of this genus grow in volcanic springs at temperatures between 75° and 80°C and at a pH between 2 and 3.	 *Sulfolobus* being infected by bacteriophage
Nanoarchaeota This group currently contains only one species, *Nanoarchaeum equitans*.	*Nanoarchaeum equitans* This species was isolated from the bottom of the Atlantic Ocean and from a hydrothermal vent at Yellowstone National Park. It is an obligate symbiont with *Ignicoccus*, another species of archaea.	 *Nanoarchaeum equitans* (small dark spheres) are in contact with their larger host, *Ignicoccus.*
Korarchaeota Members of this phylum, considered to be one of the most primitive forms of life, have only been found in the Obsidian Pool, a hot spring at Yellowstone National Park.	No members of this species have been cultivated.	 This image shows a variety of korarchaeota species from the Obsidian Pool at Yellowstone National Park.

Glossary

- **Acidophile**

Organism with optimal growth pH of three or below

- **Alkaliphile**

Organism with optimal growth pH of nine or above

- **Ammonification**

Process by which ammonia is released during the decomposition of nitrogen-containing organic compounds

- **Anaerobic**

Refers to organisms that grow without oxygen

- **Anoxic**

Without oxygen

- **Antibiotic**

Biological substance that, in low concentration, is antagonistic to the growth of prokaryotes

- **Biofilm**

A microbial community growing together on a surface, often held together with a gummy matrix

- **Biological nitrogen fixation**

Conversion of atmospheric nitrogen into ammonia exclusively carried out by prokaryotes

- **Bioremediation**

Use of microbial metabolism to remove pollutants

- **Biotechnology**

Any technological application that uses living organisms, biological systems, or their derivatives to produce or modify other products

- **Black Death**

Devastating pandemic that is believed to have been an outbreak of bubonic plague caused by the bacterium *Yersinia pestis*

- **Botulism**

Disease produced by the toxin of the anaerobic bacterium *Clostridium botulinum*

- **CA-MRSA**

MRSA acquired in the community rather than in a hospital setting

- **Capsule**

External structure that enables a prokaryote to attach to surfaces and protects it from dehydration

- **Chemotroph**

Organism that obtains energy from chemical compounds

- **Conjugation**

Process by which prokaryotes move DNA from one individual to another using a pilus

- **Cyanobacteria**

Bacteria that evolved from early phototrophs and oxygenated the atmosphere; also known as blue-green algae

- **Decomposer**

Organism that carries out the decomposition of dead organisms

- **Denitrification**

Transformation of nitrate from soil to gaseous nitrogen compounds such as N_2O, NO and N_2

- **Emerging disease**

Disease making an initial appearance in a population or that is increasing in incidence or geographic range

- **Endemic disease**

Disease that is constantly present, usually at low incidence, in a population

- **Epidemic**

Disease that occurs in an unusually high number of individuals in a population at the same time

- **Extremophile**

Organism that grows under extreme or harsh conditions

- **Foodborne disease**

Any illness resulting from the consumption of contaminated food, or of the pathogenic bacteria, viruses, or other parasites that contaminate food

- **Gram negative**

Bacterium whose cell wall contains little peptidoglycan but has an outer membrane

- **Gram positive**

Bacterium that contains mainly peptidoglycan in its cell walls

- **Halophile**

Organism that require a salt concentration of at least 0.2 M

- **Hydrothermal vent**

  Fissure in Earth's surface that releases geothermally heated water

- **Hyperthermophile**

  Organism that grows at temperatures between 80–122 °C

- **Microbial mat**

  Multi-layered sheet of prokaryotes that may include bacteria and archaea

- **MRSA**

  Methicillin-resistant *Staphylococcus aureus* very dangerous *Staphylococcus aureus* strain resistant to multiple antibiotics

- **Nitrification**

Conversion of ammonium into nitrite and nitrate in soils

- **Nitrogen fixation**

Process by which gaseous nitrogen is transformed, or "fixed" into more readily available forms such as ammonia

- **Nodule**

  Novel structure on the roots of certain plants (legumes) that results from the symbiotic interaction between the plant and soil bacteria, is the site of nitrogen fixation

- **Nutrient**

Essential substances for growth, such as carbon and nitrogen

- **Osmophile**

Organism that grows in a high sugar concentration

- **Pandemic**

  Widespread, usually worldwide, epidemic disease

- **Peptidoglycan**

Material composed of polysaccharide chains cross-linked to unusual peptides

- **Phototroph**

  Organism that is able to make its own food by converting solar energy to chemical energy

- **Pilus**

Surface appendage of some prokaryotes used for attachment to surfaces including other prokaryotes

- **Pseudopeptidoglycan**

  Component of archaea cell walls that is similar to peptidoglycan in morphology but contains different sugars

- **Psychrophile**

Prganism that grows at temperatures of -15 °C or lower

- **Radioresistant**

  Organism that grows in high levels of radiation

- **Resuscitation**

  Process by which prokaryotes that are in the VBNC state return to viability

- **S-layer**

Surface-layer protein present on the outside of cell walls of archaea and bacteria

- **Serotype**

  Strain of bacteria that carries a set of similar antigens on its cell surface, often many in a bacterial species

- **Stromatolite**

  Layered sedimentary structure formed by precipitation of minerals by prokaryotes in microbial mats

- **Teichoic acid**

  Polymer associated with the cell wall of Gram-positive bacteria

- **Thermophile**

  Organism that lives at temperatures between 60–80 °C

- **Transduction**

  Process by which a bacteriophage moves DNA from one prokaryote to another

- **Transformation**

  Process by which a prokaryote takes in DNA found in its environment that is shed by other prokaryotes

- **Viable-but-non-culturable (VBNC) state**

  Survival mechanism of bacteria facing environmental stress conditions

- **Zoonosis**

  Disease that primarily infects animals that is transmitted to humans

- **Proteobacteria**

Is a phylum of gram-negative bacteria that are classified as alpha-, beta-, gamma-, delta- and epsilonproteobacteria.

- **Alphaproteobacteria** are **oligotrophs**.

The taxa chlamydias and rickettsias are **obligate intracellular pathogens,** feeding on cells of host organisms; they are metabolically inactive outside of the host cell. Some Alphaproteobacteria can convert atmospheric nitrogen to nitrites, making nitrogen usable by other forms of life.

- **Betaproteobacteria** are **eutrophs**.

They include human pathogens of the genus *Neisseria* and the species *Bordetella pertussis*.

- **Gammaproteobacteria**

Are the largest and the most diverse group of Proteobacteria. Many are human pathogens that are aerobes or facultative anaerobes. Some Gammaproteobacteria are **enteric** bacteria that may be coliform or noncoliform. *Escherichia coli*, a member of Gammaproteobacteria, is perhaps the most studied bacterium.

- **Deltaproteobacteria**

Make up a small group able to reduce sulfate or elemental sulfur. Some are scavengers and form myxospores, with multicellular fruiting bodies.

- **Epsilonproteobacteria**

Make up the smallest group of Proteobacteria. The genera *Campylobacter* and *Helicobacter* are human pathogens.

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Abstract

This document provides a synthesis of the fundamental principles of microorganism classification, addressing systematics, bacterial and archaeal diversity, as well as the main differentiation criteria used in microbiology. The objective is to offer a structured and updated overview of microbial taxonomy, based on modern approaches and internationally recognized references. The first section introduces the foundations of microbial systematics, detailing key concepts, taxonomic approaches, and classification criteria. The second section describes the major bacterial and archaeal groups, highlighting their physiological, morphological, and ecological characteristics. The third section focuses on the classification of major bacterial phyla according to Bergey's Manual, a cornerstone reference in microbiology, with particular emphasis on Proteobacteria and their classes (Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Epsilonproteobacteria). The fourth section explores the diversity and classification of archaea, presenting the five major phyla (Euryarchaeota, Crenarchaeota, Korarchaeota, Nanoarchaeota, Thaumarchaeota) and their ecological significance. By integrating theoretical foundations with recent taxonomic advances, this work highlights the richness and complexity of the microbial world. It provides an essential framework for understanding the organization of microbial biodiversity and the evolutionary relationships between bacteria and archaea, while opening perspectives for their exploitation in scientific and biotechnological applications.

Keywords

Bacterial taxonomy ; Microbial systematics ;Bergey's Manual ; Proteobacteria ; Archaea ; Microbial diversity ; Classification ;Microbial biotechnology ; Microorganisms