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Course Handout

Systematics of Prokaryotes

(Bacteria and Archaea)

Presented by

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Intended for students:

- 3rd year License in Microbiology

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Preface

The Course Handout, "Systematics of Prokaryotes (Bacteria and Archaea)," is designed to provide a comprehensive and up-to-date understanding of the classification and diversity of the microbial world for third-year students in Microbiology and Microbial Biotechnology programs. Recent years have seen significant evolution of the field of prokaryotic systematics, allied with modern molecular techniques and increasing knowledge of evolutionary relationships among microorganisms.

The goal of this course is to give students the fundamental knowledge and tools they need to navigate the tumultuous and ever-changing world of bacterial and archaeal taxonomy. It consists of five chapters, each decanted to explain a fundamental facet of prokaryotic systematics in the Course Handout.

Chapter I: This combination of historical development of prokaryotic classification; importance of systematics; fundamentals and methods of prokaryotic taxonomy identification; and categorization is intended to lay a foundation from which introduction to Systematics of Prokaryotes proceeds.

Chapter II: The modern, multifaceted approach to prokaryotic taxonomy, known as polyphasic taxonomy, is covered in Current Approach of Bergey's Manual of Systematic Bacteriology. Helping to integrate elements of phenotypic, genotypic across phylogenetic data for the classification of microorganisms is the emphasis, with the Bergey's Manual of Systematic Bacteriology being presented as a strong reference.

Chapter III: Comprehensive coverage of Major bacterial phylums with distinctities, their ecological and Biotechnology significance. Students will have a useful understanding of the amazing diversity and adaptability of the bacterial domain.

Chapter IV: In depth, *Proteobacteria* explores the *Proteobacteria*, one of the most diverse and ecologically significant bacterial phyla.

Chapter V: studies Archaea as unique domain, often referred to as the "third domain of life," is covered in This fascinating group of microorganisms is explored with respect to its distinctive characteristic, evolutionary relationships and ecological and biotechnological importance.

Alegria, MAI 2024 **M. El-Hadj DRICHE**

Chapter IV: ***Archaea***

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General

Introduction

1-Introduction

Prokaryotic systematics has known considerable development since its creation. Man has tried to classify living organisms, including prokaryotes, since the time of Aristotle. Still, the first formal classification did not appear until the 18th century when Carl Linnaeus developed it with binomial nomenclature, which is still used today. Nonetheless, from this proposition, more prokaryotic groups have identity issues due to their microscopic size, such as their metabolic capabilities (Sapp, 2005; Rosselló-Móra and Amann, 2015). It was only with the beginnings of the use of microscopy in the late 19th century and its subsequent development by Robert Koch and his contemporaries that bacterial culture techniques were finally developed that became possible to isolate and identify bacteria based on their morphology and physiological traits. However, the methods were limited to observable characteristics because the bacteria had to be culturable (Gradmann, 2001;Whitman, 2016).

It was the beginning of the molecular era, which brought about a complete change. Carl Woese's studies in rRNA sequencing in the 1970s highlighted the evolutionary gap between bacteria and archaea. This led to a new classification where the three domains of life were defined (Woese & Fox, 1977). Therefore, this system separated prokaryotes into two domains (archaea and bacteria), and is now considered the biological classification base (Kitahara and Miyazaki, 2013). In addition, the resulting advancement of the polymerase chain reaction (PCR) methodology facilitated the expeditious amplification and sequencing of distinct regions of DNA obtained from very small samples. This innovation would later provide a basis for metagenomics, through which direct sequencing of DNA from environmental samples could be possible without cultivation, providing a unique opportunity for scientists to study the genetic diversity of the members constituting the microbial communities in their natural habitats (Handelsman et al., 1998; Yarza etal., 2014).

On the other hand, the recent combination of genomic data with computer tools has made it possible to improve phylogenetic trees, which describe relationships not on phenotypic characteristics but on a genetic level by considering evolution (Koonin et al ., 2012).

In recent years, there has been an increase in the significance of prokaryotic systematics, which investigates the classification, nomenclature, and evolutionary interactions among prokaryotic microorganisms. The increase can be related to the growing understanding of the critical role microbes play in diverse ecosystems and the potential medical, environmental, and technological applications of microbiology (Pace, 2009).

Furthermore, significant developments have been made in this area by developing new methodological strategies and analytical instruments to analyze the enormous amounts of data produced by high-throughput DNA sequencing technologies. There are many thousands of different species and variants have been laboriously identified, and yet more remain out there to be found and described (Burstein et al., 2017; Konstantinidis et al., 2017).

As a solution to this problem, scientists have developed a multitude of techniques and strategies, including genomic, transcriptomic, and proteomic data available for microorganisms. This has really made it possible to draw the whole structure to understand the genetic relationship between prokaryotes. It will also allow for the reconstruction of the phylogenetic tree of these organisms and carry out comparative genomic research for both fundamental and accessory regions (Parks et al., 2017; Madigan et al., 2019).

Introduction To Systematics of Prokaryotes

1.-Overview of the taxonomy of prokaryote

Taxonomy (from the greek words taxis=arrangement or order, and nemein=to distribute or govern) is the science of the classification of various living organisms. In the case of prokaryotic taxonomy, it is the science of classification and nomenclature of bacteria and archaea that has recently made profound progress. The primary role of prokaryotic classification is to define a robust and useful system that can be applied to the enormous diversity of microscopic life to place them in a natural group that follows their evolutionary lines and the characteristics they share (Parker et al., 2015). At the highest level, the three-domain system divides all life into the domains archaea, bacteria, and eukaryotes, showing a deep separation between these three fundamental branches of the tree of life (Woese et al., 1990). In the fields of archaea and bacteria, taxonomists are increasingly developing precise classifications of prokaryotes into phyla, classes, orders, families, genera and species. the International Committee on Systematics of Prokaryotes (ICSP), has a journal specializing in the field of bacterial taxonomy, this is the International Journal of Systematic and Evolutionary Microbiology or IJSEM. This committee deals with everything related to bacterial taxonomy, and any classification is only valid if it is approved by this committee and published in its journal.

2-Definitions

2.1.-Systematic

Systematic is the branch of biology concerned with the study of the diversity of life and its evolutionary relationships. It aims to understand the evolutionary history and patterns of relationships among organisms. 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 (Borrell, B. J. (2021).

2.2.-Taxonomy

Taxonomy is the specific branch of systematics that focuses on the classification of organisms, , naming ,and identification. It involves the establishment of a standardized naming system (i.e., binomial nomenclature) and the development of hierarchical taxonomic ranks to organize the diversity of life. aims to provide a standardized and organized system for naming and classifying organisms based on their shared characteristics and evolutionary relationships. It encompasses

various levels of classification, including domain, kingdom, phylum, class, order, family, genus, and species (Konstantinidis et al., 2017).

2.2.1-Classification

Classification is the arrangement and categorization of organisms into hierarchically ordered groups or categories based on their similarities and differences. The process also involves the classification and naming of organisms into the defined categories called the taxonomic rank: domain, kingdom, phylum, order, class, family, genus, and species, based on similarities and differences. Moreover, many criteria can be used to classify organisms, such as morphology, genetics, behavior, and ecological characteristics. Therefore, the last hierarchical grouping of the groups, from general to specific, defines a taxonomic hierarchy (Borrell, 2021).

2.2.2.-Nomenclature

Nomenclature is the 2nd branch of taxonomy, which names taxonomic groups according to published rules. It assigns a name to these groups according to a system binomial established by Carl Linnaeus in which a Latin genus name precedes the species name. It aims to unify the international scientific language by giving taxa identical names and allowing them to be known by any microbiologist in the world. In addition, bacterial nomenclature is regulated by the International Committee on the Systematics of Prokaryotes (ICSP) that takes charge of the nomenclature and taxonomy of Bacteria and Archaea. In addition to supervising the publication of IJSEM and the International Code of Nomenclature of Bacteria, the ICSP provides direction to a number of subcommittees tasked with establishing and revising criteria for the classification of novel species within the various Bacteria and Archaea groups (Trüper,1999; Parker et al., 2019).

When a new isolate of bacteria or archaea is obtained from nature, the degree to which it differs from existing taxa must be determined in order to classify it as a new taxon. In order to obtain formal confirmation of the taxonomic status of a new genus or species, it is necessary to publish a comprehensive description of the unique characters and traits of the organism, accompanied by the proposed nomenclature. In addition, viable cultures of the organism must be deposited in at least two global cultural collections (**Table 1**).

Table 1: Some international microbial culture collections

Collection Name	Country	Website
American Type Culture Collection (ATCC)	United States	www.atcc.org
Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ)	Germany	www.dsmz.de
Microbial Culture Collection at the National Institute of Environmental Studies (NIES)	Japan	mcc.nies.go.jp
Culture Collection University of Gothenburg (CCUG)	Sweden	www.ccug.se
China General Microbiological Culture Collection Center (CGMCC)	China	www.cgmcc.net
Belgian Coordinated Collections of Microorganisms (BCCM)	Belgium	bccm.belspo.be
CABI Bioscience	United Kingdom	www.cabi.org

Validation of the recently proposed names through publication in the International Journal of Systematic and Evolutionary Microbiology (IJSEM), which kwon as the official publication on the taxonomy and classification of microbial eukaryotes, archaea, and bacteria, facilitates their incorporation into taxonomic reference sources. Updated Prokaryotic Nomenclature (http://www.dsmz.de) and List of Prokaryotic Names with Status in Nomenclature (http://www.bacterio.net) are two websites providing lists of valid and approved bacterial names.

Furthermore, phenotypic and genotypic characteristics of microorganisms can be determined without the necessity of cultivating an isolated strain through the utilization of molecular and genomic techniques. However, in the absence of an isolate that can be deposited in two international culture collections, it is not possible to validly name a new species of microorganism in accordance with the Bacteriological Code. But in cases where an organism has been completely characterized but does not yet have a culture or pure culture, a provisional taxonomic designation may be applied to culture.

On the other hand, the scientific names of bacteria come from Greek and/or Latin or another language to which Latin prefixes or suffixes are added. Some names recall a disease or injury, others a scientist, and still others a metabolism or a Geographical location; Oren and Garrity; 2021).

There are several rules in the Binomial Nomenclature of the International Code of Nomenclature of Prokaryotes (also known as the International Code of Bacterial Nomenclature), but we cite some of them as follows:

-**The scientific name of a prokaryotic species** consists of two parts: the genus name and the species epithet, written in Latin or Latinized form. For example, *Escherichia coli*, where *Escherichia* is the genus name and *coli* is the species epithet.

-**Orthography and etymology**: Names must be written in the Latin alphabet, with the first letter of the genus name capitalized and the species epithet written in lowercase. Names are derived from Latin, Greek, or other languages, with the appropriate Latin suffixes and endings used.

-**Priority of publication:** The first validly published name for a taxon has priority and must be used, unless there are specific reasons to reject it. Subsequent names for the same taxon are considered synonyms and should not be used.

-**Type strains:** The naming of a new species or subspecies must be based on a designated type strain or type specimen, which serves as the reference for that taxon.

The type strain or specimen must be deposited in at least two publicly accessible culture collections in different countries.

-**Valid publication:** For a name to be considered validly published, it must be proposed in a journal or other publication that meets the criteria specified in the code.

The publication must include a description of the taxon and comply with other requirements, such as the effective date of publication.

2.2.3.-Identification

Identification is an essential part of taxonomic classification, which involves comparing the properties of an unidentified specimen to the diagnostic characteristics utilised to establish distinct taxonomic groups. This process is to assign the organism to the appropriate taxonomic rank, which may include domain, kingdom, phylum, class, order, family, genus, and species. in addition, this step frequently depends on the evaluation of diagnostic keys and morphological, physiological, and genetic attributes of the unidentified organism in comparison to the information contained in taxonomic references, including identification manuals, monographs, and online databases (Tedersoo et al., 2018). However, the degree of certainty of taxonomic classifications can be significantly influenced by the choice and efficacy of identification tools, including PCR primers that target the 16S rRNA gene (Klindworth et al., 2013).

The identification process usually includes the following steps (Puillandre et al., 2012; Klindworth et al., 2013; Yarza et al., 2014; Tedersoo et al., 2018):

1. Observation and data collection about the organism include its shape, physiology, genetics, and other distinguishing features.

2. Comparison with classification reference using the organism's observed characteristics with descriptions and diagnostic keys available in taxonomic resources, such as identification guides, monographs, or online databases.

3. Determination of taxonomic position using the observed similarities and differences, the organism is then assigned to the appropriate taxonomic rank (e.g., domain, kingdom, phylum, class, order, family, genus, species).
4. Verification and validation confirms the identification by checking other reliable sources or consulting taxonomists, especially for difficult or ambiguous cases.

In practice, determining the genus and species of a newly discovered prokaryote is based on polyphasic taxonomy. This approach includes the characters phenotypic, phylogenetic and genotypic.

2.2.3.1.-Phenotypic characters

A phenetic system groups living organisms according to the similarity of their phenotypic characteristics. This classification system succeeded in bringing order to the biological diversity and to clarify the function of morphological structures. Organisms which share many characters thus form a single phenetic group or taxon.

2.2.3.2.-Phylogenetic characters

The classification of bacteria and archaea is based on various phylogenetic characters. The base composition of DNA, with the content of guanine and cytosine (G+C%), is an important marker (Mesbah et al., 1989). Analysis of 16S/18S ribosomal RNA gene sequences is the reference method for establishing phylogenetic relationships (Olsen et al., 1994) and defining the domains *Bacteria, Archaea and Eukarya* (Woese et al., 1990). The structure and composition of the cell wall, membrane lipids, flagella and pili are also discriminating characters (Jarrell & McBride, 2008; Berry & Pelicic, 2015). Metabolic and genomic traits, such as metabolic pathways, transport capacities, enzymes and gene composition, also provide phylogenetic clues. The combined use of these different markers, in a molecular and genomic approach, makes it possible to precisely establish the evolutionary relationships and the classification of bacteria and archaea (Madigan et al., 2018).

The validity of this approach is now widely accepted and the number of 16S and 18S ribosomal RNA sequences deposited in international databases such as GenBank is very large and constantly increasing. For 16S rRNA sequences (bacteria and archaea) the RDP (Ribosomal Database Project) database contained more than 5.5 million 16S sequences. The SILVA database, which is one of the most comprehensive for rRNA sequences, included more than 6 million 16S sequences.

However, for 18S rRNA sequences (eukaryotic), The SILVA database included more than 2 million 18S sequences.

The GenBank database, managed by the NCBI (National Center for Biotechnology Information), contained several million 16S and 18S sequences during the same period.

2.2.3.3.-Genotypic characters

Genotypic classification is based on the analysis and compare different molecular markers such as individual genes or entire genomes. Since the guanine and cytosine content (G+C%) of the DNA has some important phylogenetic value, there is a vast difference that exists between the bacterial and archaeal groups (Mesbah et al., 1989). Since the 1970s, it has been adopted as a standard that bacteria and archaea sharing at least 70% of the homology of their genomes are part of the same species (Wayne et al., 1987; Stackebrandt and Goebel, 1994). The proportion of DNA-DNA hybridization between strains of bacteria has been measured for a long for the purpose of definition of species, with 70% homology as a guideline value for species delimitation (Brenner et al., 1969). Finally, the analysis of the proteome profile may also reflect additional phylogenetic data (Vandamme et al., 1996).

3.- Hierarchical classification (or Taxonomic ranks)

Hierarchical classification is a system in prokaryotic taxonomy that organize bacteria and archaea into progressively more inclusive groups or levels based on their evolutionary and shared characteristics. These levels, named taxonomic ranks, are the essential elements of the classification of biological organisms, and the species is the most fundamental taxonomic unit (i.e. group) in bacterial taxonomy (Whitman, 2015; Parte et al., 2019). Subsequently, groups of species are organized into genera (genus), which are further subdivided into families (Familia), orders (Ordo), classes (Classis), classes (Phylum), and domains (or kingdoms, the highest level). Nevertheless, subcategories exist within these overarching classifications (Parte, 2018 Oren & Garrity, 2021).

The major taxonomic ranks and their definitions from highest to lowest level are presented in **Table 2.**

Table 2: Hierarchical classification of microorganisms: Taxonomic ranks and Definitions

Taxonomic rank	Definition
Domain	It is the highest taxonomic rank, which divides all living organisms into three basic groups: archaea, bacteria, and eukaryotes, which represent the three basic branches on the tree of life, reflecting major evolutionary differences (Woese et al., 1990).
Kingdom	It represents the highest level in the groups of taxonomic classification, which is divided into the most basic and diverse life forms. The kingdom represents the broadest division within the tree of life, separating organisms into several large and distinct groups (Cavalier-Smith, 2004).
Phylum	It is the basic unit of taxonomy that includes classes of organisms based on their close relationship in character states and evolutionary closeness (Hugenholtz, 2002)
Class	is a taxonomic rank that groups one or more orders that share certain fundamental characteristics. Classes group closely related orders within a phylum (Yarza et al. 2014)
Order	It is a taxonomic unit consists of one or more families that show significant similarities and are grouped together as closely related families within a class (Yarza et al. 2014).
Family	A taxonomic unit that groups genera with known relationships within an order (Whitman et al. 2018).
Genus	Is a taxonomic rank that groups closely related species that share many common characteristics. The genus name forms the first part of the binomial nomenclature (Tindall et al., 2010)
Species	It is defined as all stains that share several stable characteristics and differ significantly from others, or by genetic similarity (70% or more), estimated by hybridization experiments (Rosselló-Móra and Amann, 2015)
Subspecies	It is a taxonomic rank used to classify strains that are not sufficiently isolated to be designated as separate species, but are closely related and share distinct characteristics within the same species (Cohan, 2019).
Type strain	It is generally defined as the first strain thoroughly studied and characterized compared to other strains. It is characterized by a bank code and an accession number (for example, *E. coli* ATCC 25922) and is generally used to define the characteristics of the species (Stackebrandt et al., 2002).
Strain	It is a unique organism or isolate from a pure culture that has phenotypic and genetic characteristics from other populations within a taxonomic category. However, strains within a species can differ slightly from each other in several ways (Whitman, 2015).

Table 2: Hierarchical classification of microorganisms: Taxonomic ranks and Definitions (continued)

Taxonomic rank	Definition
Biovar	It is a variant of prokaryotic strains and differ among each other in a series of biochemical or physiological differences (Garrity et al. (2001).
Morphovar	It is distinct subtype of strains based on its appearance
Serovar	It is strain delineated by its antigenic properties showing a difference when compared to others.
Isolate	It is a pure culture of a microorganism, typically a bacterial or archaeal strain, obtained from a specific environmental sample or host (Lagier et al., 2015)
Colony	It is a visible mass of microbial cells growing on a solid growth medium, typically used to obtain pure cultures of a specific bacterial or archaeal strain (Brock, 1987)

On the other hand, in microbiology, distinct suffixes are appended to the names of the various taxonomic ranks or levels of classification.

We present in Table 2, hierarchical structure in taxonomy, the frequent suffixes used for bacterial and archaeal taxa:

Table 3: Microbial taxonomic ranks and their corresponding suffixes

Rank	Suffix	Examples
Domain	No suffix	***Bacteria***, *Archaea*
Phylum	*-aeota/-ota*	***Proteobacteria***, Firmicutes, *Euryarchaeota*
Class	*-ia*	***Gammaproteobacteria***, *Methanobacteria*
Order	*-ales*	***Enterobacteriales***, *Bacillales*, *Methanobacteriales, Actinomycetales*
Family	*-aceae*	***Enterobacteriaceae***, *Bacillaceae, Methanobacteriaceae*
Genus	No suffix	***Escherichia***, *Bacillus, Methanobacterium*
Species	No suffix	***Escherichia coli***, *Bacillus subtilis, Methanobacterium formicicum*

Further details on the taxonomic approach will be discussed in more detail in Chapter 3.

4-System of classification of living organisms

4.1-Classification system with Five kingdoms

The first classification system is developed by Robert H. Whitaker in the 1960s. This system aims to classify all living organisms into five distinct kingdoms according to at least three criteria: 1) the prokaryotic or eukaryotic cell type, (2) the level of organization: unicellular or multicellular, (3) the type of nutrition. The Five kingdoms (Whittaker, 1960) as follows:

1-Monera: Prokaryotic microorganisms including bacteria and cyanobacteria.

2-Protists: Eukaryotic microorganisms that do not fit into the other four kingdoms. This class includes many unicellular organisms such as protozoa, algae, and some fungi-like organisms.

3-Fungi: Organisms that feed on organic matter and have cell walls made of chitin. This kingdom includes fungi, yeasts, and molds.

4-Plants: Multicellular photosynthetic organisms capable of producing energy through photosynthesis. This kingdom includes plants ranging from mosses and ferns to flowering plants.

5-Animalia: heterogeneous multicellular organisms that lack cell walls. This kingdom includes all animals, from simple invertebrates to complex mammals.

However, this system, with five kingdoms, is not accepted by many Microbiologists for the reasons following: a- there is a lack of distinction in the kingdom of *Monerea* between bacteria and archaea. b- Protista presents too much diversity. c-The kingdoms *Protista, Plantea* and *Fungi* are poorly defined: brown algae are not close to plants well that this system places them in the *Plantea*.

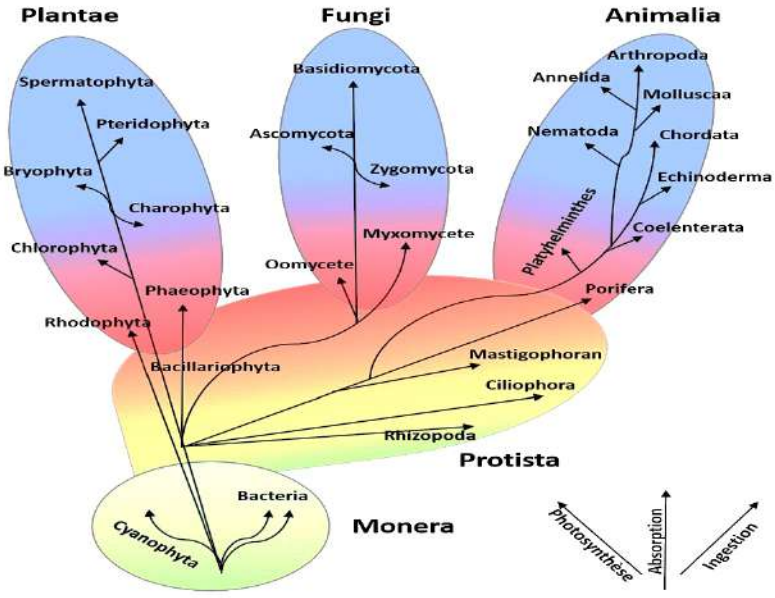

Figure 4. Diagram representing the phylogenetic tree with five kingdoms (***Monera, Protista, Plantae, Fungi and Animalia)*** proposed by Robert H. Whittaker.

1.2-Classification system with Six Kingdom

The Six Kingdoms classification system is a hierarchical classification model that divides organisms into six major groups, based on their evolutionary lines of fundamental differences in terms of cell biology, metabolism and ecology (Woese et al., 1990; Cavalier-Smith, 2004). It has provided a better understanding of the evolutionary relationships between large groups of organisms (table 4).

Table 4 : Six-Kingdom system and their characteristics (Woese et al., 1990; Cavalier-Smith, 2004

Kingdom	Characteristics	Examples
Animalia (Animals)	- Multicellular, heterotrophic organisms Eukaryotic cells,- Lack cell walls, Locomotion and sensory perception	Mammals, birds, reptiles, amphibians, fish, insects, etc.
Plantae (Plants)	- Multicellular, autotrophic organisms - Eukaryotic cells, Presence of cell walls, Photosynthesis	Flowering plants, ferns, mosses, green algae, etc.
Fungi	- Multicellular, heterotrophic organisms - Eukaryotic cells,- Presence of cell walls,- Absorption of nutrients	Mushrooms, molds, yeasts, etc.
Protista	- Diverse group of eukaryotic, unicellular organisms- Lack tissue differentiation	Protozoa, slime molds, some algae, et
Archaea	- Unicellular, prokaryotic organisms - Distinct from Bacteria in cellular structure and metabolism	Methanogens, extremophiles,
Bacteria	- Unicellular, prokaryotic organisms - Diverse in terms of morphology and metabolism	Cyanobacteria, *Proteobacteria*, firmicutes, etc.

Most of the differences between the six kingdoms system and the earlier five kingdoms system are as follows:-

-**The Monera kingdom** (prokaryotes) is split into the *Archaea and Bacteria* kingdoms to reflect their distinct evolutionary lineages and molecular characteristics.

-This classification better reflects the fundamental differences between the three domains of life: *Archaea, Bacteria, and Eukarya.*

4.3. Classification system with seven-kingdom

The seven-kingdom system was established by biologist Thomas Cavalier-Smith. This system divided organisms into seven totally different kingdoms, reflecting the basic differences in cellular, biochemical, and evolutionary characteristics (Cavalier-Smith, 1998, 2004). This system differentiates between *Plant*, *Potozoa* and *Chromista*, facilitating a more effective representation of the evolution and diversity of eukaryotes (Cavalier-Smith, 1998, 2004). It is considered an improvement of the system on six levels, representing the fidelity of evolutionary relationships. The seven kingdoms are as follows (Figure 2).

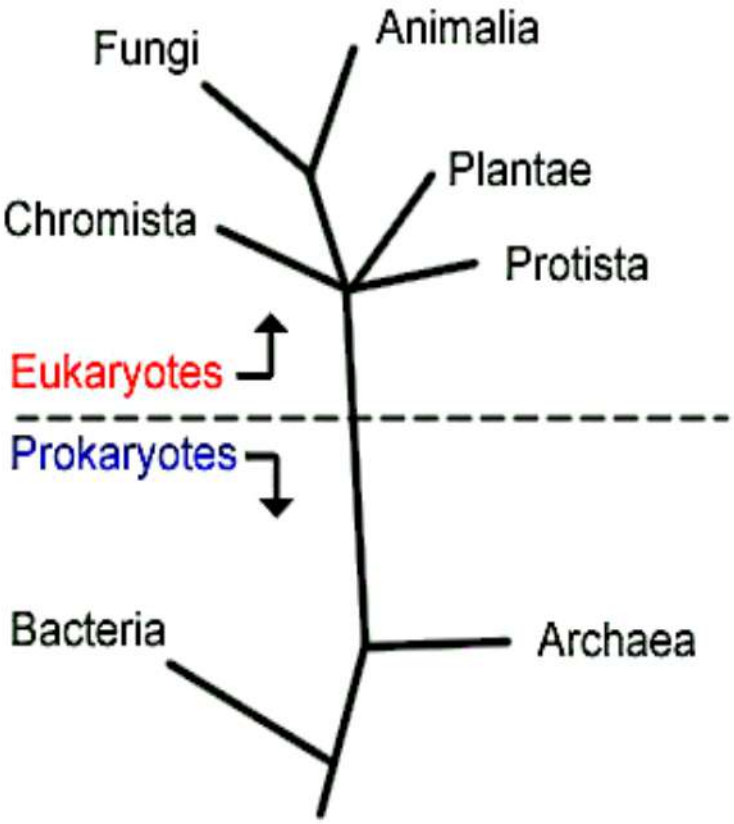

Figure 2: Seven-kingdom system of organisms based on their genetic and biochemical differences: **1-Archaea** are single-celled prokaryotic with membrane lipids. **2-Bacteria**: single-celled prokaryotic, offer remarkable morphological and metabolic diversity. **3 Protozoa:** Unicellular eukaryotic (amoebae, ciliates, and spores) 4-***Chromista***: Single-celled or multicellular eukaryotic, (brown algae and oomycetes). 5-***Plants***: multicellular, photosynthetic eukaryotic (plants, green algae, and bryophytes) ***6-Fungi***: heterotrophic eukaryotic organisms, including fungi, yeasts, and mold-like organisms.

4.4. Classification system with tree Domain

American microbiologist Carl Woese and his associates propose a new system for classifying organisms into three distinct domains: ***Archaea***, ***Bacteria***, and ***Eukaryotes*** (Figure 3), based primarily on a comparative analysis of 16S/18S ARN ribosomal sequences, departs from the traditional model that divides organisms into two regions (prokaryotes and eukaryotes) (Woese et al., 1990). This system reflects the fundamental evolutionary differences between these three major groups of life, which have been identified through molecular analysis (Pace, 1997). He profoundly influenced taxonomy and the understanding of the evolution of organisms.

In contrast to the conventional model that categorizes organisms into two prokaryotes and eukaryotes, this novel system, created by American microbiologist Carl Woese and his colleagues, sparate them into three distinct domains: Archaea, Bacteria, and Eukaryotes, utilizing a comparative analysis of 16S/18S ARN ribosomal sequences (Woese et al., 1990). As determined by molecular analysis, this system reflects the fundamental evolutionary distinctions among these three main categories of life (Pace, 1997). His impact on taxonomy and the comprehension of organismal evolution was profound.

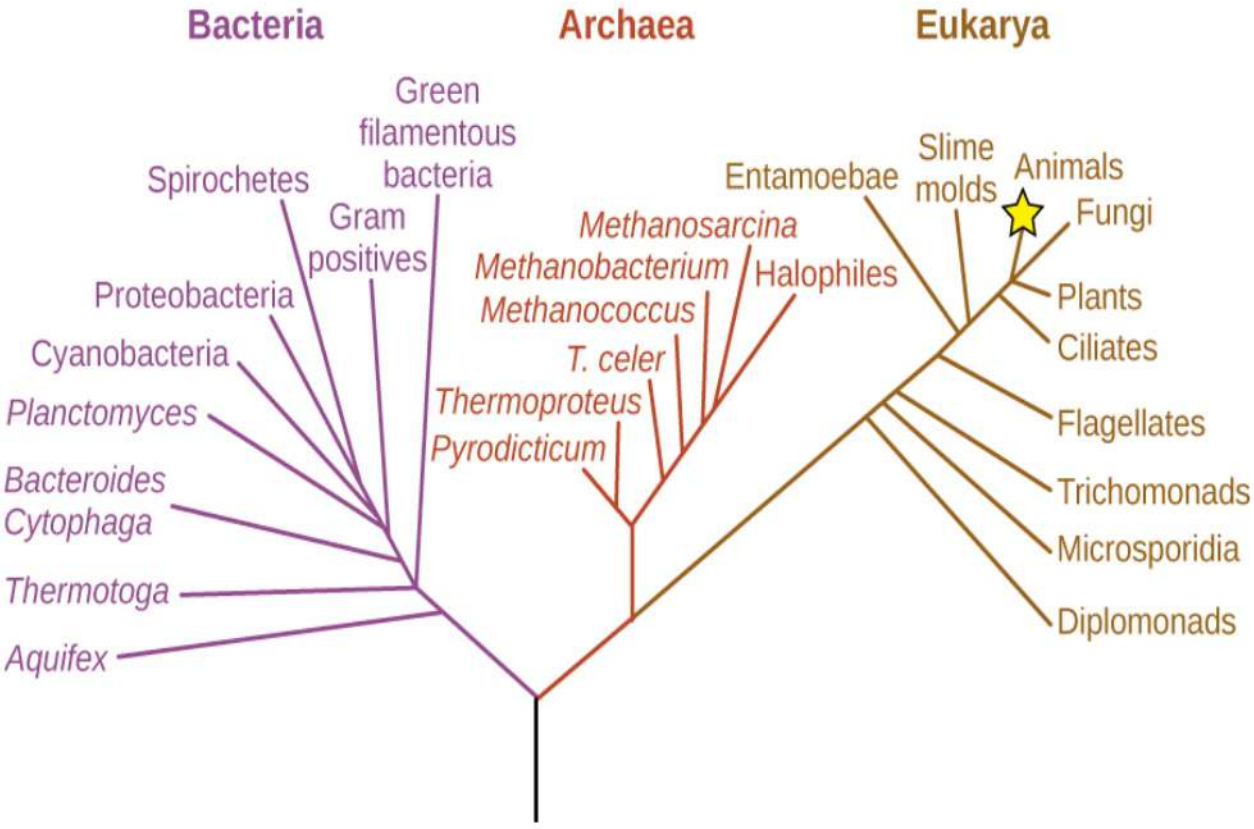

Figure 3: Universal phylogeny is based on the analysis of ribosomal ARN sequences 16S/18S, which was proposed by Carl Woese and other collaborators (Woese et al., 1990)

Chapter II

Polyphasic taxonomy: current approach of Bergey's Manual of Systematic Bacteriology (2nd edition)

1.-Overview of Bergey's Manual of Systematic Bacteriology

Bergey's Manual of Systematic Bacteriology is a major taxonomic reference for classifying and describing *Bacteria* and *Archaea*. The second edition, published between 2001 and 2012, was significantly updated compared to the first. This edition adopts a polyphasic approach, integrating a broad range of phenotypic (Cell morphology, Gram stain, Mobility, Spore formation , and physiological and biochemical proprieties, chemical taxonomic (Cell wall composition: peptidoglycan type, lipids, fatty acids, quinones), ecological characteristics (Habitats and ecology, Symbiotic or pathogenic interactions), genetic, and genomic characteristics (analysis of 16S rRNA gene sequences for evolutionary relationships, Genetic and genomic, etc) to classify prokaryotes (Whitman, 2015; Oren and Garrity, 2014). Parameters include cell morphology, physiological and biochemical properties, cell wall composition, fatty acid profiles, DNA G+C content, 16S rRNA sequence analysis, and genomic data (Parte et al., 2020; Oren & Garrity, 2021).

This polyphasic approach allows for a more robust and reliable classification of bacteria and archaea, considering their multiple aspects (Whitman et al., 2018; Oren & Arbel-Goren, 2019). The guide describes the different taxa, from species to domain, with information on their morphology, physiology, ecology, and evolution (Parte, 2018; Parte et al., 2020).

2. Five important volumes of Bergey's Manual

The second edition of Berge's manual is more different the first edition using phylogenic analyses. It does not include all the prokaryotes of clinical importance, as the first edition. Pathogenic species are placed phylogenetically int the published volumes. The 2nd edition of Bergey's Manual is organized into five volumes, each concentrating on various groupings of prokaryotes (table 5).

Table 5 : Description and Characterization of the five volumes of Bergey's Manual

Volume and Title	Characterization	Example Groups
Volume 1: **The *Archaea* and the Deeply Branching and Phototrophic Bacteria**	Covers the taxonomic classification and biology of Archaea and the earliest-branching bacterial phyla, including phototrophs.	***Archaea*** (*Crenarchaeota, Euryarchaeot*a) Deeply **branching bacteria** (e.g., *Aquificae, Thermotogae*) **Phototrophic bacteria** (*Cyanobacteria, Chloroflexi*)
Volume 2: **The *Proteobacteria***	Describes the largest and most diverse bacterial phylum, the Proteobacteria, which includes many clinically and environmentally important groups.	***Alpha-proteobacteria*** (*Rhizobiales, Caulobacterales)* ***Beta-proteobacteria*** (*Burkholderiales, Neisseriales)* ***Gamma-proteobacteria*** *(Enterobacterales, Pseudomonadales*)

Table 5: Description and Characterization of the five volumes of Bergey's Manual (continued)

Volume and Title	Characterization	Example Groups
Volume 2: **The Firmicutes**	Covers the Firmicutes phylum, which includes important Gram-positive bacteria, such as the *Bacillus, Clostridium,* and Lactobacillus groups.	*Bacilli* (*Bacillales, Lactobacillales*) *Clostridia* (*Clostridiales, Negativicutes Thermoanaerobacterales*)
Volume 4 The Bacteroidetes,*Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria,Dictyoglomi, Gemmatimonadetes, Lentisphaerae,Verrucomicrobia, Chlamydiae, and Planctomycetes*	Covers a diverse range of bacterial phyla, including the Bacteroidetes, Spirochaetes, Tenericutes, and several other less well-known groups.	Bacteroidetes (*Bacteroidales, Flavobacteriales*) Spirochaetes (*Spirochaetales*) Tenericutes (*Mycoplasmatales, Acholeplasmatales*)
Volume 5 **The *Actinobacteria***	Focuses on the *Actinobacteria* phylum, which includes many important soil bacteria, as well as clinically relevant genera such as *Mycobacterium* and *Streptomyces*.	*Actinobacteria Actinomycetales, Bifidobacteriales*) *Acidimicrobiia* (*Acidimicrobiales*) *Coriobacteriia* (e.g., *Coriobacteriales*)

3. Modern taxonomy approach (Polyphasic Taxonomy)

3.1. Definition

Modern bacterial taxonomy adopts a multiphasic approach, which combines diverse sources of information to reliably classify and identify bacteria (Oren & Garrity, 2014; Whitman, 2015). It uses phenotypic, environmental, and physiological data, chemical taxonomic markers, and genetic data, as well as phylogenetic and evolutionary data. Modern bacterial taxonomy takes a multiphasic approach, combining diverse sources of information to reliably classify and identify bacteria (Oren & Garrity, 2014; Whitman, 2015). It combines multiple lines of evidence, including phenotypic, environmental, and physiological, chemical taxonomic markers, and genetic, as well as phylogenetic and evolutionary data, to achieve a comprehensive and robust taxonomic classification. It includes traditional morphological and physiological characteristics, as well as modern molecular and genomic techniques.

3.2.- Phenotypic Taxonomy (numerical taxonomy)

Phenotypic classification studies observable characteristics of bacteria, such as their morphology and physiological and biochemical properties. These features are used for routine identification and provide phylogenetic information. It uses a small number of essential characters, such as morphology, demonstration of a biochemical character, and habitat. However, it only reflects a reduced amount of information; the important characteristics are subjective and depend on environmental conditions (Vandamme & Peeters, 2014; Konstantinidis et al., 2017).

3.2.1-Morphological characters

Morphological characters play an essential role in the classification and identification of microorganisms Observing and analyzing cellular morphology constitutes a valuable tool, especially for more complex organisms such as filamentous bacteria or protists (Durkin et al., 2006; Madigan et al., 2015).). Additionally, they play an essential in microbial taxonomy for several important reasons (Chun and Rainey, 2014; Rosselló-Móra and Amann, 2015).

1-Ease of observation and analysis: Cell morphology is relatively simple, especially in eukaryotic microorganisms and the most complex bacteria/archaea. Also, optical and electron microscopy techniques allow microbial cells' shape, size and arrangement to be observed in detail.

2-Taxonomic relevance: Cellular morphology often reflects fundamental differences in the structure and physiology of microorganisms. These morphological characters, therefore, constitute relevant taxonomic markers for discriminating large microbial groups.

3-Quick identification: Observation of cell morphology allows rapid and preliminary identification of microorganisms. This is particularly useful for microbes that are difficult to cultivate in the laboratory.

4-Link with phylogeny: Some morphological traits may be conserved during evolution and reflect phylogenetic relationships. They, therefore, provide information on the evolutionary history of microorganisms.

Thus, despite the limits of the phenotypic approach alone, morphological analysis remains an essential tool in modern microbial taxonomy, in addition to genomic and molecular data. It makes it possible to characterize more precisely the diversity of the most complex microorganisms, such as filamentous bacteria, thermophilic archaea (Chun and Rainey, 2014).

Although phenotypic classification has long been the primary approach, it has some limitations, including a) Phenotypic variation within the same species, b) Evolutionary closeness that can mask relationships, and 3) Difficulty classifying some microorganisms that are difficult to culture. For these reasons, the modern bacterial taxonomy now incorporates other data types,

such as genetic markers and phylogeny, to obtain a more robust and reliable classification. (Whitman, 2015; Konstantinidis et al., 2017).

The most morphological characters used in the identification of microorganisms are given in Table 6.

Table 6: Principal morphological characters used in the classification and identification of microorganisms.

Morphological Character	Description	Importance in Taxonomy
Cell shape	Cocci (spherical), Bacilli (rod-shaped), Spirilla (spiral), Filamentous, Irregular	Provides information about the basic cellular architecture and can help differentiate between major bacterial groups.
Cell arrangement	Includes single cells, pairs, chains, clusters, e	Reflects the mode of cell division and can be useful for identifying certain bacterial genera.
Cell size	Diameter or length of cells (in µm)	Can be a distinguishing feature between closely related taxa and help identify specific microorganisms.
Cell wall structure	Gram-positive, Gram-negative, presence of sheaths,	Provides insights into the cell envelope composition and is a key characteristic for bacterial classification.
Spore formation	Ability to form endospores	Indicates the presence of certain bacterial genera, such as *Bacillus* and *Clostridium*, and their ability to survive harsh environmental conditions.
Motility	Presence and type of flagella or other motility structures	Can be a distinguishing feature for certain bacterial groups and provides information about their lifestyle and ecological roles.
Pigmentation	Production of various pigments	Can be a useful characteristic for the identification of specific microorganisms, particularly photosynthetic bacteria and some fungi.
Filamentous Growth	Presence of long, branching filaments	Characteristic of certain bacteria (e.g., actinomycetes) and fungi, and can provide insights into their ecological niches and physiology.
Cell Inclusions	Storage granules, gas vesicles, etc.	Can be diagnostic features for the identification of specific microbial groups and their adaptations to various environments

3.2.2.-Physiological and metabolic characteristics

Physiological and metabolic traits are essential in classifying and identifying microorganisms Analysis of these functional characteristics provides complementary information to the morphological and genetic characters, their adaptation to different environments and their ecological interactions. In addition, studying optimal growth conditions, such as temperature, pH or salinity, makes it possible to distinguish between large microbial groups, such as psychrophilic, thermophilic or halophilic (Whitman, 2015; Oren and Garrity, 2021). These physiological adaptations often reflect fundamental differences in cell physiology and environment. Furthermore, examining metabolic pathways, utilization of carbon and energy sources, and production of enzymes and secondary metabolites provides valuable clues about the functional capabilities of microorganisms (Whitman et al., 2018; Oren & Arbel-Goren, 2019). These metabolic traits are particularly useful for identifying microbial species.

Thus, studying physiological and metabolic properties is increasingly important, especially for characterising microorganisms difficult to culture or identify by classical molecular methods (Whitman, 2015). It contributes to a better understanding the microbial world's diversity, ecology and evolution.

On the other hand, these physiological and metabolic characteristics and morphological features provide a comprehensive data set for classifying and identifying microorganisms. In addition, they can be beneficial for distinguishing between closely related taxa and understanding the ecological adaptations and functional roles of different microbial groups (Chun & Rainey 2014; Vandamme & Peeters, 2014).

For bacterial strains isolated from hospitalized patients characterized by rapid growth; their identification must be rapid to fund an appropriate treatment. The most preferred method for identification in these cases is biochemical identification galleries 'Biomerieux's API galleries', including API 20E for the identification of *Enterobacteriaceae* and other non-fastidious Gram-negative bacilli, API 20NE: for the identification of non-fastidious Gram-negative bacilli, non-enterobacteria, Staph API for the identification of staphylococci, Coryne API for the identification of corynebacterial, API 20 Strep for the identification of streptococci, API Candida: for the identification of yeasts of the *Candida* genus.

The resulting biochemical profile are interpreted using numerical codes and then compared against a database for identification at the genus and species level of bacteria. However, this classification is limited to identifying certain specific strains, and the error rate of galleries varies between 5 and 20% depending on the galleries considered. Also, identification systems are closed with limited databases and require updating. New species are absent from the gallery database.

The principal physiological and metabolic traits used in the classification and identification of microorganisms are given in Table 7.

Table 7: Physiological and metabolic characteristics used in the classification and identification of microorganisms (Holt, 1994)

Physiological/ Metabolic Characteristic	Description	Importance in Taxonomy
Temperature Range	Psychrophiles,mesophiles, thermophiles, etc.	Indicates the optimal growth temperature range and can be diagnostic for microbial groups.
pH Range	Acidophiles, neutrophiles, alkaliphiles, etc.	Provides information about the organism's tolerance to different pH conditions and can help differentiate between taxa.
Oxygen Requirements	Aerobes, anaerobes, facultative anaerobes	Reflects the organism's adaptations to different oxygen levels and is a key characteristic for bacterial classification.
Nutrient Requirements	Autotrophs, heterotrophs, mixotrophs	Indicates the organism's carbon and energy sources and can be useful for identification and ecological classification.
Substrate Utilization	Carbohydrates, lipids, proteins, etc.	Provides information about the metabolic capabilities of the organism and can be used for differentiation between related taxa.
Enzyme Production	Hydrolytic enzymes, oxidative enzymes, et	Can be diagnostic features for certain microbial groups and their ecological roles.
Pigment Production	Carotenoids, melanins, chlorophylls, etc.	Can be a distinguishing characteristic for photosynthetic bacteria, certain fungi, and other pigment-producing microorganisms.
Antibiotic Susceptibility	Resistance or sensitivity to various antibiotics	Can be a useful marker for the identification of specific microbial strains and for understanding their ecological adaptations.
Toxin Production	Exotoxins, endotoxins, etc.	Can be a characteristic of pathogenic microorganisms and is important for clinical identification and diagnosis.
Quorum Sensing	Production of signaling molecules	Reflects the organism's ability to coordinate behaviors in a population-dependent manner and can be a relevant feature for some microbial taxa.

3.2.3-Ecological characterizations

In addition to conventional morphological, physiological, and metabolic characteristics, current microbial taxonomy increasingly places significance on ecological characterizations. This includes examining the interactions between microorganisms, their reactions to environmental stresses, and their functional significance within ecosystems. Such ecological information is of great value for helping classify and identify microorganisms (see table 8), Amaral-Zettler et al., 2017; Fierer et al., 2021). Additionally, ecological characteristics of microbial populations, such as their sensitivity to contamination or adaptation to extreme conditions, can be used to distinguish them in specific conditions (Barberán et al., 2014). These characteristics frequently represent fundamental genetic and evolutionary distinctions. Furthermore, this ecological information contained in conventional methodologies improves the overall categorization of microorganisms by considering the complex dynamics that occur within ecosystems (Barberán et al., 2016).

Table 8: some ecological characteristics used in the classification and identification of microorganisms (Medini et al., 2008; Cavicchioli, 2011; Konstantinidis et al., 2017)

Ecological Characteristic	Description	Importance in Taxonomy
Habitat	Aquatic (marine, freshwater),terrestrial, host-associated (skin)	Provides information about the organism's preferred living environment and can be used to differentiate between microbial groups adapted to different ecological niches.
Trophic Level	Autotrophs, heterotrophs, mixotrophs	Indicates the organism's role in the ecosystem, such as primary producer, decomposer, or symbiont, and can be a distinguishing feature.
Metabolic Capabilities	Aerobic, anaerobic, chemotrophic, phototrophic, etc.	Reflects the organism's adaptations to different energy sources and redox conditions, which can be relevant for ecological classification.
Symbiotic Relationships	Mutualism, commensalism, parasitism	Can be a characteristic of certain microbial groups, such as nitrogen-fixing bacteria or pathogenic microorganisms,
Biogeography	Cosmopolitan, , thermophilic, psychrophilic, etc.	Indicates the organism's geographic distribution and adaptations to specific environmental conditions, useful for taxonomic placement.
Bioremediation Potential	Ability to degrade or transform pollutants	Can be a relevant characteristic for the classification of microorganisms with applications in environmental biotechnology.

Ecological Characteristic	Description	Importance in Taxonomy
Biotechnological Applications	Production of enzymes, biofuels, antibiotics, etc.	Provides information about the organism's potential for industrial and commercial use, which can be a taxonomically relevant feature.
Pathogenicity	Ability to cause disease in hosts	Can be a distinctive feature for the identification of clinically relevant microorganisms, such as bacterial and fungal pathogens.

3.2.4. Serological characteristics

Serological characteristics also play an important role in the classification and identification of microorganisms. Analysis of surface antigens and immune reactions provides complementary information to other taxonomic approaches (Janda & Abbott, 2007; Bottone, 2015). Some cellular antigens, lipopolysaccharides, surface proteins or cell wall components, can be used as serological markers to differentiate between microbial populations. For example, *Salmonella* serotypes are determined by the antigenic composition of their surface (Logan and de Vos, 2009; Reissbrodt, 2004). In addition, serological tests, such as agglutination or immunofluorescence techniques, can detect cross-reactivities between microbial antigens and specific antibodies. These serological reactivity profiles provide insight into evolutionary associations and relationships between species (Janda & Abbott, 2007). However, serological data should be interpreted with caution, as cross-reactions can sometimes mask subtle differences between specific microbial species or strains. Therefore, its use must be combined with other classification methods (Logan & De Vos, 2009). Thus, the integration of serological characteristics, as well as morphological, physiological, genetic and environmental analyses, contributes to a more robust and reliable microbial classification (Janda & Abbott, 2007; Bottone, 2015).

Table 9: some commonly used serological characteristics in bacterial taxonomy along with examples

Serological Characteristic	Description	Example
Serotyping Grimont, & Weill, 2007).	Determining specific antigens on the surface of bacteria to classify them into different serotypes or serovars	*Escherichia coli:* O157:H7

Serological Characteristic	Description	Example
Agglutination Cheesbrough, 2006)	Clumping of bacteria when exposed to specific antibodies or antiserum, aiding in identification and classification	*Salmonella enterica* Typhi
Western Blotting (Harlow and Lane, 1988)	Detecting and characterizing bacterial proteins using specific antibodies to identify unique protein patterns	*Borrelia burgdorferi* Flagella protein (p41)
Enzyme-Linked Mégraud & Lehours, 2007).	Detecting specific bacterial antigens or antibodies using	*Helicobacter pylori:* Detection of H. pylori
Immunosorbent Assay(ELISA) Crowther, 2000	enzyme-linked antibodies or antigens for classification and identification	antigens using ELISA
Coagglutination (Facklam, 2002)	Agglutination reaction between the bacteria and specific antibody-coated particles, aiding in identification and differentiation	*S.aureus*: Detection of coagulase using coagglutination test
Ouchterlony Doub (Duthie et al., 2011)	Analyzing antigen-antibody interactions through	*Bordetella pertussis* Analysis ofpertussis
Latex Agglutination Mackie et al., 1996)	Detecting specific bacterial antigens through agglutination reactions using latex particles coated with antibodies	*S.pneumoniae:* Detection of pneumococcal antigens using latex agglutination test

3.2.5.-Chimiotaxonomy

Chemotaxonomy plays an increasing role in modern microbial taxonomy, which studies the chemical composition of microorganisms. It consists of the analysis of lipids, fatty acids, pigments, sugars, and other cellular components that provide chemical markers that can be used to differentiate and classify microbial groups (Tyndall et al., 2010; Stackebrandt & Schumann, 2014). Membrane fatty acid profiles are powerful tools for identifying and characterizing bacteria. Some lipid signatures are specific to taxonomic groups (Kämpfer & Glaeser, 2019). For example, quinones, menaquinones, and lipoquinones were important in distinguishing archaea and bacteria. These compounds in electron transport chains reflect fundamental differences in cell physiology (Stackebrandt & Schumann, 2014).

In addition, chemical taxonomy data offer supplementary comprehensions in addition to the conventionally employed morphological, genetic, and metabolic characteristics. However, the

precision of microbial chemical profiles has been considerably improved by the development of innovative analytical techniques such as mass spectrometry. This has led to increasing chemical classifications used in classifying bacteria and archaea (Kampfer & Glaeser, 2019).

Cell Wall Component	Chemical Markers	Taxonomic Significance
Peptidoglycan Analyzed by HPLC or GC-MS	- Muropeptide composition (e.g., amino acids, sugars) - Degree of crosslinking - Presence of specific structural modifications	- Distinguishes between Gram-positive and Gram-negative bacteria - Provides information about phylogenetic relationships within bacterial groups
Teichoic Acids Analyzed by GC-MS or HPLC- SM	- Wall teichoic acids - Lipoteichoic acids - Composition and structure of the polymers	- Characteristic of Gram-positive bacteria - Differences in teichoic acid composition can differentiate between bacterial genera and species
Mycolic Acids Analyzed by GC-MS HPLC	- Structure and chain length of hydroxylated, long-chain fatty acids	- Diagnostic marker for the phylum Actinobacteria, *Corynebacteriales* (e*Mycobacterium*,Corynebacterium)
Glycopeptides Analyzed by HPLC-SM	- Composition and structure of glycosylated peptides	- Found in the cell walls of certain Gram-positive bacteria, such as *Actinomyces, Bifidobacterium*, etc
Lipopolysaccharides (LPS) Analyzed by HPLC , NMR	- O-antigen polysaccharide - Core oligosaccharide - Lipid A	- Characteristic of Gram-negative bacteria - Differences in LPS structure can differentiate between bacterial genera and species
Arabinogalactans Analyzed by HPLC-MS	- Composition and structure of the polysaccharide	- Present in the cell walls of some *Actinobacteria*, such as *Mycobacterium*

HPLC: High-Performance Liquid Chromatography; GC-MS: Gas Chromatography-Mass Spectrometry ; NMR:Nuclear Magnetic Resonance Spectroscopy; MS: Mass Spectrometry

For the identification and classification of many microbial genera and species, as well as to better understand their evolutionary relationships and adaptations, analysis of these cell wall components and their associated chemical markers provides essential taxonomic information (Stackebrandt & Schumann, 2014).

The use of chemotaxonomic markers for the identification and classification of some *Actinobacteria* are given in table 11.

Table 11: chemotaxonomic markers for the identification and classification of some *Actinobacteria* (Stackebrandt et al., 1997; Goodfellow & Fiedler, 2010; Gao and Gupta, 2012).

Actinobacterial Genus	Cell Wall Composition	Lipid Composition	Other Markers
Mycobacterium	- Mycolic acids Arabinogalactan	- Branched, saturated, and hydroxylated fatty	- Carotenoid pigments - Trehalose
Corynebacterium	- Mycolic acids Arabinogalactan	- Branched, saturated, and hydroxylated fatty acids Menaquinones	- Carotenoid pigments - Trehalose
Nocardia	- Mycolic acids	- Branched, saturated, and hydroxylated fatty acids Menaquinones	- Carotenoid pigments
Streptomyces	- meso-Diaminopimelic acid	- Branched, saturated, and hydroxylated fatty acids - Menaquinones	- Production of secondary metabolites
Frankia	- meso-Diaminopimelic acid	- Branched, saturated, and hydroxylated fatty acids - Menaquinones	- Nitrogen fixation
Micrococcus	- meso-Diaminopimelic acid in peptidoglycan	- Branched, saturated, and hydroxylated fatty acids - Menaquinones	- Carotenoid pigments
Arthrobacter	- meso-Diaminopimelic acid in peptidoglycan	- Branched, saturated, and hydroxylated fatty acids - Menaquinones	- Carotenoid pigment

3.2.6.- Numerical classification

Numerical classification, also called numerical or phenotypic classification, is an approach to microbial classification based on statistical analysis of microorganisms' phenotypic characteristics. It consists of: "Group organisms into taxa based on their overall similarities, using statistical techniques to analyze large numbers of objectively measurable traits." (Snaith and Sokal,1973).

The characteristics retained are considered of equal value. They are quantified numerically to establish taxonomic distances which reflect both the similarity (resemblance) and relationships of evolutionary ancestry between the confronted organisms. Binary quantification (0 or 1, i.e. absence or presence) of similarities and differences then makes it possible to characterize the taxa by a coefficient of similarity, calculated in various ways, depending on the choice of selected characters and the coding and processing applied to the data collected.

The principal steps in the numerical classification of microorganisms are as follows: (Medini et al., 2008; Tindall et al., 2010; Legendre & Legendre, 2012)

1-Data collection and preparation sampling: isolation of microbial strains, Measurement of phenotypic characteristics (morphological, physiological, biochemical), and

2-Data coding: Transformation of characters into numerical data.

3-Similarity analysis: Calculation of similarity coefficients between strains and Choice of coefficients adapted to the type of data (binary, quantitative, etc.)

4-Grouping (clustering): Application of numerical classification algorithms (simple, complete links, Ward, etc.) and construction of dendrograms representing similarities between strains

6.Identification of taxonomic groups: Definition of operational taxonomic units (OTUs) based on similarity levels, and comparison with reference strains for species naming.

6-Validation and interpretation: Evaluation of the robustness of the groups formed (analysis of variance, Jaccard index, etc.) et biological interpretation of results in relation to phenotypic characteristics.

Classification results is generally of the ascending hierarchical type, based on a general similarity evaluated by comparing many characteristics, each having the same weight. The micro-organisms are then assembled into several "Custers" groups or "sub-clusters" sub-groups according to the similarities defined by a similarity index.

The most commonly used in Microbiology being those of Sokal and Michener and Jaccard.

1-Sokal and Michener coefficient (Simple Matching Coefficient):

Considers both the presence and absence of traits in the pairwise comparison of strains.

Formula: $S = (a + d) / (a + b + c + d)$

Where. **a** = number of characters present in both strains; **b** = number of characters present in the first strain but absent in the second; **c** = number of characters present in the second strain but absent in the first ; **d** = number of characters absent in both strains

2-Jaccard coefficient:

Contrary to the Sokal and Michener coefficient, the Jaccard coefficient only considers the presence of characters, ignoring the absence of characters.

Formula: $J = a / (a + b + c)$

Where a = number of characters present in both strains. b = number of characters present in the first strain but absent in the second; c = number of characters present in the second strain but absent in the first.

The principal difference is that the **Sokal and Michener coefficient** gives equal importance to the presence and absence of characters, while the Jaccard coefficient focuses only on shared characters. The choice between these two coefficients depends on the type of data and the taxonomic objective. **The Jaccard coefficient** is often preferred when the absence of characters is not considered a relevant similarity. Their joint use can also provide additional information. The number of characters retained must be significant (Legendre and Legendre, 2012).

In practice, the number of compared tests is between 30 and 300 characters which can be of various natures: genomic parameters, classic phenetic characters (morphology, physiology, structure, metabolism), chemical components (peptidoglycan, LPS, etc.). The results of numerical taxonomic studies are usually displayed in the form of a tree-like diagram called a dendrogram, which shows the relationships between the operational taxonomic units (OTUs) based on their overall similarity. Organisms with the greatest similarity are grouped into groups called" phenons", which can be species, genera, etc. in numerical taxonomy (Tindall et al., 2010; Legendre and Legendre, 2012).

It is generally accepted that a level of similarity (groups of similar OTUs) greater than 80% belong to the same species (Sokal and Michener index). Concerning the Jaccard index, those above 70% belong to the same type.

For example, the morphology and physiological characteristics of four isolates (Ag12, Ag13, Ag32 and Ag20) analyzed by Sokal and Michener coefficient indicated that these isolates belong to the genus *Bacillus,* and appeared closely related to *B. pumilis.* They showed a level of similarity above 80%, indicating that they belong to the same species, *B. pumilis* (Figure 4).

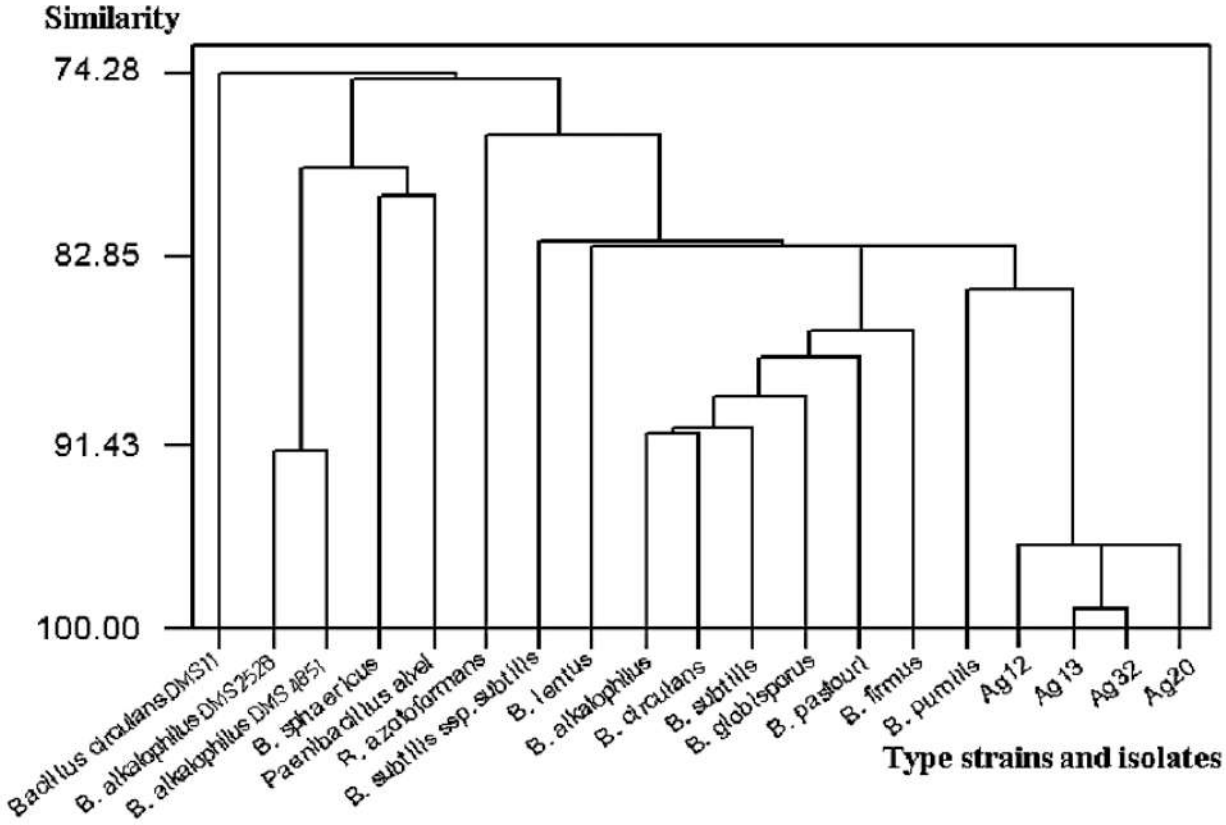

Figure 4: Dendrogram illustrating the relationships between specific *Bacillus* isolates and type strains according to their phenotypic characteristics (Azeri et al., 2010).

3.3. Molecular taxonomy

3.3.1 Introduction

The molecular taxonomy of microorganisms has considerably developed in recent years due to advances in genetic sequencing and phylogenetic analysis techniques. This approach is essentially based on the comparative analysis of reference gene sequences, such as 16S rRNA in bacteria and archaea and protein sequences. In addition, to reconstruct phylogenies, different genes and proteins can be used. These molecules are called phylogenetic markers. Their chosen depend to the mutation rate that can be more or less high. Genes with a low mutation rate allow access to the distant past while genes with high mutation rates allow the study of nearby divergence events (Vandamme & Peeters, 2014; Yarza et al., 2014).

Moreover, the speed of evolution varies greatly depending on the genes considered. These speed differences depend on the probability that the mutation will arise and on its compatibility with the survival of the organism (Rossi-Tamisier et al., 2015; Chun & Rainey, 2014).

On the other hand, this molecular approach that combine between the molecular and phenotypic analyses called the "polyphasic", providing a more robust and reliable taxonomy (Vandamme & Peeters, 2014). The storage of molecular data in international databases, including GenBank, EMBL, and DDBJ, facilitates nomenclature standardization in accordance with the International Code of Nomenclature for Prokaryotes (Oren et al., 2015). Molecular classification has thus changed microbial taxonomy by making it more objective and in acceptance with the phylogenetic relationships between microorganisms.

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3.3.2.- Characteristics of molecular chronometers markers used in molecular taxonomy

There are several criteria when choosing molecular chronometers (or markers) for microbial taxonomy. The most of them are as follows:

a)-Gene function and conservation:

The genes used as markers must be universal and conserved in the microorganisms studied.

b)- Sequence variability:

The molecular marker should be appropriate for the taxonomy rank studied. A hypervariable region is preferred when conducting species-level identification. Conversely, a more conserved region explains higher taxonomic levels (e.g., family).

c)-Availability of reference sequences:

It is necessary to have many reference sequences in public databases such as GenBank, EMBL, or DDBJ.

d)-Simple to amplify or reproduce:

The molecular marker should be easily amplifiable and sequenceable in diverse isolates.

h-Discriminatory power:

Taxa should be well-discriminant at the selected taxonomic level; generally, it is only at this level that the sequence similarity can ideally be defined to differentiate species.

g)-Compatibility with other taxonomic approaches:

The molecular marker should be compatible with phenotypic, genomic, and similar classification methods.

For that reason, the 16S rRNA gene is the most reference molecular marker used in the identification and phylogenetic classification of *Archaea* and *Bacteria* (Yarza et al., 2014). By aligning and comparing these sequences, it is possible to construct phylogenetic trees with the evolutional relationships between the microorganisms.

3.3.3.-Example of molecular markers used in molecular taxonomy

Several molecules have been proposed as molecular markers, each providing more or less relevant information depending on the bacterial groups studied and the types of relationships studied (old and recent). The most of them are given in Table 12.

Table 12: Example of nucleic acid molecular chronometers used in molecular taxonomy

Molecular markers	Characterization
16S ribosomal RNA (rRNA) gene (Yarza et al., 2014)	The 16S rRNA gene is widely used for bacterial taxonomy due to its presence in all bacteria and its relatively slow rate of evolution. Specific regions of the 16S rRNA gene sequence are used to determine phylogenetic relationships among bacteria.
Internal Transcribed Spacer (ITS) region (Schoch et al., 2012)	The ITS region, which includes the ITS1, 5.8S rRNA gene, and ITS2, is commonly used for fungal taxonomy. The ITS region exhibits a high degree of variation among fungal species, making it useful for species-level identification.
D1/D2 region of the 26S rRNA gene (Kurtzman & Robnett, 1998).	The D1/D2 region of the 26S rRNA gene is used for the identification and classification of yeasts and other fungi.
Cytochrome c oxidase subunit I (COI) gene: (Hebert et al., 2003)	The COI gene, also known as the "DNA barcode," is widely used for the identification and classification of animal species, including some microorganisms like protists.

RNA polymerase subunit B (rpoB) gene (Mollet et al., 1997)	The rpoB gene is used as a complementary marker to the 16S rRNA gene in bacterial taxonomy, particularly for resolving relationships within closely related species.
Elongation factor Tu (*tuf*) gene (Koseki et al., 2021)	The *tuf* gene is used as an alternative marker for bacterial taxonomy, especially for species identification and phylogenetic analysis of certain bacterial groups.
DNA gyrase subunit B (*gyrB*) gene: (Yamamoto & Harayama ,1995).	The gyrB gene is another protein-coding gene used for bacterial taxonomy, particularly in resolving relationships among closely related species or strains.
Multilocus Sequence Typing (MLST) (Maidenet al., 1998)	MLST involves the sequencing and analysis of multiple housekeeping genes to determine the allelic profiles of bacterial strains, which can facilitate their identification and classification

3.3.4. Comparative sequences and Phylogenic analyses

Comparative analysis of molecular marker sequences, particularly genes encoding 16S ribosomal RNA, is essential to modern microbial taxonomy (Singer et al., 2016). The alignment and comparison of these sequences make it possible to construct phylogenetic trees reflecting the evolutionary relationships between microorganisms (Parks et al., 2018). Taxonomic units are delineated using sequence similarity thresholds; for bacterial and archaeal species, a similarity threshold of 97% is frequently accepted (Rosselló-Mora & Amann, 2001; Konstantinidis & Tiedje, 2005).

Taxonomic units are delineated using sequence similarity thresholds; for bacterial and archaeal species, a similarity threshold of 97% is frequently accepted (Rosselló-Mora & Amann, 2001; Konstantinidis & Tiedje, 2005). Nevertheless, there is a continuing discussion about these thresholds, as many researchers have suggested more precise values depending on taxonomic groups (Stackebrandt & Goebel, 1994; Kim et al., 2014). Additionally, at the level of species and genera, phylogenomic analysis employing average nucleotide identity (ANI) or core gene sets provides more precise taxonomic resolution (Chun et al., 2018; Konstantinidis et al., 2017).

Therefore, comparative approaches related to nucleotide sequences are indispensable to establishing the taxonomy of microorganisms objectively consistent with their evolutionary relationships (Kim et al., 2014; Parks et al., 2018).

Generally, it is accepted that below 97% homology two bacteria cannot belong to the same species. Thus, it is not useful to carry out DNA/DNA hybridizations below of this threshold. If the homology percentage is > 97%, the placement of wo strains in the same species or not is based on the results of DNA/DNA hybridization. However, two species can have very similar 16S rRNA sequences and still be very different by DNA/DNA hybridization

For example, the species *Bacillus cereus* and the species *Bacillus anthracis* are two bacteria of the genus *Bacillus* which present a very high similarity in their 16S rRNA sequences, reaching more than 99% identity However, these two species are very distinct genetically, as shown by DNA-DNA hybridization values, less than 70%, indicating that they are indeed two different species (Daffonchio et al., 1998;Ash et al., 1991).

3.3.4.1.-Principal steps in comparative sequences of 16S rRNA sequences and phylogeny analysis

The principal steps in comparative analysis of 16S rRNA sequences in molecular taxonomy and phylogenic analysis are as follows:

a-16S rRNA amplification and sequencing:

-Extracting total DNA from the studied organisms from a pure isolate
-Amplification of the 16S rRNA gene by PCR using universal primers.
-The sequence of the amplicons is obtained, generally by Sanger or next-generation sequencing.

b)-Restoring reference sequences:

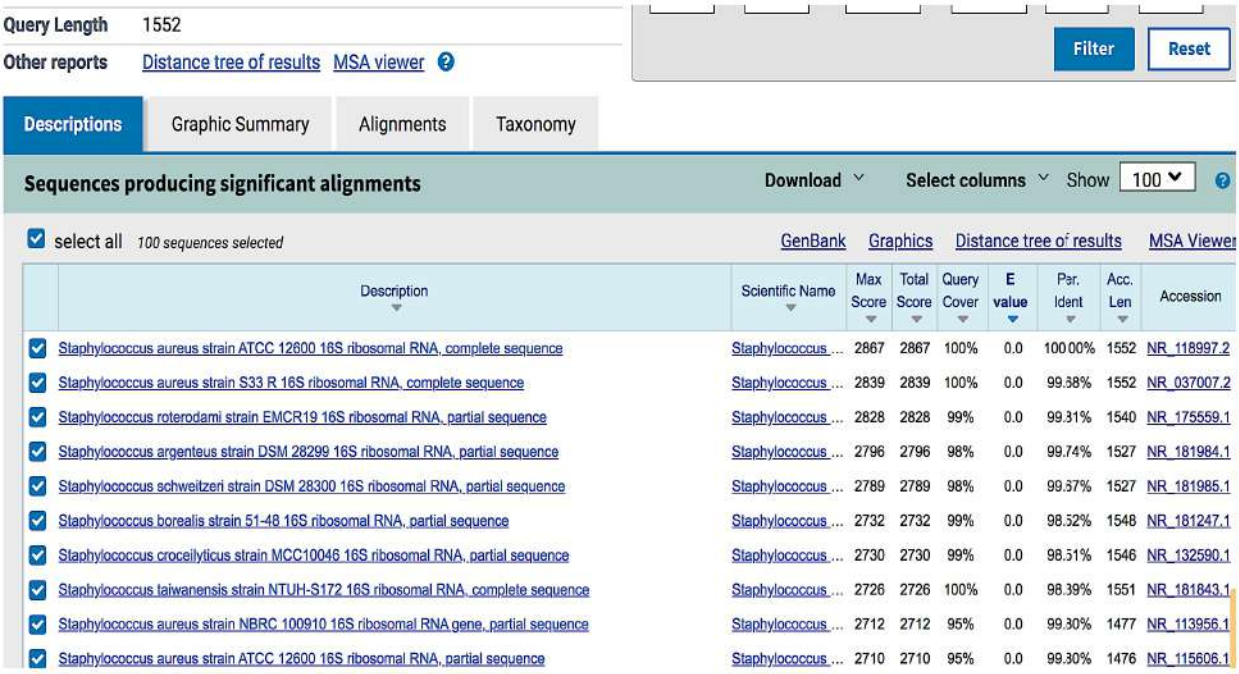
-Access to publicly available databases, such as GenBank, RDP (Ribosomal Database Project),
-Recovery of 16S rRNA sequences from reference strains representing different taxa.

c)-Multiple alignments of sequences:

-The acquired sequences should be aligned with reference sequences utilizing bioinformatics tools, including MUSCLE, CLUSTAL, or Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome)

d)-Sequence similarity analysis:

-Calculate the percent sequence identity between different organisms (Figure 4.1)



Query Length 1552

Other reports Distance tree of results MSA viewer

Filter Reset

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Select columns — Show 100

select all 100 sequences selected — GenBank | Graphics | Distance tree of results | MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Staphylococcus aureus strain ATCC 12600 16S ribosomal RNA, complete sequence	Staphylococcus ...	2867	2867	100%	0.0	100.00%	1552	NR_118997.2
Staphylococcus aureus strain S33 R 16S ribosomal RNA, complete sequence	Staphylococcus ...	2839	2839	100%	0.0	99.58%	1552	NR_037007.2
Staphylococcus roterodami strain EMCR19 16S ribosomal RNA, partial sequence	Staphylococcus ...	2828	2828	99%	0.0	99.31%	1540	NR_175559.1
Staphylococcus argenteus strain DSM 28299 16S ribosomal RNA, partial sequence	Staphylococcus ...	2796	2796	98%	0.0	99.74%	1527	NR_181984.1
Staphylococcus schweitzeri strain DSM 28300 16S ribosomal RNA, partial sequence	Staphylococcus ...	2789	2789	98%	0.0	99.37%	1527	NR_181985.1
Staphylococcus borealis strain 51-48 16S ribosomal RNA, partial sequence	Staphylococcus ...	2732	2732	99%	0.0	98.52%	1548	NR_181247.1
Staphylococcus croceilyticus strain MCC10046 16S ribosomal RNA, partial sequence	Staphylococcus ...	2730	2730	99%	0.0	98.51%	1546	NR_132590.1
Staphylococcus taiwanensis strain NTUH-S172 16S ribosomal RNA, complete sequence	Staphylococcus ...	2726	2726	100%	0.0	98.39%	1551	NR_181843.1
Staphylococcus aureus strain NBRC 100910 16S ribosomal RNA gene, partial sequence	Staphylococcus ...	2712	2712	95%	0.0	99.30%	1477	NR_113956.1
Staphylococcus aureus strain ATCC 12600 16S ribosomal RNA, partial sequence	Staphylococcus ...	2710	2710	95%	0.0	99.30%	1476	NR_115606.1

Figure 4.1: example for the Blast result of 16S ribosomal RNA of the type strain of *Staphylococcus aureus* ATCC 12600. *Other types of strains showed a high level of similarity (between 98.52% and 99.81%), but they are validated as different species according to AND/DNA hybridization experiments (<70%).*

e)- Phylogenetic tree construction

Phylogenetic trees are graphical representations of evolutionary relationships between different organisms or taxonomic groups. They are widely used in modern taxonomy to understand the evolutionary history of life. They are illustrated in the form of branched diagrams that connect nodes (figure X). The nodes represent taxonomic units such as species or genes; the external nodes at the end of the branches represent living (extant) organisms. The length of the branches represents the number of molecular changes that have taken place between the two nodes.

Many softwares can be used to construct phylogenetic trees such as MEGA, PAUP, RAxML, or MrBayes, etc, using appropriate evolutionary models and methods for phylogenetic analysis like maximum likelihood, neighbor-joining, or Bayesian inference (see example, Figure 5).

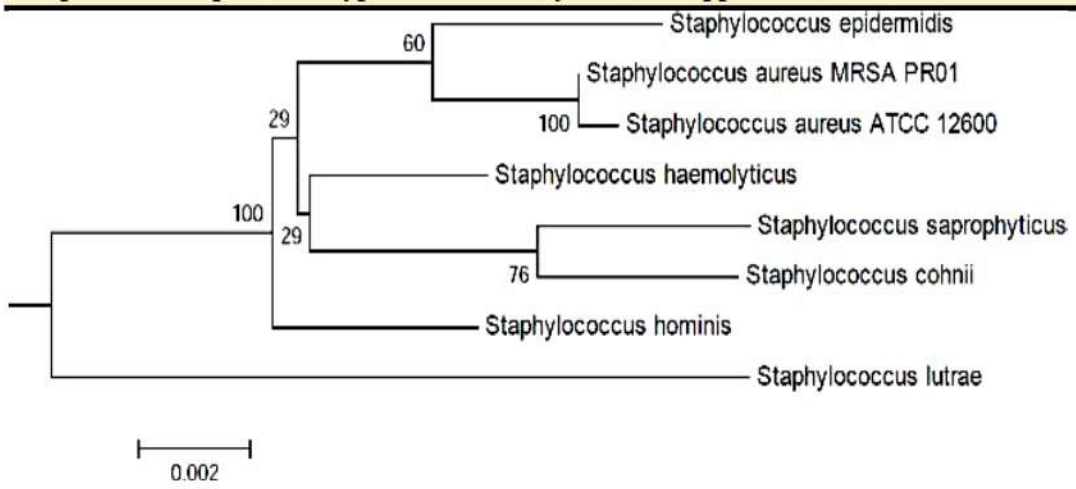


Figure 5; The phylogenetic tree illustrates the classification of *Staphylococcus aureus* strain PR01 in relation to other strains of the same type that belong to the *Staphylococcaceae* family (Lee et al., 2014)

The strains and their respective GenBank accession numbers for 16S rRNA genes are as follows: ATCC 12600, L36472 for *S. aureus;* ATCC 15305, AP008934 for *S. saprophyticus*; ATCC 14990, D83363 for S. epidermidis; DSM 20328, X66101 for S. hominis; CCM2737, X66100 for *S. haemolyticus;* and ATCC 49330, AB009936 for *S. cohnii*. The sequences in the tree are aligned by the RDP aligner, and a distance matrix is generated using the Jukes-Cantor corrected distance model and alignment model positions, omitting the need for alignment inserts.

f)-Definition of taxonomic units:

-Species and genera identification thresholds for 16S rRNA sequence similarity should be established at a range of 97-99% identity.

g)-Compatibility with additional studies:

-Polyphasic approach combining molecular, phenotypic and genomic data.
-Through this comparative analysis of 16S rRNA sequences, bacteria, and archaea are identified and categorized.

3.3.4.2.-Limitations of the 16S rRNA sequencing technique in molecular taxonomy

The principal limitations of the 16S rRNA sequencing technique in molecular taxonomy are as follows:

1)-Limited taxonomic resolution:

The 16S rRNA gene is relatively conserved, which can make discrimination between some closely related species difficult (Janda & Abbott, 2007)

2)-Presence of multiple 16S operons:

Some bacteria may have several slightly different copies of the 16S gene in their genome. This complicates the interpretation of results and taxonomic assignment (Větrovský & Baldrian, 2013).

3)-Lack of resolution at the species level:

For some taxonomic groups, 16S sequence similarity thresholds do not allow reliable species delimitation. Other approaches, such as ANI (Average Nucleotide Identity), are necessary (Rosselló-Mora, R., & Amann, 2001).

4)- Difficulty of identifying complex societies:

In some cases, the results of bacterial community analyzes of 16S RNA sequences may incorrectly reflect true diversity (Carini et., 2016).

5)-Incomplete databases:

Some 16S ARN sequence databases continue insufficient for some microbial groups, which may lead to incorrect results. Consequently, complementary techniques, including metagenomics, are often necessary (Rappé & Giovannoni, 2003).

Although 16S rRNA sequencing continues to be an important reference method in molecular taxonomy, it is imperative to combine its capabilities with other techniques to achieve a more detailed and effective classification of microorganisms (Janda and Abbott, 2007).

3.3.4. ADN/ADN hybridization technique

The ADN/ADN hybridization technique has long been considered the reference method for determining the boundaries of bacterial organisms.This method is based on the principle that for each of the apparent coatings in particular, an amount of ADN/ADN hybridization higher than 70% must be provided, with a ΔTm (melting temperature difference) of less than 5 °C (Stackebrandt and Goebel, 1994; Rosselló-Mora et Amann, 2001).

The protocol measures the degree of relatedness between the ADN genes of both types of bacteria. In addition, the ADN is similar, and the amount of hybridization will be high. This technique allows assistance in determining genetic relationships between organisms and identifying bacterial species from their phenotypes (Rossello-Mora et Amann, 2001; Konstantinidis et Tiedje, 2005).

However, the ADN/ADN hybridization technique imposes certain limitations, including relative complexity, which makes it incapable of reproducing and resolving difficult space-level taxonomy (Goris et al., 2007). They may be replaced by modern genomic and novel approaches such as average nucleotide identity (ANI) (Konstantinidis et Tiedje, 2005; Chun et al., 2018).

Chapter III

Major groups of Bacteria

1.-Introduction

Based on the comparison of 16S DNA sequences, the phylogenetic analysis made it possible to divide the bacterial domain into 24 phyla and several classes and understand their evolutionary relationships (Woese, 1987; Prescott, 2008). These bacteria are grouped into two major groups based on their Gram staining. The group of gram-negative bacteria, which is the most diverse with phyla 21, is the most diversified group, which is that of Proteobacteria, with 5 classes (will be studied in the next chapter). However, a significant phylum is that of Firmicutes, composed of Gram-positive bacteria, often with low GC content, including genera such as *Bacillus* and *Clostridium* (Woese, 1987; Ludwig et al., 2009). The *Actinobacteria* phylum, also Gram-positive but rich in GC, plays an essential role in several domains (Woese, 1987; Gao & Gupta, 2012).

The principal phyla (groups) of the domain *Bacteria* are given in Table 13 and their phylogenetic relationships analysis in Figure 6.

Table 13: Representative groups of the domain *Bacteria*

Taxonomic Rank	Representative class or Genera
Gram Negative bacteria	
Phylum *Aquificae*	*Aquifex, Hydrogenobacter*
Phylum *Thermotogae*	*Thermotoga, Geotoga*
Phylum *Thermodesulfobacteria*	*Thermodesulfobacterium*
Phylum *Deinococcus-Thermus*	*Deinococcus, Thermus*
Phylum *Chrysiogenetes*	*Chrysogenes*
Phylum *Chloroflexi*	*Chloroflexus, Herpetosiphon*
Phylum *Thermomicrobia*	*Thermomicrobium*
Phylum *Nitrospira*	*Nitrospira*
Phylum *Deferribacteres*	*Geovibrio*
Phylum *Cyanobacteria*	*Prochloron,Oscillatoria, Anabaena, Nostoc,*
Phylum *Chlorobi*	Chlorobium, Pelodictyon
Phylum *Proteobacteria*	Class: *Alph, Beta Gamma, Delta and Epsilon of Proteobacteria*
Phylum *Planctomycetes*	*Planctomyces, Gemmata*
Phylum *Chlamydiae*	*Chlamydia*
Phylum *Spirochaetes*	*Spirochaeta, Borrelia, Treponema, Leptospira*
Phylum *Fibrobacteres*	*Fibrobacter*
Phylum *Bacteroidetes*	*Bacteroides, Porphyromonas, Prevotella, Flavobacterium*
Phylum *Fusobacteria*	*Fusobacterium, Streptobacillus*
Phylum *Verrucomicrobia*	*Verrucomicrobium*
Phylum *Dictyoglomi*	*Dictyoglomus*
Phylum Gemmatimonadetes	*Gemmatimonas*
Gram positive bacteria	
hylum Firmicutes (low GC Gram-Positive Bacteria)	Class I. *Clostridia*, Class II. *Mollicutes*, Class III. *Bacilli*
Phylum *Actinobacteria* (High GC Gram-Positive Bacteria)	Class I *Actinobacteria*

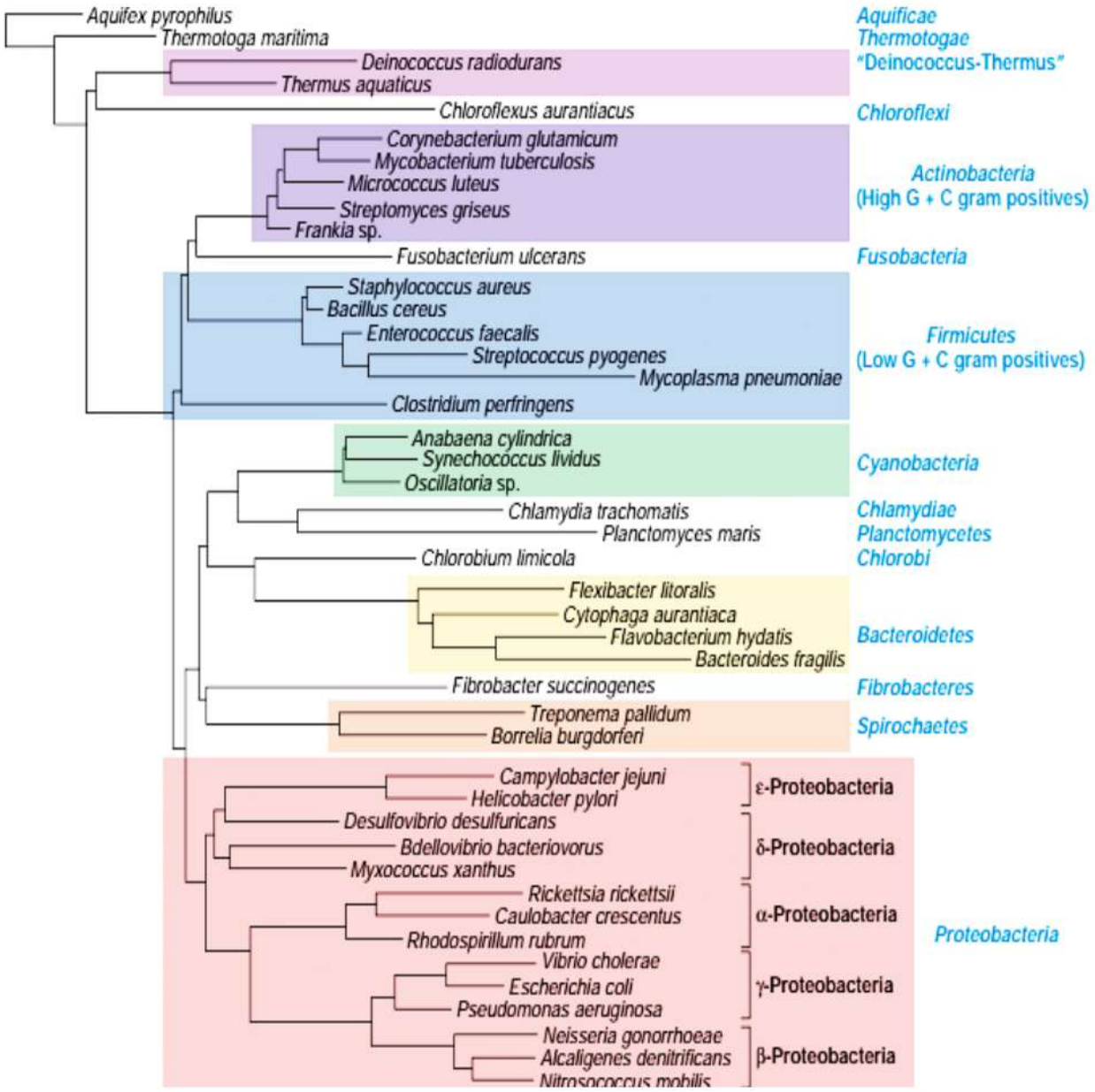


Figure 6: Phylogeny tree of the domain *Bacteria* based on the comparisons of 16S rRNA sequences of the most phyla showed the phylogenetic relationships between the phyla of gram negative and gram-positive bacteria (Willey et al., 2008).

2.- Photosynthetic bacteria

There are indeed three groups of gram-negative photosynthetic bacteria: the purple bacteria, the green bacteria, and the cyanobacteria. These groups are classified based on their photosynthetic pigments and other physiological and biochemical characteristics (Willey et al., 2008).

The phylum *Cyanobacteria*, also known as oxyphotobacteria, comprises Gram-negative bacteria capable of oxygenic photosynthesis, producing oxygen as a byproduct (Raven, 2017),

both *Chlorobi* (green sulfur bacteria) and *Chloroflexi* (green non-sulfur bacteria) phyla that perform anoxygenic photosynthesis, using compounds other than water as electron donors (Bryant & Frigaard, 2006; Hug et al., 2016).

Furthermore, certain genera belonging to the phylum *Proteobacteria*, including Alpha-, Beta-, and Gamma-proteobacteria, contain purple bacteria that participate in anoxygenic photosynthesis by utilizing bacterial chlorophyll and carotenoids (Imhof et al., 2017).

Moreover, these photosynthetic bacteria are frequently found in many ecosystems, where they play essential roles in biogeochemical processes on a global scale, primary production, and transfer of energy (Falkowski et al., 2008).

In contrast, photosynthetic bacteria exhibit distinct phylogenic relationships. The phylum *Chloroflexi* is very close to the phylum *Deinococcus-Thermus*. However, the phyla *Chlorobi* and *Cyanobacteria* are close to Bacteroidetes and *Chlamydia*, respectively (see Figure X).

The most characteristics of the Major Groups of Gram-negative Photosynthetic Bacteria are given in Table X.

Cyanobacteria differ fundamentally from other photosynthetic bacteria in that they are capable of anoxic photosynthesis. They have photosystems I and II, use water as an electron donor, and produce oxygen during photosynthesis. In contrast, purple-green bacteria and anoxygenic aerobic photosynthesis have a single photosystem and practice anoxygenic photosynthesis because they are unable to use water as an electron source. They use other reductase molecules as electron donors.

2.1.- 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 respective hues are chocolate brown and verdant green. *Pelodictyon, Chlorobium, and Prosthecochloris* are representative genera (Imhoff, 2014; Overmann, 2008)

Contrary to other groups, the green sulfur bacteria are a small group of photolithoautotrophs that use hydrogen sulfide, elemental sulfur, and hydrogen as electron sources. The elemental sulfur produced by sulfide oxidation is deposited outside the cell. The organism's photosynthetic pigments reside in ellipsoidal vesicles known as chlorosomes or chromium. These vesicles are attached to plasma membrane but not continuous with it (Bryant & Frigaard, 2006; Madigan et al., 2018).

Physiologically, the green sulfur bacteria are distinguished by their capacity to engage in anoxygenic photosynthesis, in which they utilize reduced sulfur compounds such as sulfide or, alternatively, water as electron donors (Imhoff, 2003). They have chlorosomes, which are light-harvesting complexes containing bacteriochlorophylls c, d, or e and which impart a discernible green hue (Overmann & Garcia-Pichel, 2013).

On the other hand, these bacteria are discovered in various sulfur-rich anoxic environments, including sulfur-rich marine sediments, anoxic zones of stratified lakes, and hot springs (Overmann, 2008). They play a role in primary production in these ecosystems and play essential functions in the global sulphur cycle (Frigaard & Bryan 2008). Important genera in this taxonomic class are *Chlorobium*, *Chlorobaculum, and Chloroherpeton*. Additionally, the potential biological remediation of toxic compounds and effluent treatment using specific species of *Chlorobi* is currently being investigated (Gaisin et al., 2015).

2.2.- The phylum *Chloroflexi* (green non-sulfur bacteria)

The phylum Chloroflexi is composed of photosynthetic and non-photosynthetic bacteria. Based on 16S rRNA studies, it is divided into classes: *Chloroflexia, Thermomicrobia* and *Anaerolineae*. This phylum is not closely related to any other bacterial group and is a deep and ancient branch of the bacterial tree (Figure X). Members of this phylum have a varied morphology, including notably filamentous, slippery, and thermophilic bacteria often isolated from neutral to alkaline hot springs, where they develop in the form of orange-reddish mats, generally associated with cyanobacteria. They mainly use bacteriochlorophyll c and a, which gives them a distinct green color, like green sulfur bacteria with small chlorosomes and bacteriochlorophyll c, found in the plasma membrane. Additionally, their metabolism is more similar to that of non-sulfur purple bacteria. They can carry out anoxygenic photosynthesis using pigments different from other photosynthetic bacteria or they grow aerobically as a chemoheterotroph ((Willey et al., 2008; Madigan et al., 2018).

Ecologically, members of Chloroflexi are usually found in association with cyanobacteria in aquatic environments rich in organic matter, such as freshwater and wastewater. They can also be present in soils and other habitats. They can use a wide variety of carbon and energy sources, and some are even capable of photosynthesis even in the presence of low light levels. They play an important role in aquatic and terrestrial ecosystems. Their ability to photosynthesize in varied environments and their adaptation to specific ecological conditions make them intriguing organisms to study (Claus & Berkeley,1986; Madigan et al., 2018).

2.3-The phylum *Cyanobacteria*

The phylum *Cyanobacteria* is the largest and most diverse group of photosynthetic bacteria. It occupies a unique place in the history of life on Earth. These photosynthetic Gram-negative bacteria are considered the precursors of aerobic life on our planet (Schopf, 2000; Tomitani et al., 2006). They present diversity in the G C content of the group, ranging from 35 to 71, and have more than 62 species and 24 genera.

Morphologically, *Cyanobacteria* are diverse, ranging from simple unicellular forms to complex filamentous or colonial organisms (Whitton & Potts, 2012). They have subcellular structures to house their photosynthetic pigments, such as chlorophyll a, phycocyanins and carotenoids (Whitton, 2012).

Metabolically, *Cyanobacteria* can oxygenic photosynthesis, using water as an electron donor. This process, which appeared several billion years ago, allowed the progressive production of oxygen and the radical transformation of the primitive. Many cyanobacteria are obligate photolithoautotrophs, and a few can grow slowly in the dark, such as chemoheterotrophs, by oxidizing glucose and a few other sugars. Under anoxic conditions, *Oscillatoria limnetica* oxidizes hydrogen sulfide instead of water and carries out anoxygenic photosynthesis, much like the green photosynthetic bacteria atmosphere (Olson & Blankenship, 2004).

In addition, the photosynthetic system closely resembles the eukaryotes because they have chlorophyll a and photosystems I and II, performing oxygenic photosynthesis. Indeed, the cyanobacteria were once known as "blue-green algae." Like the red algae, cyanobacteria use phycobiliproteins as accessory pigments. Photosynthetic pigments and electron transport chain components are in thylakoid membranes lined with particles called phycobilisomes. However, some species have also developed atmospheric nitrogen fixation capabilities by forming specialized cells called heterocysts (Whitton, 2012).

Cyanobacteria also have biotechnological solid potential. Their ability to fix CO2 and produce molecules of interest makes them promising organisms for producing biofuels and bioproducts (Olson & Blankenship, 2004; Raymond et al., 2002). They are also used in phytoremediation to treat wastewater. They occupy a central place in many aquatic and terrestrial ecosystems (Whitton & Potts, 2012). They constitute the basis of many food chains and actively participate in biogeochemical cycles. However, certain species can produce toxins and cause nuisance in natural environments (Whitton, 2012).

The morphology and characteristics of the most important genera of cyanobacteria are given in Figure 7 and Table 14.

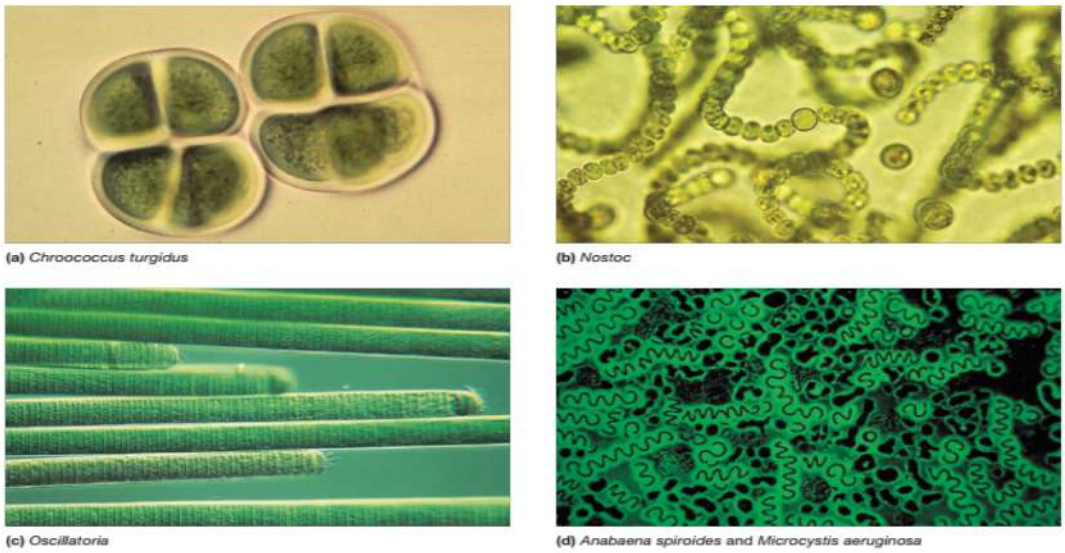


Figure 7: Representative cyanobacteria (Willey et al., 2008).
(a) Chroococcus turgidus, two colonies of four cells each (x600); (b) Nostoc with heterocysts (X550). (c) Oscillatoria trichomes seen with Nomarski interference-contrast optics (x 250). (d) The cyanobacteria Anabaena spiroides and Microcystis aeruginosa. The spiral A. spiroides is covered with a thick g.

Table 14: Some genera of *Cyanobacteria* and their characteristics

Genus	Characteristics
Anabaena	- Filamentous cyanobacteria, Form heterocysts for nitrogen fixation. - Produce various secondary metabolites, including toxins - Found in freshwater environments
Nostoc	- Filamentous, branched cyanobacteria - Able to form resistant spores called akinetes - Heterocysts allow nitrogen fixation - Commonly found in terrestrial and aquatic habitats
Oscillatoria	- Filamentous cyanobacteria with oscillating/gliding motility - No true branching or heterocysts - Produce various pigments like chlorophyll a and phycoerythrin - Thrive in benthic or mat-forming environments
Synechococcus	- Unicellular, picocyanobacteria - Widespread in marine and freshwater ecosystems - Important contributors to primary productivity - Lack specialized structures like heterocysts
Microcystis	- Colonial cyanobacteria, forming irregular aggregates - Produce hepatotoxins like microcystins - Responsible for harmful algal blooms in eutrophic waters - Adapted to a wide range of light and nutrient conditions

2.4.-Non-sulphurous purple bacteria

All non-sulfur purple bacteria are *alpha-proteobacteria*, except the exception of *Rhodocylus*, a *beta-proteobacterium* (Madigan et al., 2018).

Physiologically, non-sulfurous purple bacteria are distinguished by their ability to carry out anoxygenic photosynthesis without the release of oxygen (Imhoff, 2014). Their photosynthetic pigments, which mainly consist of carotenoids and bacterial chlorophylls a or b., give them this distinctive purple color. In contrast to green sulfur bacteria, they do not use sulfur as an electron donor but prefer organic compounds (Madigan et al., 2018). However, some non-sulfur purple bacteria can survive in aerobic or anaerobic conditions. Their capacity for both photosynthesis and anoxygenic respiration enables them to grow in diverse ecological conditions. (Yurkov & Csotonyi, 2009).

Ecologically, purple non-sulfur bacteria are found in various aquatic and terrestrial ecosystems (Pfennig,1989). They are found in lakes, marshes, sediments, soil, and even in certain extreme environments, such as hydrothermal sources. This diversity of habitats reflects the great ecological adaptability of these microorganisms. They play key roles in biogeochemical cycles, notably in the degradation of organic matter and the recycling of nutrients (Imhoff, 2014). In addition, non-sulfurous purple bacteria present also biotechnological solid potential. Their ability to fix CO2 and produce hydrogen makes them promising organisms for producing biofuels and bioproducts. They are also being studied for their use in phytoremediation to treat wastewater (Madigan et al., 2018). The most important genera and their characteristics are given in Table 15

Table 15: Some genera of non-sulphurous purple bacteria and their characteristics

Genus	Characteristics
Rhodobacter	- Aerobic photoheterotrophic bacteria - Rod-shaped cells, Pigments: carotenoids and bacteriochlorophylls - Found in lakes and rivers
Rhodopseudomonas	- Facultatively anaerobic photoheterotrophic bacteria - Rod-shaped or vibrioid cells - Similar pigments to Rhodobacter -Play important roles in C and N cycles
Rhodospirillum	-Anaerobic or microaerophilic photoheterotrophic bacteria - Spiral-shaped or vibrioid cells - Pigments mainly composed of carotenoids - Present in diverse aquatic environments
Rhodocyclus	- Facultatively anaerobic photoheterotrophic bacteria - Coccoid or short rod-shaped cells - Pigments based on bacteriochlorophylls - Often found in sediments and anoxic zones

2.5. Purple Sulfur Bacteria

Purple sulfur bacteria are Gamma proteobacteria, strict anaerobes, generally photolithoautotrophic and divided into two families: *Chromatiaceae* and *Ecthothiorhodospiraceae* in the order *Chromatial*. The family *Ecthothiorhodospiraceae* has eight genera. However, most purple sulfur bacteria are found in the family *Chromatiaceae*, which has 26 genera (see some important genera in table X). They have various forms, from single-celled cocci to filamentous as well as branched forms (Willey et al., 2008).

As for the physiological and metabolic characteristics, purple sulfur bacteria can perform anoxygenic photosynthesis, which does not lead to the release of oxygen Photosynthetic pigments, consisting mainly of carotenoids and bacterial chlorophyll, give them their characteristic purple or reddish-purple colour (Imhof, 2014). However, they utilise autotrophic sulphur compounds for electron donation during photosynthesis, including hydrogen sulphide (H2S) and elemental sulphur (Madigan et al., 2018). They exhibit ecological diversity by residing in terrestrial and aquatic habitats, with a particular preference for sulfur-rich, anoxic, or partially anoxic regions.They play crucial roles in various biogeochemical processes (Pfennig, 1989).

On the other hand, purple sulfur bacteria are very interested in biotechnology. They fix CO2 and produce biomass, and their potential for bioremediation and biofuel production has stimulated numerous research efforts (Madigan et al., 2018).

Table 16: Some genera of non-sulphurous purple bacteria and their characteristics

Genus	Characteristics
Chromatium	- Obligately anaerobic, phototrophic bacteria - Large, ovoid or rod-shaped cells - Utilize sulfide, thiosulfate, or elemental sulfur as electron donors - Produce globules of elemental sulfur as photosynthetic byproduct.
Allochromatium	- Obligately anaerobic, phototrophic bacteria - Ovoid to rod-shaped cells - Oxidize sulfide, thiosulfate, and other reduced sulfur compounds - Accumulate elemental sulfur inside or outside the cells
Thiocapsa	- Anaerobic, phototrophic bacteria - Spherical to slightly irregular cells - Capable of utilizing a wide range of reduced sulfur compounds, - Can form characteristic reddish-purple cell aggregates or colonies
Lamprocystis	- Phototrophic, obligately anaerobic bacteria - Spherical to slightly ovoid cells - Oxidize sulfide, thiosulfate, and other sulfur compounds - Produce intracellular sulfur globules during photosynthesis
Thiopedia	- Phototrophic, anaerobic bacteria - Flat, plate-like cell arrangement in packets or sheets - Utilize sulfide, thiosulfate, and elemental sulfur as electron donors - Characteristic

2.6.-Heliobacteria

Heliobacteria are a distinct branch of anoxygenic photosynthetic bacteria with a wide diversity of photosynthetic bacteria belonging to the phylum Firmicutes. Members of *Heliobacteria* have several remarkable features (table 17):

1)-Anoxygenic photosynthesis: perform photosynthesis that does not release oxygen, and their photosynthetic pigments are based on bacteriochlorophyll g (Madigan et al., 2018).

2)- Distinctive morphology: these bacteria have a distinctive rod-shaped appearance with rounded ends (Imhof, 2014).

3)-Metabolic diversity: They are completely anaerobic and are often found in environments rich in organic matter, such as soil, sediments, or rice fields, but some species can also be aerobic (Satley and Madigan, 2006).

4) Environmental roles: Other types of bacteria are rarely associated with photosynthesis but play an essential environmental role in the harmful ecosystems in which they exist (Madigan and Ormerod, 1995). They indicate the degradation of organic matter and the recycling of nutrients.

5)-Interests in biotechnology: The study of solar bacteria also includes interest in biotechnology. Its diverse metabolism and ability to produce hydrogen by organisms promotes applications such as biogenic carbohydrate production (Madigan et al., 2018).

Table 17: Some genera of *Heliobacteria* and their characteristics

Genus	Characteristics
Heliobacterium	- Strictly anaerobic, photoheterotrophic bacteria - Rod-shaped cells with rounded ends - Photosynthetic pigments based on bacteriochlorophyll g - Can grow chemoheterotrophically in the dark
Heliophilum	- Anaerobic, photoheterotrophic bacteria - Rod-shaped cells with pointed ends - Utilize a variety of organic compounds as carbon and energy sources - Can also fix atmospheric nitrogen
Heliorestis	- Anaerobic, photoheterotrophic bacteria - Vibrioid or spirillum-shaped cells - Produce hydrogen as a byproduct of photosynthesis - Commonly found in anoxic sediments and soils
Thermothor	- Thermophilic, anaerobic, photoheterotrophic bacteria- Rod-shaped or coccoid cells - Adapted to grow at high temperatures (up to 65°C) - Utilize a limited range of organic compounds

3.- Autotrophic bacteria

3.1.- Introduction

Autotrophic bacteria include a large and heterogeneous group of microorganisms. The main characteristic of these bacteria includes the capacity for synthesizing their organic compounds from inorganic substances. They play an important role in many biogeochemical cycles, such as the cycling of carbon and fixation of nitrogen, which altogether are essential to the life support systems of ecosystems (Sorokin and Muyzer, 2011; Madigan et al., 2018).

3.2.-Morphology of autotrophic bacteria

Autotrophic bacteria present large morphological variation. They can be found in different forms, unicellular or filamentous, in a great variation of shapes, sizes, and arrangements. Some autotrophic bacteria have distinct cell structures, for example, sheaths or special appendages. For example, filamentous cyanobacteria usually develop chains of cells around it with an envelope for protection. Other autotrophic bacteria may have specialized structures such as stalks or holdfasts for attachment (Rippka et al., 2018; Madigan et al., 2018).

3.3.-Classification of autotrophic bacteria

Autotrophic bacteria are divided into many taxonomic groups based on similarity displayed by the grouping and their evolutionary affinities. They include the phylum of autotrophic bacteria is *Cyanobacteria*, which includes well-known genera such as *Anabaena, Microcystis*, and *Synechococcus*; and, some group of *Proteobacteria,* including the genera *Nitrosomonas and Nitrobacter,* involved in nitrification belonging to those involved in nitrification (Soo et al., 2014; Daims et al., 2016).

3.4.-Metabolism of autotrophic bacteria

In autotrophic bacteria, different metabolic pathways are involved in carbon fixation. Among the important pathways, the most commonly found pathway in most autotrophic organisms, besides *Cyanobacteria* and some *Proteobacteria*, is the Calvin-Benson cycle. Other autotrophic bacteria use alternative pathways such as the reductive citric acid cycle (reverse of the Krebs cycle) or the 3-hydroxypropionate/4-hydroxybutyrate cycle. Autotrophic bacteria making use of such pathways can assimilate carbon inorganic compounds, for example, carbon dioxide in the form of organic compounds (Berg, 2011; Hugler & Sievert, 2011).

3.5.-Ecology of autotrophic bacteria

Autotrophic bacteria inhabit in many environments, including aquatic systems, soil, and extreme environments. The autotrophic bacteria, mainly cyanobacteria, contribute to the primary and oxygen production in the aquatic environment through anoxic photosynthesis. Some autotrophic bacteria are also found in nitrogen and sulfur cycles and are accomplished by nitrification for nitrogen and sulfur oxidation, respectively (Falkowski et al., 2008; Kirchman, 2018).

The most genera of autotrophic bacteria and their characteristics are given in Table 18.

Table 18: most genera and their morphology, metabolism and ecological characteristics

Genus	Morphology	Metabolism Type	Ecological Role	Typical Habitat
Cyanobacteria	Diverse; can be unicellular or filamentous	Photoautotrophic	Primary producers, nitrogen fixation	Freshwater, marine, terrestrial
Nitrosomonas	Rod-shaped	Chemoautotrophic	Ammonia oxidation	Soil, freshwater
Nitrobacter	Rod-shaped	Chemoautotrophic	Nitrite oxidation	Soil, freshwater
Chlorobi	Spherical or rod-shaped	Photoautotrophic	Sulfur cycling	Anoxic freshwater and marine sediments
Chloroflexi	Filamentous	Photoautotrophic	Decomposition, organic matter cycling	Hot springs, sewage sludge

4.- Gram-negative bacteria other than *Proteobacteria*

4.1.- Phylum *Chlamydiae*

Chlamydiae are an obligate intracellular bacteria with a distinctive growth cycle, and constitute a different category of Gram-negative bacteria. In contrast to most bacteria, *Chlamydia* can not multiply outside of their host cell. To survive and proliferate, they must penetrate and control the cellular machinery of their eukaryotic hosts (Greub, 2009; Sachse et al., 2015).

The phylum *Chlamydiae* is classified in four distinct orders: *Waddliiales, Chlamydiales, Parachlamydiales,* and *Criblamydiales.* Chlamydiae, which includes a few medically important species, is the most well-known and extensively researched genus. For example, *C. trachomatis,* infects humans and mice, causing trachoma, nongonococcal urethritis in the man; *C. psittaci* that causes psittacosis (poumo) in humans, the parrots, turkeys, sheep, cattle and cats, and *Chlamydia pneumoniae* is a common cause of human pneumonia. Others. Genera such as *Parachlamydia,*

Wadalia, and *Symcania* have a wide variety of hosts, including birds, mammals, amoebae, and protists. (Horn, 2008; Greub, 2010; Kuo et al., 2015).

Morphologically, the chlamydiae are Gram-negative bacteria, a small group of non-motile coccoid bacteria, non-motile, coccoid, varying in size from 0.2 to 1.5 µm. They can only reproduce in cytoplasmic vesicles of host cells, according to a developmental cycle which includes the formation of two types of cells: elementary bodies and reticle bodies. Although their envelope resembles that of other Gram-negative bacteria, their cell wall is different because only devoid of muramic acid and peptidoglycan layer (Sachse et al., 2015; Madigan et al., 2018).

4.2.- Phylum *Rickettsiae*

The rickettsiae are Gram-negative bacteria, known for their intimate associations with eukaryotic hosts, especially arthropods and vertebrates. Two major orders constitute them: *Rickettsiales* and *Holosporales*. Rickettsiales contains the most known and medicinally significant genera, including *Ehrlichia, Rickettsia, and Orientia* (Dumler, 2010; Gillespie et al., 2012).

Physiologically, they are obligate intracellular parasitism with a small cell size (0.3 to 0.5 um) due to their simplified genomes and specialized intracellular lifestyle, and unable to reproduce outside the confines of the eukaryotic host cell. In addition, *Rickettsiae* can infect a wide range of eukaryotic hosts, such as arthropods like ticks, fleas, and lice, and vertebrates such as humans and other mammals (Raoult & Roux, 1997; Perlman et al., 2006).

The genus *Rickettsiae* is ecologically significant due to its importance in the ecology of arthropod and vertebrate hosts. Many Rickettsia species can be transmitted by ticks, fleas, or parasites, and causing major mortality and morbidity in both animals and humans, including typhus and Rocky Mountain spotted fever (Dumler, 2010; Fournier et al., 2021).

4.3.- Phylum Planctomycetes

Planctomycetes are gram negative bacteria, characterized by their distinctive cell morphologies, which possess an intracellular membrane system that divides the cell into multiple compartments, a feature that is rare among prokaryotes, and do not contain peptidoglycan (Fuerst & Sagulenko, 2011). They are currently divided into several classes, including *Planctomycetia, Phycisphaerae,* and *Verrucomicrobiae*. Each class contains many genera and species, each of which possesses unique ecological and physiological properties (Table 19) (Dedysh & Ivanova, 2019).

Planctomycetes exhibit a wide range of metabolic capabilities, including the degradation of complex biopolymers such as chitin and cellulose, in addition to aerobic and anaerobic respiration. The latter process aids in the circular motion of organic substances within aquatic ecosystems.

They are chemotrophs, which means they can utilize an extensive variety of organic compounds for energy. Certain organisms within this taxonomic group demonstrate mixotrophic abilities, participating in both heterotrophy and autotrophy (Strous et al., 1999; Wiegand et a., 2020).

In addition, planktomycetes have ecological importance and biotechnological potential. They assure, among other vital functions, the cycling of carbon, nitrogen, as well as in a great number of other types of aquatic ecosystems. They form relationships with other organisms, such as sponges, which may provide metabolic or structural benefits to their hosts (Lage & Bondoso, 2014).

Table: 19: Most genera of planctomycetes and their characteristics (Fuerst & Sagulenko, 2011; Dedysh & Ivanova, 2019)

Genus	Morphology	Metabolism	Ecological Role
Planctomyces	Budding bacteria with intracellular membranes	Chemoheterotrophic, potential mixotrophs	Involved in organic matter degradation, nitrogen cycling
Gemmata	Envelope-possessing intracellular symbionts	Mixotrophic, capable of anammox	Potential symbionts, involved in nitrogen cycling
Rhodopirellula	Peptidoglycan-less bacteria	Chemoheterotrophic	Found in marine environments, potential role in carbon cycling
Isosphaera	Spherical cells with intracellular membranes	Chemoheterotrophic	Found in freshwater environments, potential role in carbon cycling
Pirellula	Budding bacteria with intracellular membranes	Chemoheterotrophic	Found in marine environments, potential role in carbon cycling

4.4.- Phylum Spirochaetes

Spirochaetes are Gram negative bacteria, anaerobic, facultative anaerobic or aerobic, that contains chemoheterotrophic distinct by their structure and their mobility mechanism. They are grouped into a single order, *Spirochaetales*, divided into three families (*Spirochaetaceae, Serpulinaceae, and Leptospiraceae)* and 13 genera. In addition, *Spirochetes* form symbiotic associations with other organisms and are found in very diverse places such as in the hind intestine, in the digestive system of molluscs (*Cristispira*) and mammals, in the oral cavities of animals (Radolf et al., 2012; Brinkman et al., 2013).

Morphological and ecological characteristics, spirochaetes are long, thin bacteria shaped like a flexible propeller long, flexuous and very spiraled. They are all movable by means of an axial filament. Those are aerobic or anaerobic inhabitants of soils and aquatic environments. Members of the genus *Spirochaeta* live free and often grow in marine, anoxic and rich in sulfide. Certain species of the genus Leptospira develop in water loaded with oxygen and in moist soil (Faine et al., 1999; Charon & Goldstein, 2002; Brinkman et al., 2013).

The most important genera of spirochaetes are given in Table 20.

Table 20 : some of the most common genera of Spirochaetes and their characteristics (Faine et al., 1999; Charon & Goldstein, 2002; Radolf et al., 2012 ; Brinkman et al., 2013).

Genus	Morphology	Motility	Habitat	Notable Features
Borrelia	Thin, helical spirochetes	Flagella and axial filaments	Ticks, lice, and mammals	Pathogenic species cause Lyme disease (Borrelia burgdorferi), relapsing fever, and other tick-borne illnesses
Treponema	Thin, flexible spirochetes	Axial filaments	Oral cavity, gastrointestinal tract, and genitourinary system	Pathogenic species include Treponema pallidum, causing syphilis, and Treponema denticola, periodontal disease
Leptospira	Thin, coiled spirochetes	Hooked ends	Soil and water environments	Pathogenic species cause leptospirosis, a zoonotic disease transmitted through contaminated water or contact with infected animals
Spirochaeta	Long, flexible spirochetes	Periplasmic flagella	Aquatic environments	Some species are involved in anaerobic degradation processes, such as fermenting carbohydrates and producing hydrogen
Brachyspira	Short, coiled spirochetes	Axial filaments	Intestinal tract of animals	Pathogenic species are associated with intestinal diseases in animals, including swine dysentery and avian intestinal spirochetosis
Cristispira	Spiral-shaped spirochetes	Unknown	Marine environments	Marine species associated with the gastrointestinal tract of fish, potentially playing a role in fish health and digestion

4.5.- Phylum Bacteroidetes

The phylum Bacteroidetes contains a large group of Gram-negative, non-spore-forming, rod-shaped bacteria, Strict anaerobes or facultative aerobic organisms, these bacteria inhabit detritus, animals, and human and soil environments, as well as the digestive tracts of humans and animals (Thomas et al., 2011). Some important genera that belong to this phylum include *Bacteroides, Prevotella, Porphyromonas* and *Flavobacterium*. These bacteria play a major role in the degradation of complex organic compounds such as cellulose, chitin, and proteins (Reichardt et al., 2014). Many species are involved in food digestion and carbohydrate metabolism in animals. However, some members are also opportunistic pathogens and can cause infections in humans (Brock, 2008). The phylum Bacteroidetes displays high metabolic and ecological diversity, and its members are abundant in many ecosystems, especially those rich in organic matter (Krieg et al., 2010).

4.- Phylum Firmicutes (low GC Gram-Positive Bacteria)

The Firmicutes are large group of Gram positive that includes very diverse bacteria, from pathogenic species such as *Staphylococcus* to bacteria involved in fermentation such as *Lactobacilli*. They are part of the domain *Bacteria* and are classified in the phylum Firmicutes, characterizing by its low GC. This phylum is divided into three classes: *Clostridia, Bacilli, Mollicutes*, and *Negativicutes* and 10 orders and 34 families (Figure 8). Each class contains different genera and species of Firmicutes. They are distinguished by some common characteristics, such as the presence of a thick cell wall composed mainly of peptidoglycan. This cell wall gives them increased resistance to adverse environmental conditions. In addition, Firmicutes are generally Gram-positive bacteria with varied cellular forms (cocci, bacilli, filaments). Some form resistant spores (e.g. *Bacillus, Clostridium*) (Claus & Berkeley, 1986; Garrity et al., 2005; Madigan et al., 2018).

Physiologically, Firmicutes present a great diversity of physiologies and lifestyles. They can be aerobic, anaerobic, fermentative or respiratory. Some are autotrophs, others are heterotrophs. Many Firmicutes species are capable of forming endospores, which are hardened structures that allow them to survive in adverse environmental conditions for long periods of time (Madigan et al., 2015; Hisamatsu et al. 2020).

Ecologically, Firmicutes are found in a wide range of ecological habitats, including plants, animals, sediments and water. Some fixed plants inhabit the digestive tract of animals as symbionts or symbiotes, effectively facilitating the digestion of food. Pathogenic organisms have the ability

to cause disease in plants, animals, or humans. Some bacteria are used more in industrial processes, including the cheese and alcoholic beverage industry (Aberrantigan et al., 2015; Irwin et al., 2013).

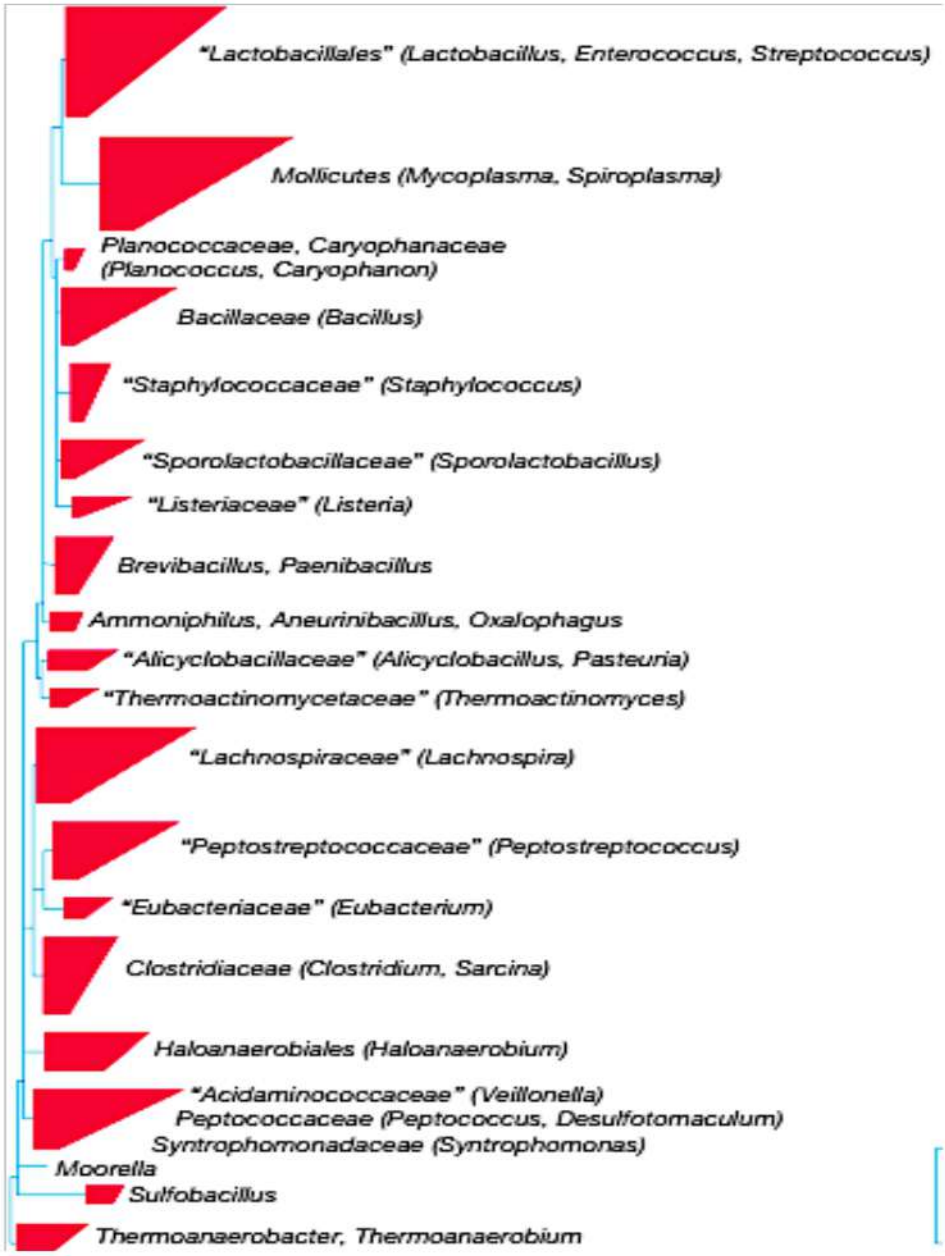


Figure 8: Phylogenetic relationships in the phylum Firmicutes (Low GC% Gram Positives).

4.1.-Class mollicutes (the mycoplasmas)

The Mollicutes, also known as the "mycoplasmas," constitute a unique class within the Firmicutes phylum. These bacteria are characterized by the absence of a cell wall, a feature that sets them apart from most other bacteria (Razin et al., 1998).

Mollicutes are known for their lack of a cell wall, instead relying on a flexible plasma membrane for structural support. They also have some of the smallest cell sizes and genome sizes known among free-living organisms, typically ranging from 0.2 to 0.8 µm in diameter and 0.58 to 1.38 Mb in genome size (Himmelreich et al., 1996). Due to their cell wall-deficient nature, molluscs can adopt a variety of pleomorphic shapes, ranging from spherical to filamentous forms (Waites & Talkington, 2004).

The class Mollicutes is also divided into several orders, families, and genera. These include *Mycoplasma*, which contains the genus *Mycoplasma* with several pathogenic species, and the insect-associated *Entomoplasmatales* and *Acholeplasmatales*, which are commonly found in the environment which are anaerobic molluscs found in the digestive tract of animals (Weisburg et al., 1989; Gasparić, 2010);

Molluscs occupy diverse ecological niches and play different roles, from commensalism and mutualism to pathogenicity. Many mollicutes form symbiotic relationships with their hosts, while others are known to cause diseases in humans, animals and plants (Waites & Talkington, 2004). Most important genera including pathogenic species are given in Table 21.

Table 21: major genera and species within the Mollicutes class

Genus	Characteristics
Mycoplasma	- Lack cell wall - cause diseases in humans, animals, and plants - Examples: *M. pneumoniae*, *M. genitalium*, *M. mycoides*
Ureaplasma	- Belong to the family *Mycoplasmataceae* - Associated with infections of the urogenital tract in humans - Examples: *U. urealyticum, U. parvum*
Acholeplasma	- Found in the environment (soil and plants) - Not known to be pathogenic - Examples: *Acholeplasma laidlawii, Acholeplasma hippikon*
Spiroplasma	- Helical, motile cell morphology - Some species are plant pathogens - Examples: *Spiroplasma citri, Spiroplasma apis*
Entomoplasma	- Associated with insects, including honeybees and leafhoppers - Examples: *Entomoplasma freundtii, Entomoplasma luminosum*
Mesoplasma	- Also associated with insects, - Examples: *Mesoplasma florum,*
Anaeroplasma	- Anaerobic Mollicutes found in the gastrointestinal tracts of animals - Examples: *Anaeroplasma abactoclasticum, Anaeroplasma bactoclasticum*

4.2.- Class *Clostridia*

Clostridiums 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 (Wiegel et al., 2006). Their evolutionary relationships are shown in Figure 09. The phylum Clostridia is divided into three orders and 11 families (Table 22). 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 compounds (Wiegel et al., 2006). 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 (Paredes-Sabja et al., 2011). Clostridia play important roles in the cycling of organic matter and nutrients in various ecosystems, especially in anaerobic environments such as the rumen, sediment, and human intestine (Flint et al., 2012).

They can break down a variety of organic molecules and are forced anaerobes that get their energy from fermentation or anaerobic respiration (Wiegel et al., 2006). This includes *Clostridium difficile*, a major cause of antibiotic-associated diarrhea and colitis (Hensgens et al., 2012); *Clostridium tetani*, the causative agent of tetanus (Pascual, 2015); and *Clostridium botulinum*, which produces the most potent biological toxins known and can cause severe illness and botulism (Montecucco & Rasotto, 2015).

Table 22: characteristics of some important orders and genera of the class *Clostridia*:

Order/Genus	Characteristics
Clostridiales *Clostridium*	- Obligate anaerobes - Able to form endospores - Diverse metabolic capabilities, including fermentation and anaerobic respiration - Include pathogenic species like *C. difficile, C. tetani,*
Peptoclostridium	- Anaerobic, spore-forming bacteria - Involved in the degradation of proteinaceous substrates - Some species are associated with the human gut microbiome
Ruminococcus	- Anaerobic, Gram-positive bacteria - Found in the gastrointestinal tracts of various mammals, including humans - Play roles in the digestion of cellulosic materials
Thermoanaerobacterales Thermoanaerobacter	- Thermophilic, anaerobic *Clostridia* - Capable of fermenting a variety of carbohydrates - Found in high-temperature environments, such as hot springs and geothermal areas

Order/Genus	Characteristics
Caldicellulosiruptor	- Extremely thermophilic, anaerobic bacteria - Able to degrade lignocellulosic biomass - Potential applications in biofuel production from plant-derived feedstocks
Halanaerobiales *Halanaerobium*	- Halophilic (salt-tolerant) anaerobic *Clostridia* - Adapted to saline environments, such as hypersaline lakes and marine sediments - Able to ferment a range of organic compounds
Halothermothrix	- Thermophilic, halophilic anaerobic *Clostridia* - Thrive in high-temperature, high-salinity environments - Involved in the degradation of complex organic matter in saline habitats
Oscillospirales *Oscillospira*	- Anaerobic, spiral-shaped bacteria - Abundant members of the gut microbiome in various mammals, including humans Potential roles in gut health and nutrient cycling

4.3.- Class Bacilli

Bacilli form a important class in the phylum Firmicutes. They are Gram-positive bacteria with diverse forms (cocci, rods, spore-forming cocci, and non-spore-forming rods). Taxonomic diversity and taxonomy. The class of bacilli is also divided into two ordre *Bacillales* and *Lactobacillales*. , and contains 17 families and more than 70 Gram-positive genera.

In addition to the genus *Bacillus*, the order *Bacillales* contains *Paenibacillus* and *Lysinibacillus* (Logan & De Vos, 2009). On contrary, the order *Lactobacilli* consists of genera that produce lactic acid, including *Enterococci, Streptococci*, and *Lactobacilli*, all of which are significant in the human gastrointestinal microbiome and dietary fermentation (Pot et al., 1994).

Bacilli have a variety of metabolic capabilities, which enable them to survive in a wide range of environments. Bacilli can use both aerobic and anaerobic respiration, allowing them to adapt to a wide range of anoxic conditions (Lechner et al., 1998). Some bacilli, including *Streptococci* and *Lactobacilli*, are known for their ability to metabolize a number of organic substrates into beneficial compounds including lactic acid and other fermented foods (Giraffa et al., 2010).

However, some species of *Bacillus*, among others, can fix atmospheric nitrogen, thus helping to cycle nutrients in terrestrial ecosystems (Heulin et al., 1987). Due to their ecological importance and applications in biotechnology, bacilli play critical roles in various ecosystems and have great potential in biotechnology. Many bacilli, especially lactic acid-producing species, are essential members of the human and animal gut microbiome, contributing to digestive processes, nutrient absorption, and regulation of the immune system (Giraffa et al., 2010). Some bacilli, such as *Bacillus* species, form beneficial relationships with plants, providing compounds that promote growth or protect against pathogens (Beneduzi et al., 2012). Bacilli are also widely used in the

production of fermented foods, probiotics, enzymes, biofuels, and other valuable biotechnology products (Schallmey et al., 2004).

4.3.1- Order *Bacillales*

4.3.1.1--Family *Bacillaceae*

Bacillaceae is a member of the order *Bacillales*, which is classified within the phylum Firmicutes and is composed of Bacilli. These bacteria are distinguished by their ability to produce endospores. They are obligate aerobes, obtaining the majority of their energy from aerobic respiration. Their ability to form resistant endospores allows them to survive in adverse environmental conditions. They have remarkable metabolic capacities, and found in a wide variety of environments. Some species can promote plant growth, either by fixing atmospheric nitrogen or by producing growth compounds (Logan & Vos, 2009 ; Vaishampayan, 2010).

In terms of ecological importance and biotechnological applications, *Bacillaceae* play important ecological roles and are of great biotechnological interest. Certain Bacillus species contributing to soil fertility and plant protection (Vaishampayan et al., 2010; Yoon et al., 2007). Many species are also exploited in the production of enzymes, biopesticides, probiotics and biofuels. Although most *Bacillaceae* are harmless, some species such *as B. anthracis* and *B. cereus* can cause serious illness in humans and animals Becker et al., 2014). The main genera, species and their characterizations are given in Table 23.

Table 23 : Characteristics of some important genera of the family *Bacillaceae*:

Genus	Characteristics
Bacillus	- 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., B. *subtilis, B. cereus,*
Paenibacillus	- Closely related to *Bacillus*, but with some distinct phylogenetic and physiological differences - Able to form endospores - Known for nitrogen fixation and plant growth-promoting activities
Lysinibacillus	- Separated from the genus *Bacillus* based on the presence of diaminopimelic acid in the cell wall - Involved in the degradation of complex organic compounds
Anoxybacillus	- 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

4.3.1.2.-Family des *Staphylococcaceae*

The family *Staphylococcaceae* belongs to the order *Bacillales*, within the class *Bacilli*. This family is mainly represented by the *genus Staphylococcus,* whose morphological and metabolic characteristics are described in Table X (Logan & Vos, 2009).

Morphology, bacteria of this family are Gram-positive, non-sporulating cocci, generally arranged in clusters resembling bunches of grapes. Their cell wall is composed of peptidoglycan and may contain teichoic acid. They are generally aerobic or facultatively anaerobic, and can utilize a wide variety of carbon and energy sources, including sugars, organic acids and amino acids (Otto, 2018). Some species are catalase-positive and can produce lactic acid as a product of fermentation. However, this family contains commensal species, as well as major opportunistic pathogens in humans and animals (Becker et al., 2014).

Table 24: Most important characteristics of the genus *Staphylococcus*

Characteristics	Genus Staphylococcus
Morphology	Gram-positive cocci, non-spore-forming, clustered in grape-like arrangements
Metabolism	Aerobic or facultatively anaerobic, can utilize a wide variety of carbon sources
Habitat	Skin, nasal passages, and mucous membranes of humans and animals
Representative species	- *Staphylococcus aureus:* important pathogen, produces numerous toxins - *Staphylococcus epidermidis*: commensal species, can be opportunistic
Importance	- Pathogenicity (skin infections, septicemia, food poisoning) - Biotechnological applications (enzyme production, bioproducts)

4.3.1.3.-Family *Listeria*

The family *Listeriaceae* also belongs to the order *Bacillales*, within the class *Bacilli*. This family is mainly represented by the genus *Listeria* (Allerberger & Wagner, 2010). Its morphological and metabolic characteristics are detailed in table 25.

Morphologically, bacteria of the family *Listeriaceae* are Gram-positive, non-sporulating bacilli, generally mobile thanks to the presence of peritrichous flagella (Farber & Peterkin, 1991). Their cell wall is composed of peptidoglycan and may contain teichoic acid. *Listeriaceae* are generally

aerobic and chemoorganotrophic, capable of utilizing a wide variety of carbon sources, including carbohydrates, amino acids and organic acids (Vazquez-Boland et al., 2001). Some species can grow at relatively low temperatures, giving them the ability to grow in refrigerated environments.

However, the Listeria genus is the largest in the family *Listeriaceae*. It includes several species, with *Listeria monocytogenes* as the main pathogenic species for humans and animals (Allerberger & Wagner, 2010).

The most important characteristics of the genus *Listeria* are given in Table 25.

Table 25: Characteristics of the genus *Listeria* and its species

Characteristics	Genus Listeria
Morphology	Gram-positive rods, non-spore-forming, motile
Metabolism	Aerobic, chemoorganotrophic, can grow at low temperatures
Habitat	Environment (soil, wastewater), food products, animal microbiota
Representative species	- *Listeria monocytogenes*: important foodborne pathogen, responsible for listeriosis - *Listeria innocua:* non-pathogenic species, often used as a study model
Importance	- Pathogenicity (serious infections, especially in vulnerable populations) - Food contamination, major concern in food safety

4.3.2- Order *Lactobacillales*

Bacteria of the order *Lactobacillales* are Gram-positive bacilli or cocci, generally non-sporulating, which produce lactic acid as a single end or intermediate product. Their cell wall is composed of peptidoglycan and may contain teichoic acids. Some species can form chains or pairs of cells (Pot et al., 1994).

Additionally, *Lactobacillales* are primarily anaerobic or microaerophilic, with lactic acid production as the primary metabolite. They can utilize a wide variety of substrates, including sugars, amino acids, and organic acids, depending on the species (Giraffa et al., 2010) Moreover, Bacteria of the order *Lactobacillales* play essential roles in many ecosystems. They are major players in the human and animal intestinal microbiota, contributing to digestion, nutrient absorption and immune regulation (Saavedra, 2001). They are also involved in the fermentation of many foods and drinks, such as dairy products, cold meats and alcoholic beverages (Jézéquel et al., 2013).

The most important families and genera of *Lactobacillales* are given in Table 26.

Table 26: Characteristics of some important genera of the Order *Lactobacillales*:

Family Genera	Morphology	Metabolism	Characteristics
Lactobacillaceae *Lactobacillus*	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.
Leuconostoc	Coccoid to rod-shaped, non-motile	Heterofermentative, CO2 and lactic acid production	Involved in fermentation of cabbage to sauerkraut, sourdough, and kefir. Enhances flavor and texture.
Pediococcus	Coccus, non-spore-forming, tetrads	Homofermentative, lactic acid production	Used in sausage fermentation, cheese, and sour beer production.
Streptococcaceae *Streptococcus*	Coccus, chains	Facultative anaerobic, lactic acid fermentation	Includes S. thermophilus for yogurt and cheese. Some species are pathogenic.
Lactococcus	Coccus, pairs or chains	Homofermentative, lactic acid production	Used in cheese making (e.g., cheddar, gouda).
Enterococcaceae *Enterococcus*	Coccus, pairs or short chains	Facultative anaerobic, lactic acid fermentation	Used in cheeses, indicators of water quality, potential pathogens.
Leuconostocaceae *Weissella*	Coccoid to rod-shaped, non-motile	Heterofermentative, lactic acid, acetic acid, ethanol, CO2 production	Involved in various fermentation processes, researched for probiotic properties.
Carnobacteriaceae *Carnobacterium*	Rod-shaped, non-spore-forming, motile	Facultative anaerobic, lactic acid fermentation	Found in meat, dairy products; used for biopreservation and probiotics

5.- Phylum *Actinobacteria*

Actinobacteria are Gram-positive bacteria showing exceptional diversity and very high physiological and ecological activities with essential biotechnological applications (Barka et al., 2016). 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 network branched hyphae. In addition, the Actinomycetes phylum is vast and very complex. The Bergey classifies G+C-rich bacteria phylogenetically using 16S rRNA data. Phylum *Actinobacteria* contains have their GC-rich relatives, it includes one class, five subclasses, six orders, 14 suborders and 44 Families (Goodfellow, 2012). Phylogenetic relationships between order and family of actinobacteria are given in Figure 9.

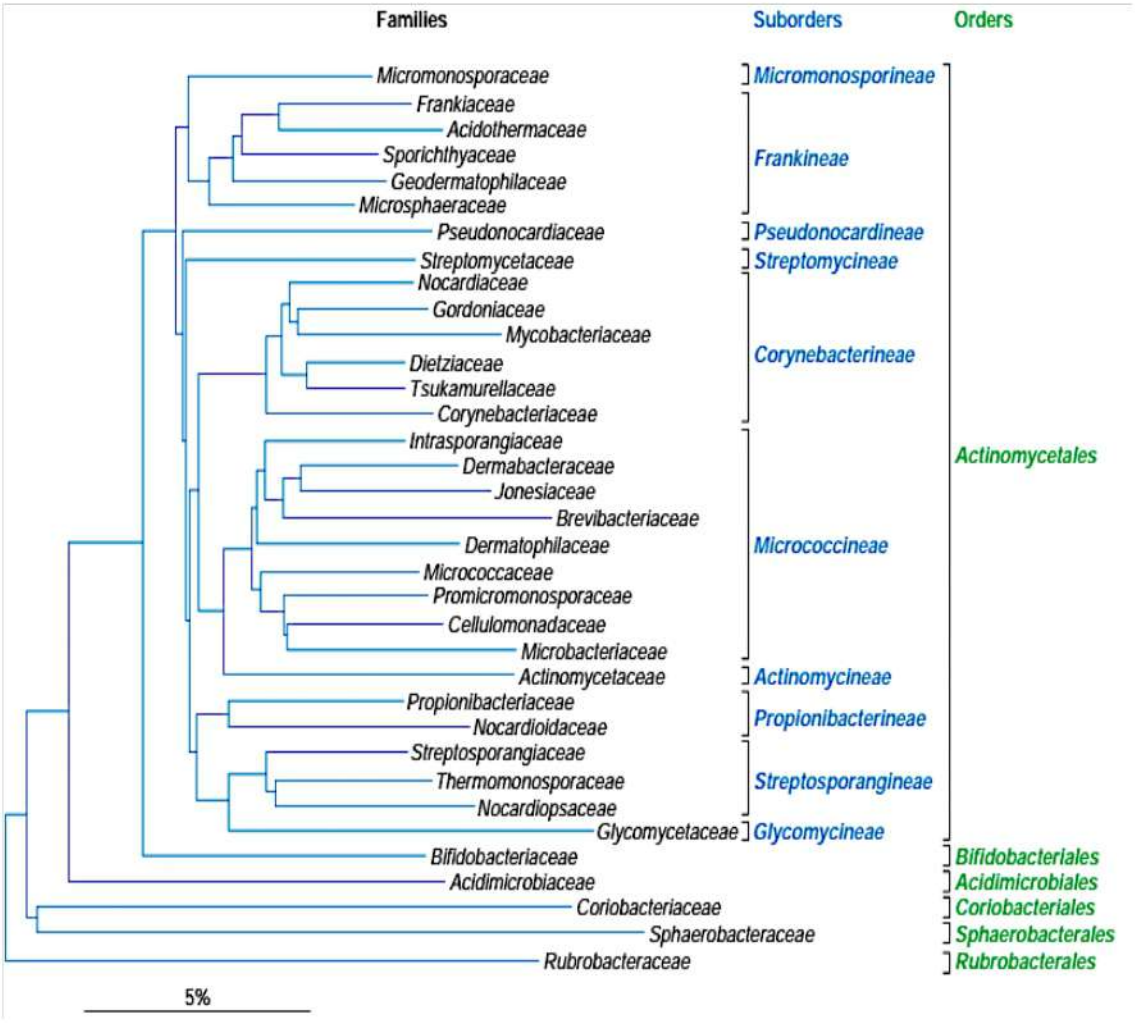


Figure 09: The phylogenetic relationships between orders, suborders, and families of phylum *Actinobacteria* based on 16S rRNA data are shown. (Willey et al., 2008).

The bar represents 5 nucleotide substitutions per 100 nucleotides.

5.1.Morphological features

Actinobacteria present the most diverse morphologies: from cocci and bacilli to complex filaments or mycelia. It is in most of the species an action of spores that accounts for their resistance to the most adverse environmental conditions (figure 10). The cell wall is normally composed of peptidoglycan. It may include other several unique structural components, such as mycolic acids(Bergey, 1989).

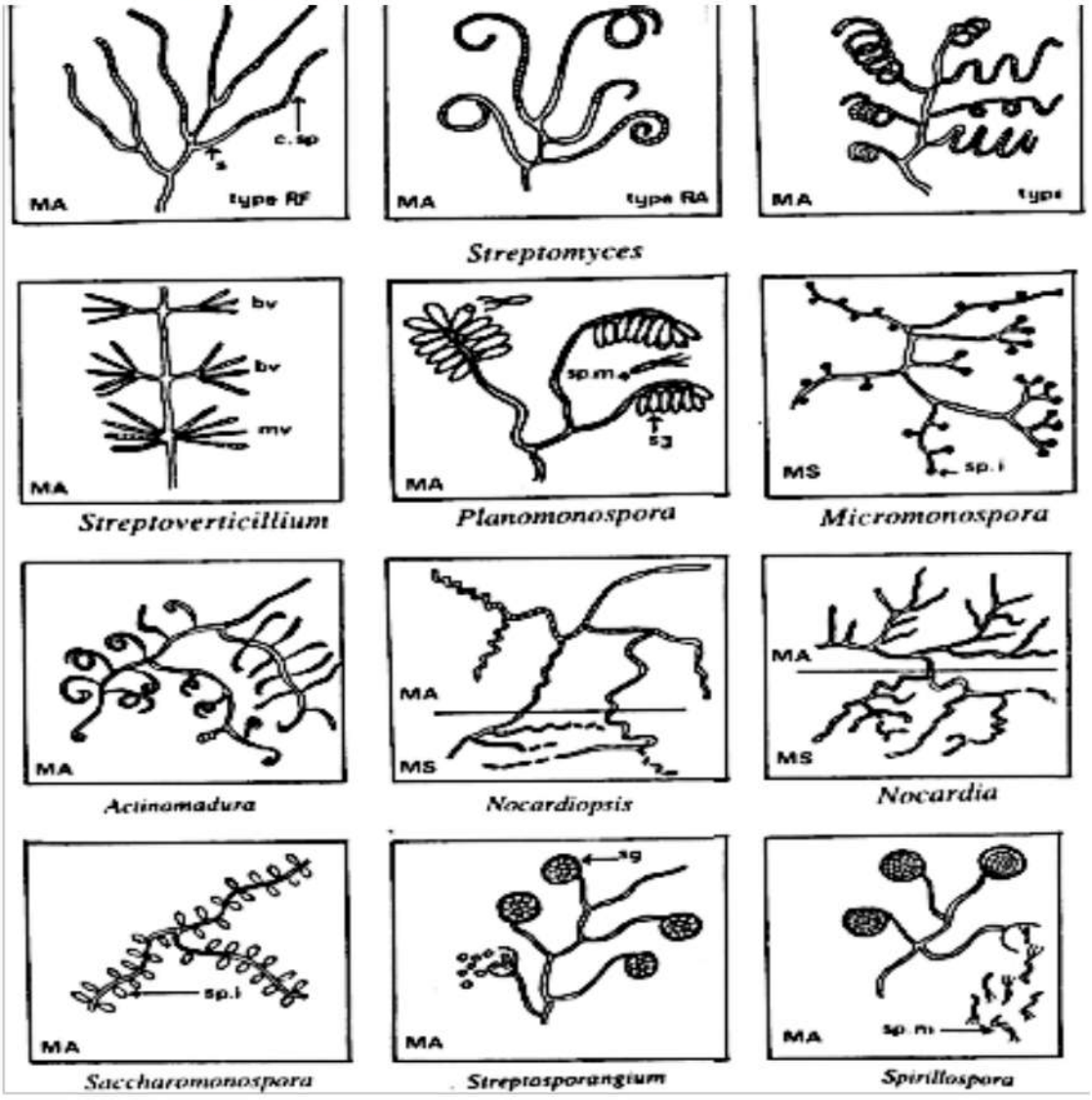


Figure 10: Micromorphology of the principle genera of actinomycetes (In Bergey, 1989).

MA, aerial mycelium; MS, substrate mycelium; RF, Rectus Flexibilis (straight spore chains at flexuous); RA, Retinaculum Apertum (hooked or closed loop chains); S, Spira (chains spiraled); s, sporophore; vs. sp.; spore chains; sp. i.; isolated spores; sp. m.; mobile spores; sg.,sporangia.

5.2.-Physiology characteristics

Actinobacteria are generally aerobes, but some are facultative or strict anaerobes. They are heterotrophic, but some species are chemotrophic. Most are capable of using a wide variety of energy sources and also of producing specific substances such as geosmin and 2-methyl isoborneol

which are responsible for the characteristic humus odor of soils (Zaitlin et al. , 2003). The majority of them prefer a neutral or slightly alkaline pH (6.8-8). They are generally mesophilic, others are thermophilic (50°C) and can go up to 60°C (Kroppenstedt and Goodfellow, 2006).

5.3.-Environmental roles and applications

Actinobacteria are found in many ecosystems, soil to aquatic environments, and also play a critical part in the human microbiome. The strains have been known to be active in degrading recalcitrant organic matter, fix nitrogen, and produce a wide range of secondary metabolites, including several clinically important antibiotics, antineoplastic agents, and other bioactive compounds (Barka et al., 2016). Numerous applications of the biotechnological field make heavy use of Actinobacteria, for example: enzyme production, biofuels, and bioprocessing.

Table 27: Characteristics of most important Genera of *Actinobacteria*:

Genus	Morphology	G+C Content	Oxygen Relationship	Other Distinctive Characteristics
Actinomycetaceae *Actinomyces*	filaments, spore-forming	55-70%	Facultatively anaerobic	Opportunistic pathogens
Bifidobacteriaceae *Bifidobacterium*	Irregular,sometimes branched rods	42-67%	Anaerobic	Commensal of the intestinal microbiota
Corynebacteriaceae *Corynebacterium*	Rod-shaped,	46-74%	Aerobic	Some species are pathogenic
Micrococcaceae *Micrococcus*	Cocci arranged in tetrads	60-75%	Aerobic	Pigmented, ubiquitous in the environment
Mycobacteriaceae *Mycobacterium*	Acid-fast, alcohol-resistant rods	57-69%	Aerobic	Pathogens such as M. tuberculosis and M. leprae
Nocardiaceae *Nocardia*	Fragmented, filaments	64-70%	Aerobic	Some are opportunistic pathogens
Propionibacteriaceae *Propionibacterium*	Rods with Y-shaped branching	53-67%	Anaerobic	Involved in propionate fermentation
Streptomycetaceae *Streptomyces*	Branched filaments, spore-forming	69-73%	Aerobic	Producers of numerous secondary metabolites

Chapter V

Phylum Of *Proteobacteria*

1-Phylum *Proteobacteria*

1- 1.- Introduction

Proteobacteria constitute one of the most important and most studied phyla of the domain *Bacteria*. This vast group brings together a great diversity of bacteria with varied morphologies, metabolisms and ecological niches. Based on the comparison of 16S rRNA sequences, the phylum *Proteobacteria* is considered monophyletic and divided into the main *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, and Epsilonproteobacteria* (Brenner et al., 2005) (Figure 11). Each of these classes brings together orders his is the largest and most diverse group of bacteria; currently there are over 500 generations.

Proteobacteria present a wide diversity of forms, ranging from bacilli and cocci to more complex forms such as spirillas and photosynthetic bacteria. Their cell wall is of the Gram-negative type, composed of a thin layer of peptidoglycan surrounded by an outer membrane (Brenner et al., 2005).

Proteobacteria colonize a wide variety of habitats, such as soil, water, animal and plant hosts. They play essential roles in many ecological processes, such as nitrogen fixation, degradation of organic matter, energy production by chemo- or photosynthesis, and pathogenicity in humans, animals and plants (Garrity et al., 2005).

Ecologically, Proteobacteria occupy a wide variety of terrestrial, aquatic and living host habitats. They play crucial roles in many biogeochemical processes such as carbon, nitrogen, sulfur and heavy metal cycles (Williams et al., 2010). Some genera like *Rhizobium, Azotobacter* and *Nitrosomonas* are involved in nitrogen fixation and nitrification. Others like *Pseudomonas*, *Xanthomonas* and *Agrobacterium* are important plant pathogens (Madigan et al., 2015).

However, Proteobacteria also include many notable human and animal pathogens such as Escherichia, Salmonella, Vibrio, Helicobacter and Neisseria (Brenner et al., 2005).

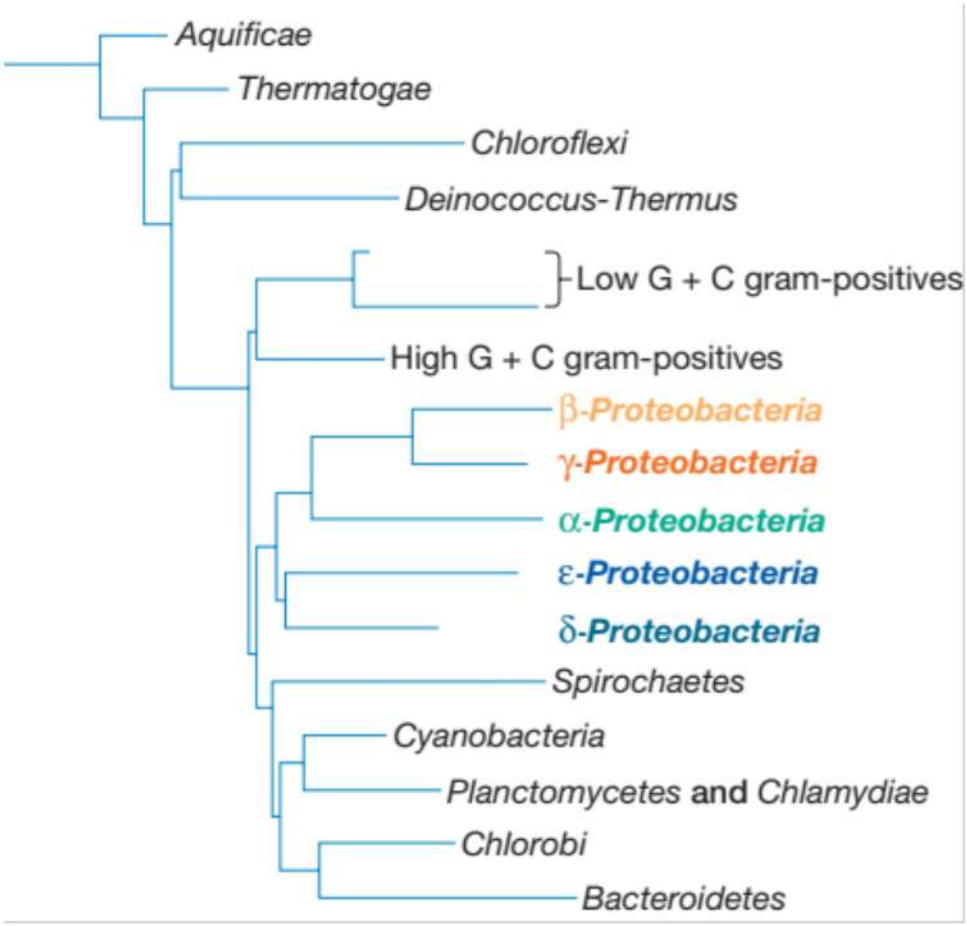


Figure 11: Phylogenetic relationships between the five classes of the phylum proteobacteria and their close phyla among prokaryotes

2- Class alpha-*Proteobacteria*

Alpha-proteobacteria form a major class within the phylum Proteobacteria. They include numerous Gram-negative bacteria exhibiting great morphological and metabolic diversity. Some are straight or curved bacilli, while others adopt spiral, vibrioid, or irregular shapes. Many Alpha-proteobacteria are motile thanks to polar or peritric flagella (Madigan et al., 2015).

Ecologically, they colonize a wide variety of terrestrial, aquatic and living host environments. Some important genera like *Rickettsia, Orientia, Anaplasma* and *Ehrlichia* are obligate intracellular pathogens affecting humans and animals (Oren, 2014). Others like *Rhizobium, Bradyrhizobium and Azorhizobium* form nitrogen-fixing symbioses with leguminous plants. The genera *Agrobacterium, Sinorhizobium* and *Mesorhizobium* include phytobacterial pathogens (Madigan et al., 2015).

Additionally, Alpha-*proteobacteria* exhibit great metabolic diversity, being capable of chemoautotrophy, chemoheterotrophy, photoheterotrophy and methanotrophy. They play crucial

roles in the biogeochemical cycles of carbon, nitrogen and sulfur Some genera like *Nitrobacter* and *Nitrosomas* are involved in nitrification (Oren, 2014).

Despite their diversity, Alpha-proteobacteria share some common characteristics such as a Gram-negative cell wall, the absence of lateral flagella, and the presence of ubiquinones and cyclopropanic fatty acids (Madigan et al., 2015). Their ecological and pathogenic importance makes them a major bacterial group.The most important genera of this class are given in Table 28.

Table 28: Characteristics of Selected Alpha-Proteobacteria Genera

Genus	Morphology	G+C (%)	Genome (Mb)	Oxygen Relationship	Other Distinctive Characteristics
Rhizobium	Rods	59-63	5-7	Aerobic	Nitrogen fixation, symbiosis with legumes
Caulobacter	Curved rods	67-69	4-5	Aerobic	Complex life cycle, budding reproduction
Azospirillum	Spirillum -shaped rods	68-70	4-5	Facultatively aerobic	Nitrogen fixation, plant growth promotion
Sphingomonas	Short rods	65-67	4-5	Aerobic	Degradation of aromatic compounds, polymer production
Bradyrhi-zobium	Rods	62-65	8-9	Aerobic	Nitrogen fixation, symbiosis with legumes
Paracoccus	Cocci	65-70	2-3	Facultatively aerobic	Denitrification, environmental stress adaptation

3- Class Beta -*Proteobacteria*

The class Beta-*proteobacteria* is one of the five main classes within the phylum Proteobacteria. This group includes a diverse group of bacteria with a wide range of metabolic capabilities and ecological roles (Garrity et al., 2005). This class is divided into several orders, including *Burkholderiales*, *Neisseriales, Nitrosomonadales, Rhodocyclales*, and others. Some of the most well-known and studied genera within this class include *Burkholderia, Polaromonas, Methylophilus*, Neisseria, and Ralstonia (Brenner et al., 2005). In addition, Bacteria of this class exhibit a variety of morphologies, occurring mostly as rods and cocci. Many species are motile, and use flagella for locomotion. The cell wall structure is typical of Gram-negative bacteria, with

a thin peptidoglycan layer surrounded by an outer membrane (Brenner et al., 2005). Furthermore, They display a wide range of metabolic capabilities. Many are aerobic or optionally anaerobic, and are able to use a variety of organic compounds as carbon and energy sources. Some genera, such as Nitrosomonas, are chemoautotrophs, oxidizing inorganic compounds such as ammonia to produce energy (Garrity et al., 2005). Moreover, they play important roles in various ecosystems. Some species participate in the nitrogen cycle, participating in nitrification or denitrification processes. Others are known for their ability to degrade xenobiotic compounds, making them valuable for bioremediation applications. Some beta-proteobacteria are also recognized as opportunistic pathogens, causing infections in humans, animals and plants (Brenner et al., 2005).

The most important genera of this class are given in Table 29.

Table 29 Characteristics of Selected Beta-Proteobacteria Genera

Genus	Morphology	G+C (mol%)	Genome	Oxygen Relationship	Other Distinctive Characteristics
Burkholderia	Rods	66-68	6-9	Aerobic	Opportunistic pathogens, degradation of organic compounds
Polaromonas	Rods	62-65	4-5	Aerobic	Psychrophiles, degradation of aromatic compounds
Methylophilus	Rods	50-52	3-4	Aerobic	Methanol metabolism, role in carbon cycle
Neisseria	Cocci	52-54	2-3	Aerobic	Human pathogens, commensals of mucous membranes
Nitrosomonas	Rods	50-52	3-4	Aerobic	Ammonia oxidation, role in nitrogen cycle
Ralstonia	Rods	66-68	5-6	Aerobic	Plant pathogens, degradation of xenobiotics

4- Class Gamma -*Proteobacteria*

The class Gamma-*proteobacteria* is the largest and most diverse within the phylum *Proteobacteria* (Brenner et al., 2005). This class is divided into many orders, such as *Enterobacteriales, Pseudomonadales, Vibrionales, Xanthomonadales,* and others. It includes well-known and widely studied genera, such as *Escherichia, Salmonella, Vibrio, Pseudomonas, and Xanthomonas* (Garrity et al., 2005). For morphological characteristics, members of this class show a wide diversity of shapes, ranging from simple bacilli and cocci to more complex spiral or curved shapes. Many species are motile, and use flagella to move (Brenner et al., 2005).

Bacteria of Gamma-proteobacteria display exceptional metabolic diversity. They can be aerobic, anaerobic or optionally anaerobic, and can use a wide range of organic and inorganic compounds as sources of carbon and energy. Some genera, such as *Pseudomonas*, are known for their ability to degrade a wide range of compounds, including xenobiotics (Garrity et al., 2005).

Moreover, Gamma-proteobacteria are ubiquitous in diverse environments, including soil, water, and the human/animal microbiome. They play essential roles in many environmental processes, such as carbon, nitrogen, and sulfur cycles. Many of them are also known to be important pathogens, causing infections in humans, animals and plants (Brenner et al., 2005).

The most important genera of this class are given in Table 30.

Table 30: Characteristics of Selected Gamma-Proteobacteria Genera

Genus	Morphology	G+C (mol%)	Genome Size (Mb)	Oxygen Relationship	Other Distinctive Characteristics
Escherichia	Rods	50-52	4-5	Facultatively anaerobic	Commensal and pathogenic, model organism
Salmonella	Rods	52-54	4-5	Facultatively anaerobic	Enteric pathogens causing salmonellosis
Vibrio	Curved rods, spirilla	38-51	3-5	Facultatively anaerobic	Marine bacteria, some species pathogenic
Pseudomonas	Rods	58-67	4-7	Aerobic	Metabolically versatile, opportunistic pathogens
Xanthomonas	Rods	63-65	4-5	Aerobic	Plant pathogens, production of xanthan gum
Acinetobacter	Cocci, rods	38-47	3-4	Aerobic	Ubiquitous, emerging opportunistic pathogens
Shewanella	Rods	44-52	4-5	Facultatively anaerobic	Metal-reducing, potential for bioremediation

5- Class Epsilon -*Proteobacteria*

The class Epsilon-*proteobacteria* is an integral part of the phylum *Proteobacteria*. Although it is the smallest of the five classes of *Proteobacteria*, it has important roles in various environments, including as pathogens and chemoautotrophic bacteria in marine ecosystems (Brenner et al., 2005).It is mainly divided into two orders: *Campylobacterales* and Nautiliales. The most

representative genera of this class are *Campylobacter, Helicobacter, Arcobacter, Sulfurimonas, Nitratiruptor, Sulfurovum and Nautilia* are given in Table 31 (Garrity et al., 2005).

For morphological characteristics, members of this class generally have a curved, spiral or vibrio-shaped morphology (Brenner et al., 2005). In addition, these bacteria show great metabolic diversity. Some are microaerophilic, others are anaerobic chemoautotrophs, using inorganic compounds such as S or H_2 as energy sources. (Garrity et al., 2005).

However, this group play key roles in diverse environments. Some species are known to be pathogenic, causing gastrointestinal infections in humans and animals. Others are found in extreme environments such as ocean hydrothermal vents (Brenner et al., 2005).

Table 31: Characteristics of Selected Epsilon-*Proteobacteria* Genera

Genus	Morphology	G+C (mol%)	Genome (Mb)	Oxygen Relationship	Other Distinctive Characteristics
Desulfovibrio	Vibrio-shaped spirilla	53-66	3-4	Anaerobic	Sulfate-reducing bacteria, involved in biocorrosion
Bdellovibrio	Vibrio-shaped predatory	49-51	3-4	Aerobic, microa-erophilic	Predatory bacteria that attack other Gram-negative bacteria
Myxococcus	Rods, gliding motility	67-71	9-10	Aerobic	Multicellular behavior, fruiting body formation
Geobacter	Rods	58-59	4-5	Anaerobic, microa-erophilic	Metal-reducing bacteria, important in bioremediation
Desulfuromonas	Rods	60-62	3-4	Anaerobic	Sulfur-reducing bacteria, involved in metal cycling
Lawsonia	Curved rods	44-45	4-5	Anaerobic	Pathogenic, causes proliferative enteropathy in pigs

6- Class *Delta -Proteobacteria*

The class Deltaproteobacteria is an integral part of the phylum Proteobacteria. Although they are relatively less well-known than other classes of Proteobacteria, they harbor bacteria that play important ecological roles, particularly in biogeochemical cycles and complex microbial interactions (Brenner et al., 2005). It is divided into several orders, such as *Desulfuromonadales,*

Desulfobacterales, Bdellovibriionales, and *Myxococcales*. Some of the most represented genera are *Desulfovibrio, Bdellovibrio, Myxococcus, Geobacter, and Desulfuromonas* (Garrity et al., 2005) are given in Table 32.

For morphological characteristics, this class display a variety of morphologies, including vibrio, spiral, rod, and shell forms. Some genera, such as *Myxococcus*, are motile and able to move by gliding (Brenner et al., 2005). In addition, these bacteria display a wide range of metabolic capabilities. Some are anaerobic, such as sulfate-reducing bacteria of the genus *Desulfovibrio*, while others are aerobic or microaerobic (Garrity et al., 2005).

Furthermore, these bacteria serve crucial roles in different ecological systems, and play a crucial role in organic matter decomposition, predator-prey interactions, and biological erosion, among other important ecological processes (Brenner et al. 2005).

Table 32: Characteristics of Selected Delta-Proteobacteria Genera

Genus	Morphology	G+C (%)	Genome (Mb)	Oxygen Relationship	Other Distinctive Characteristics
Campylobacter	Spiral, curved rods	30-36	1-2	Micro-erophilic	Pathogenic, a leading cause of gastroenteritis in humans
Helicobacter	Spiral, curved rods	34-41	1-2	Microa-erophilic	Pathogenic, causes gastritis and peptic ulcers in humans
Arcobacter	Curved, spiral rods	27-30	2-3	Aerobic, microa-erophilic	Emerging pathogen, found in food and water sources
Sulfurimonas	Curved rods	35-37	2-3	Chemolithoauto trophic, anaerobic	Sulfur-oxidizing bacteria, found in marine environments
Nitratiruptor	Curved rods	35-36	1-2	Chemolithoauto trophic, anaerobic	Nitrate-reducing bacteria, isolated from deep-sea hydrothermal vents
Sulfurovum	Curved rods	40-42	2-3	Chemolithoautotr ophic, microaerophilic	Sulfur-oxidizing bacteria, found in marine sediments and hydrothermal vents

Chapter VI

Major Phyla

Of

Archaea

1-Introduction

Archaea, a former group of prokaryotic called archeabacteria, is a domain forming one of the three domains of life, including Bacteria and Eukarya (Woese et al., 1990). Originally believed to be highly specialized extremophiles, archaea have been found in a wide range of environments, ranging from soils to oceans and even in the human gut (Spang et al., 2015; Hug et al., 2016; Raymann et al., 2017).

Archaea are also prokaryotic organisms, like bacteria, but they possess several unique characteristics that differentiate them from bacteria. Among the characteristics which appear to be unique and different are the odd and diverse nature of the structure of the cell walls, composition in lipids of membranes, and information-processing mechanisms that are more similar to eukaryotes (Albers and Meyer, 2011; Koga and Morii, 2007;).

Phylogenetic analysis was later revealed that archaea diverged early from bacteria and eukaryotes to form an independent domain of life (Spang et al., 2015). This diversity of metabolic capabilities, together with the many kinds of ecological adaptations characteristic of the archaea, including methanogens, halophiles, thermophiles, and large numbers of other special groups (Leigh et al., 2011; Bonch-Osmolovskaya et al., 2018),

Recent developments in cultivation-independent approaches, including metagenomics and single-cell genomics, have contributed to the discovery of many previously unidentified archaeal families, expanding our knowledge of the evolutionary relationships and ecological functions of these microorganisms (Hug et al., 2016; Parks et al., 2017). Archaeal importance in biotechnological applications, global biogeochemical cycles, and our comprehension of the tree of life is becoming more widely recognized (Bonch-Osmolovskaya et al., 2018; Castelle & Banfield, 2018).

2.General structure of archaea

The archaea are unicellular microorganisms, and morphologically similar to bacteria, for that reason, they were included initially among prokaryotes. At present, *Archaea* and *Bacteria* are classified as separate taxa. Their distinction is consistent with the observation that they each possess distinct and individual qualities. Some of these are summarized in table 33.

Table 33: Comparison of Bacterial and Archaeal Cells. (Albers and Meyer, 2011; Willey et al., 2019)

Characteristic	Archaea	Bacteria
Cell Envelope	- Diverse cell surface structures (S-layers, glycoproteins, pseudopeptidoglycan) Lack a rigid peptidoglycan cell wall	- Typical Gram-positive or Gram-negative cell wall composed of peptidoglycan
Cell Membrane Lipids	- Ether-linked isoprenoid lipids provide increased stability and resistance to harsh conditions	- Ester-linked straight-chain fatty acids
Genome structure	- Smaller genomes (0.5-5.8 Mbp) –Circular dsDNA - More compact with fewer non-coding regions	- Generally larger genomes (1-10 Mbp) -Circular, double-stranded (ds) DNA - More extensive non-coding regions
Plasmids present	Circular dsDNA	circular and linear dsDNA
Genetic Information Processing	- More similar to eukaryotes (e.g., histones, TATA-binding proteins, RNA polymerases),	- More prokaryotic-like (e.g., lack of histones, simpler transcription/translation machinery)
Number of RNA polymerases	Many	Only one
Major reproductive strategy	-Binary fission -Budding -Fragmentation	-Binary fission, -Budding -Fragmentation, -Spore formation
Metabolism	- chemolithoautotrophs, heterotrophs, methanogens, etc. - Adapted to extreme environments (high temperature, low pH, high salinity, etc.)	Also diverse metabolic capabilities, but generally less adapted to extreme conditions

The most important characterizations of archaea are as follows:

2.1. Morphology of archaea

The most archaea have a simple cellular organization, Gram-positive or Gram-negative. They can be spherical, rod-shaped, spiral, lobed, plate-shaped, irregularly shaped, or pleomorphic with a size ranging from 0.1 to over 15 µm. Some of them can be single cells while others form filaments (200 µm in length) or aggregates (Chaban et al., 2006).

2.2. Cell wall structure

Archaeal cell walls, particularly those classified as Gram-negative or Gram-negative, present compositionally different from the peptidoglycan-based cell walls of bacteria. The cell walls have unique compositions, including pseudopeptidoglycan, glycoproteins, and specialised macromolecules. These structures are essential for their survival, structural integrity, and interactions of archaea in their various and often extreme environmental habitats (Steenbakkers et al., 2006; Jarrell et al., 2014).They play very important roles in many respects; for example, structural integrity, survival, and interaction of archaea in their diverse extreme environmental niches (Steenbakkers et al., 2006; Jarrell et al., 2014).

The cell wall profiles of different archaea are illustrated in Figure 12. In general, archaea have a semi rigid and multilayered cell wall, composing of peptidoglycan, which is common in bacteria. In contrast, archaea possess many cell surface characteristics that provide both form and protection. The cell wall is composed of:

2.2.1-S-layer is an external cell envelope composed of protein or glycoprotein sheets that are two-dimensional and paracrystalline, forming a regular, lattice-like structure on the surface of cells. It was found in many archaea. This Layer can be in some methanogens like *Methanococcus*, *Halophilus* like *Halobactirium*, and extreme thermophiles like *Sulpholubus, Pyrodictum* (Figure 12). In addition, it can be covered by additional layers in outside of the S-layer, composed of protein sheath such as, *Methanosprillum,* or protected by a methanochondroitin layer in *Methanosarcina. Furthermore,* However, in some archaea like *Methanothermus* and *Methanopyrus*, S-layer separated from the plasma membrane by a pseudomurein, and it is absent in stain gram positive like *Methanobacterium et Halococcus* **(**Albers. And Meyer, 2011).

2.2.2. Glycoproteins

Many Gram-negative archaea possess cell wall glycoproteins. In other words, they contain proteins covalently linked to carbohydrates. Additionally, these glycoproteins may serve as cell wall structures and be involved in recognition (Jarrell et al., 2014). However, Gram-positive archaea frequently contain glycoproteins as the primary structural elements of their cell walls (Albers & Meyer, 2011) (Figure 12).

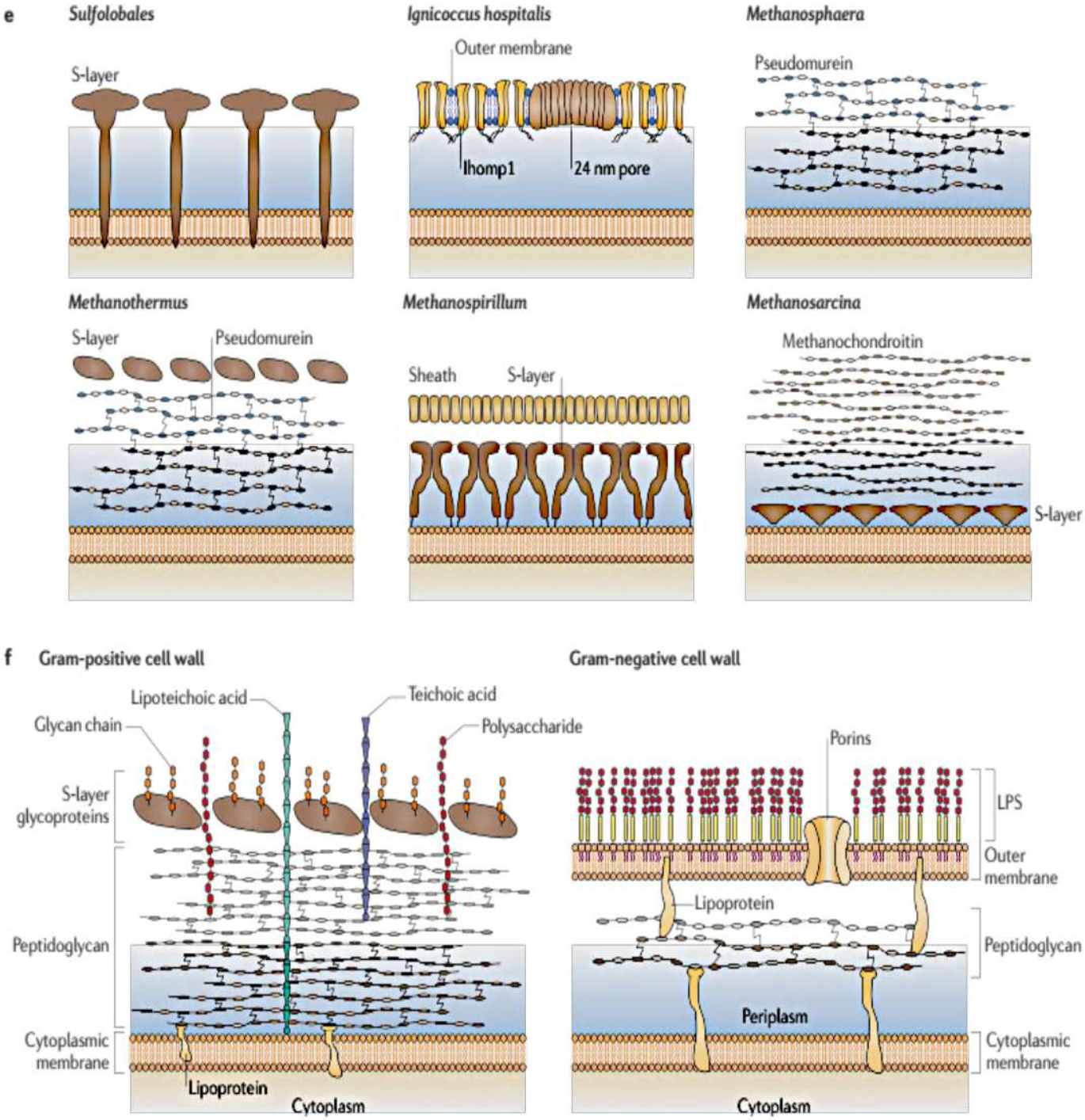


Figure 12: Cell wall profiles of different archaea (Albers. And Meyer, 2011).

e- Cell wall profiles of various archaea, presented in schematic side view. Blue represents pseudoperiplasmic space. **f)** Bacterial cell wall schematic: In some instances, surface-layer **(S-layer)** glycoproteins (also known as murein) are present as the final layer above the peptidoglycan of Gram-positive bacteria, which have a thick, amorphous, multilayered cover of peptidoglycan, teichoic, and lipoteichoic acid as their cell wall.

Gram-negative Bacteria have an asymmetrical outer bilayer membrane composed of two layers: one layer containing lipopolysaccharide (LPS) and the other layer comprising phospholipids, a gel-like substance. Layers ; **CM**, cytoplasmic

2.2.3. Pseudomurein

Archaeal cell walls, particularly those classified as Gram-negative or Gram-negative, present compositionally different from the peptidoglycan-based cell walls of bacteria which contains N-acetylmuramic acid attached to d-Nacetylglucosamine (GlcNAc) via β-1,4, the structural component of pseudomurein oligosaccharide consists of l-N-acetyltalosaminuronic acid linked to GlcNAc via β-1,3. In addition, d-amino acids are absent from the amino acid interbridge. Conversely, it is common for the interbridge to consist of three l-amino acids, namely glutamic acid, alanine, and lysine. It is interesting that despite the existence of a proposed biosynthetic pathway for pseudomurein in archaea, there is no homology between proteins produced by pseudomurein-producing archaea and bacterial proteins involved in peptidoglycan biosynthesis and assembly (Figure 13). This absence of similarity suggests that the two pathways independently formed. (Albers & Meyer, 2011; Willeyet al., 2019).

For Gram-negative archaea classified within the phylum *Euryarchaeota* have cell walls that composed of different macromolecules (Albers & Meyer, 2011) like glycan strands linked by peptide bonds, with variations in the peptide composition among different archaeal species. Some Gram-negative archaea integrate sulfolipids into their cell walls, especially those that have adapted to survive in extreme conditions. This Sulfolipids, which have sulfate groups, affixed to their lipid chains, offer supplementary stability and defense for the cell wall when exposed to abrasive environments (Koga & Morii, 2007).

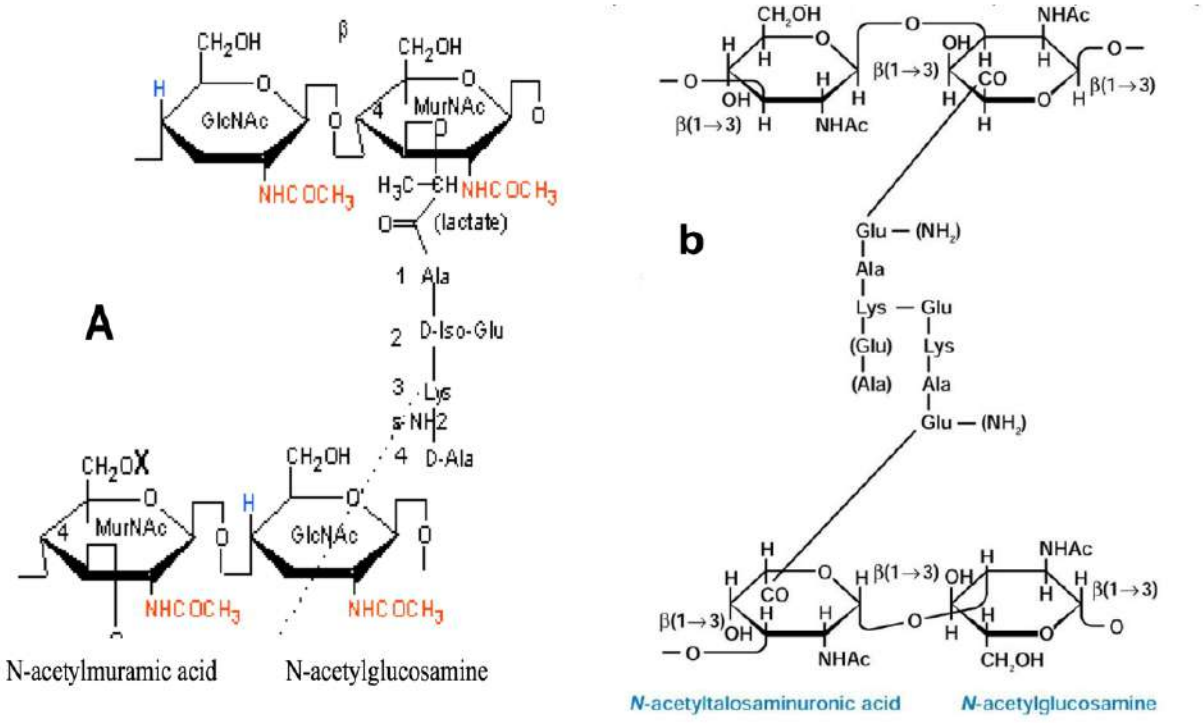


Figure 13: Difference between the composition of peptidoglycan (a) in bacteria and pseudomurein (b) in archaea (Willey et al., 2019)

Some Gram-positive archaea of the phylum *Crenarchaeota* have a cell wall layer known as pseudomurein, in place of the peptidoglycan of Gram-positive bacteria's cell walls (In contrast to the N-acetylmuramic acid and N-acetylglucosamine found in bacterial peptidoglycan, pseudouronic acid is composed of N-acetylglucosamine and N-acetyltalosaminuronic acid, which are joined by β-1,3 and β-1,4 glycosidic bonds (Willeyet al., 2019).

2.3. Archaeal lipids and membranes

The predominant component of archaeal membranes are lipids containing ether bonds, which are distinguished from the lipids of bacteria and eukaryotes formed from lipids containing ester bonds. Archaeal membrane consists of hydrocarbons that are hydrocarbon chains composed of five carbon atoms and are branched. As illustrated in figure 4.3, the hydrocarbons are linked to glycerol via ether bonds as opposed to ester bonds. Consequently, the fluidity and permeability of the membrane are influenced by the manner in which the lipids assemble. This is particularly critical for extremophilic archaea, whose permeability and membrane fluidity may be compromised by severe environments (Koga, 2012; Caforio and Driessen, 2017).

In comparison to ester connections, ether bonds exhibit enhanced resistance to chemical attack and heat. There have been identified two primary categories of archaeal lipids: di-glycerol tetraethers and glycerol diethers. The process of glycerol di-eher lipids formation involves the attachment of two hydrocarbons to glycerol (figure 4.4). The chains of hydrocarbons in glycerol diethers are typically 20 carbon atoms long. The formation of di-glycerol tetraether lipids involves the liaison of two glycerol residues via two 40-carbon long hydrocarbons (see figure 4.4, lipids 5 and 6) (Koga, 2012; Becker et al., 2016; Willey et al., 2019).

Additionally, tetraether chains are more rigid lipids than diethers. Their cyclization into pentacyclic circles allows cells to modify the total length of lipids (Figure 13). In general, di-ether and tetra-ether lipids can attach groups containing phosphorus, sulfur, amino acids, and sugars to glycerol units, similar to phospholipids known to line the membranes of bacteria and eukaryotes .Moreover, two structural variations of archaeal membrane lipids can be formed: a bilayer membrane formed from C20 diethers which produces a hydrophobic core with two hydrophilic surfaces (see Figure 14) and a monolayer membrane, exhibiting significantly enhanced rigidity, is produced when the membrane is composed of C-40 tetraethers (see figure 14). In addition, the incorporation of pentacyclic rings makes it possible to increase this rigidity. The membrane

composition of extreme thermophiles consists mainly of tetraether monolayers, consistent with their inherent requirement for stability (Koga, 2012; Becker et al., 2016; Willey et al., 2019).

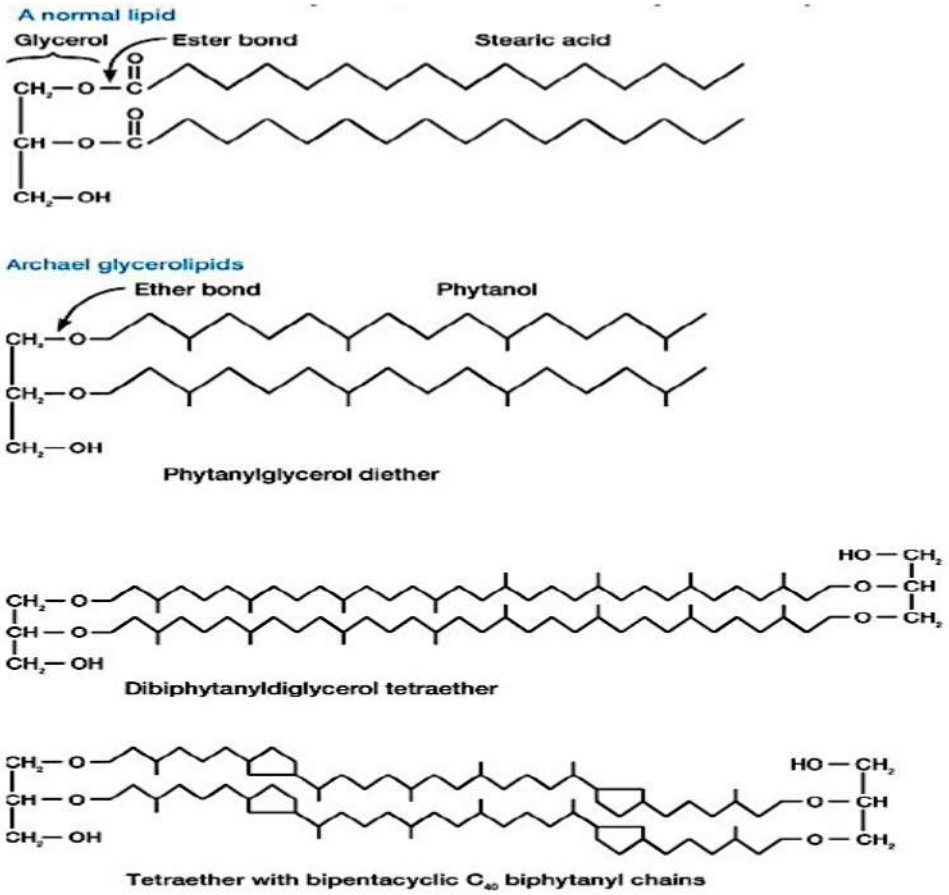


Figure 13: Examples of Archaeal Plasma Membranes (John et al., 2003)

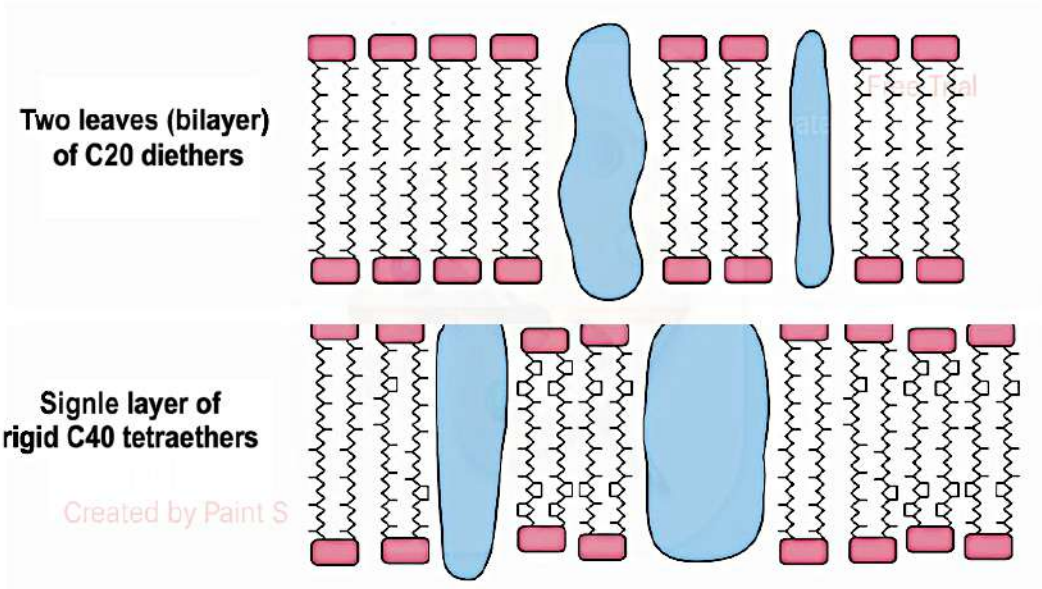


Figure 14: Examples of membranes found in archaea. (a) A bilayer of C20 diethers is attached to a membrane containing integral proteins. b) Internal proteins and C40 tetraethers constitute a rigid monolayer (John et al., 2003).

On the other hand, archaeal membranes also contain other polar lipids, such as phospholipids, sulfolipids, and glycolipids (Becker et al., 2016).

For nonpolar lipids, archaea can integrate additional nonpolar lipids, such as isoprenoid hydrocarbons, including pentacyclic hopanoids and squalenes, into their cell membranes in addition to the biphenyl chains In addition to potentially contributing to membrane properties, these more intricate nonpolar lipids may also be involved in cellular processes such as electron transport, signalling, and others (Jain et al., 2014).

In the past few years, nonpolar lipids derived from butanetriol and pentanetriol have been discovered in specific archaeal groups, including the methanogenic archaeon *Methanomassiliicoccus luminyensis*. The identification of these singular lipid structures indicates the continued ability of archaea to synthesise a wide variety of nonpolar membrane constituents (Becker et al., 2016).

3.-Physiology and metabolic diversity of archaea

Physiologically, archaea are aerobic, facultative anaerobic, or strictly anaerobic. They have very diverse metabolic capacities, from chemoautotrophs to organotrophs, using a wide variety of energy and carbon sources, including inorganic compounds, and adapted to extreme environments (high temperatures, extreme pH, high salinity, etc.) (Madigan et al., 2015). Certain archaea have a unique metabolic pathway such as methanogenesis (Thauer, 1998).

Certain archaea use the anaerobic respiration as a unique metabolic process, such as halophilic *Halobacteriales*, that can use alternative electron acceptors, like sulfate or nitrate, instead of oxygen (Madigan et al., 2018).

For metabolic diversity, archaea exhibit a wide range of metabolic capabilities, including chemolithoautotrophy, heterotrophy and methanogenesis, which has enabled them to colonize a wide range of environmental niches. The principal archaeal metabolic types follow:

3.1-Chemolithoautotrophy

Certain archaea, including specific members of the *Thaumarchaeota* and *Crenarchaeota*, can survive exclusively on inorganic compounds for carbon and energy. The biogeochemical cycling functions and various carbon and energy metabolic pathways utilized by these archaea have been

the subject of recent research (Offre et al., 2013; Kato et al., 2021). For example, some archaea including hydrogen-oxidizing *Thermococcales* and the *sulfur-oxidizing Sulfolobales* produce energy by oxidising inorganic compounds and utilise carbon dioxide as their exclusive carbon source (Madigan et al., 2018).

3.2.-Heterotrophy

Although chemolithoautotrophy is predominate among archaea, certain archaea, especially those belonging to the *Euryarchaeota*, operate as heterotrophs using a wide range of carbohydrates, proteins, and lipids as carbon and energy sources by degradation of complex organic matter, such as chitin, cellulose, and lignin (Goyal et al., 2022; Sousa et al., 2020).

3.3.-Methanogenesis

Methanogens, recognized as *Archaea* in the phylum *Euryarchaeota*, can generate methane as a metabolic product. This capability offers considerable importance in the context of the worldwide carbon cycle (Madigan et al., 2018).

4.-Genomic organization and characteristics

Genomes of archaea are usually between 0.5 and 5.8 million base pairs, which is smaller than genomes of bacteria. These organisms generally possess shorter intergenic spaces and fewer non-coding regions in their genomes (Charlebois & Doolittle, 1990). Additionally, they possess eukaryotic-like characteristics, including histones, TATA-binding proteins, and RNA polymerases). In addition, their replication, transcription, and translation molecular mechanisms are similar to those of eukaryotes rather than bacteria (Koonin, 2008).

In terms of genome structure and organization, these organisms may possess linear or circular chromosomes, in addition to extrachromosomal components like plasmids. Akin to eukaryotes, numerous archaea contain protein-coding introns in their genomes (Perler et al., 1992). The analysis of comparative genomics demonstrated that archaea exhibit a substantial degree of diversity, marked by the existence of unique phylogenetic lineages. This finding carries significant evolutionary ramifications (Forterre, 2015).

On the other hand, archaea represent the primordial domain of life, given that their origins span billions of years (Woese et al. 1990). They demonstrate considerable rates of lateral gene transfer, a process that enables the rapid acquisition of new metabolic capabilities and adaptations (Nelson-Sathi et al., 2015).

5.-Ecology and environmental roles

Archaea inhabit diverse kinds of extreme environments, such as hot thermal systems, saline lakes, acidic environments, and the anaerobic ones (Auguet et al., 2010). Their highly lipid membranes, metabolic proficiencies, and stress resistance mechanisms allows them to survive in such harsh conditions (Valentine, 2007). They may dominate microbes' communities, earthing microorganisms that continue, in a large extent, to the structure and function of the ecosystem (Cavicchioli, 2011). A great number of archaea have been extremophilic archaea and are observed to live in places where the temperature is very high, pH is low, salts are concentrated, or places that are completely anoxic (Borrel et., 2016). In addition, many archaea also inhabit soils, freshwater, and marine environments at neutral pH and moderate temperatures, where they participate in nutrient cycling and energy flow (Schleper, 2010). In some hypersaline habitats, their populations can be extremely dense, causing the brine to appear red with archaeal pigments (Oren, 2014).

Furthermore, archaea are major players in biogeochemical circulation, specifically in the global nitrogen, carbon, and sulfur cycles (Offre et al., 2013; Kuypers et al., 2018). For example, sulfur-metabolizing archaea, commonly observed in hydrothermal vents, are essential in the sulfur compound cycling process (Amend & Shock, 2001); methanogenic archaea are important players in the anaerobic degradation of organic matter, producing methane as a byproduct (Thauer et al., 2008); Ammonia-oxidizing archaea (AOA), which found in many ecosystems, play essential role in nitrification by converting ammonia to nitrite (Prosser & Nicol, 2012). However, archaea, especially uncultured archaea, play significant roles in anaerobic environments by engaging in diverse metabolic functions in anaerobic processes, such as sulfur reduction, in deep-sea sediment environments. The diversity of chemical receptors in archaea is related to habitat adaptations, with specific ligand-binding domains correlated to different environments (Dong et al., 2019).

On the other hand, archaea can establish symbiotic associations with a wide range of eukaryotic hosts, such as plants, animals, and protists (Kitzinger et al., 2019). They can provide essential metabolic and nutrients, or protect their hosts against environmental stressors (Raymann et al., 2017). They also may play different roles in host physiology and health (Vavourakis et al., 2016). In addition, archaea contribute to relatively normal marine and soil communities. In addition, some archaea are part of the human microbiome and are located in the human digestive system and oral cavity (Dridi et al., 2012).

The most important genera and their species which can be found in this extreme environment are presented in Tab 34.

Table 34: Diverse archaeal species and their ecological habitats

Species	Ecological Habitat	References
Pyrococcus furiosus	Hydrothermal vents (temperatures can exceed 100°)	Fiala and Stetter, 1986
Thermococcus litoralis	Hydrothermal vents (temperatures can exceed 100°)	Jolivet et al., 2004
Sulfolobus solfataricus	Acidic hot springs (are found in acidic sulfuric springs and mine drainages)	Zillig, 1980
Methanopyrus kandleri	Hydrothermal vents at great depths	Kurr et al., 1991
Halobacterium salinarum	Highly saline environments like salt pans	Oren, 2002
Methanobrevibacter smithii	Human gut, especially in the digestive tract	Samuel and Gordon, 2006
Methanogenium frigidum	Cold Habitat: sediment of Ace Lake in Antarctica, a cold, saline, and permanently anoxic environment, at temperatures as low as 0°C	Franzmann et al., 1997
Nitrosopumilus maritimus	Oceanic and sea sediments, involved in nitrification	Könneke et al., 2005

Some of these archaea, for instance, *Methanobrevibacter smithii,* are harbored by the human gut, and they may have some impact on the human host health and diseases' pathogenesis (Dridi et al., 2012). An indication was given that *Methanobrevibacter smithii*, for example, present in the human gut, had high energy harvest, thus weight gain, and therefore could be likely reasons for *Methanobrevibacter smithii* becoming a potential factor in metabolism and obesity (Samuel and Gordon, 2006).

6.-Classification of *Archaea*

Based on the comparison of rRNA sequences from a wide variety of organisms, the archaea represent significant diversity with differences that distinguish them from Gram positive and negative bacteria.

Archaea are currently classified into several major phyla, including *Euryarchaeota, Crenarchaeota, Thaumarchaeota, Korarchaeota,* and *Nanoarchaeota* (Spang et al., 2015; Zaremba-Niedzwiedzka et al., 2017) Figure 15.

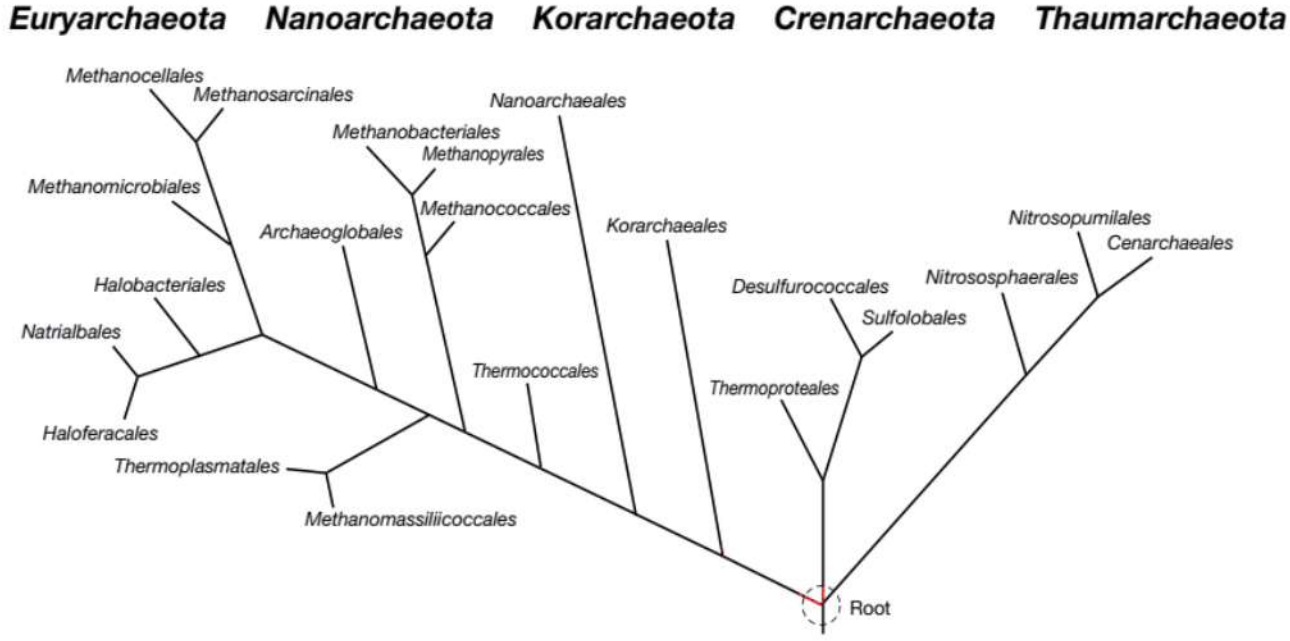


Figure 15: Archaeal phylogenetic tree based on 16S rRNA gene sequences of the five archaeal phyla and their major orders (Madigan et al., 2019)

6.1.- Phylum *Euryarchaeota*

The phylum *Euryarchaeota* comprises a diverse group of archaea, including all known methanogens, extreme halophiles, and some hyperthermophiles. Members of this phylum exhibit a wide range of metabolic capabilities, with many being strictly anaerobic, although some can tolerate low oxygen levels. This phylum includes autotrophs and heterotrophs, the latter often employing unique metabolic pathways such as modified glycolysis. These organisms inhabit a variety of ecosystems, ranging from the extreme conditions of hydrothermal vents to more commonplace areas such as cow and buffalo rumens (Adam et al., 2017; Castelle & Banfield, 2018).

Phylogenomic investigations have provided a more exhaustive comprehension of the evolutionary connections and variety within the *Euryarchaeota*. These analyses have further subdivided the

phylum into many different orders: *Methanobacteriales, Methanococcales, Methanomicrobiales, Methanosarcinales, Methanocellales*, and two methanogen taxa that are comparatively recent, *Methanofastidiosa* and *Methanomassiliicoccales* (Spang et al., 2017; Evans et al., 2015).

The **phylum** *Euryarchaeota* is a very diverse phylum with five major groups:

6.1.1.-Methanogenic group

Several organisms of *Euryarchaeota* are Methanogens, that have a wide range of adaptations and metabolic capacities, enabling their successful existence in a wide variety of anaerobic habitats. They produce methane (CH4) as an integral part of their energy metabolism (methane production is called methanogenesis). They are strict anaerobes, autotrophic when they grow on H2 and CO2, obtaining energy by converting CO2, H2, formate, methanol, acetate and other methane compounds or methane and CO2. They have an essential role in the global carbon cycle by generating methane as a metabolic product. There are five orders: *Methanobacteriales, Methanococcales, Methanomicrobiales, Methanosarcinales and Methanopyrales,* with more than 26 genera, some of them including *pseudomureine* (table 35).

Table 35: characteristics and comparative Overview of Methanogenic Orders within the Phylum of *Euryarchaeota*

Order Metabolism	Habitat	Genre	Temperature Range	References
Methanobacteriales strictly Hydrogenotrophic ($H_2 + CO_2 \rightarrow CH_4$)	Anaerobic environments Gastrointestinal tracts	*Methanobacterium, Methanothermo-bacter, Methanosphaera*	Mesophilic to thermophilic	Thauer et al. (2008) Borrel et al. (2013)
Methanococcales strictly Hydrogenotrophic ($H_2 + CO_2 \rightarrow CH_4$)	Marine, high-temperature environments	*Methanococcus, Methanotherm-ococcus, Methanotorris*	Mesophilic to thermophilic	Whitman et al. (2006),Vanwonterghem et al. (2016)
Methanomicrobiales Hydrogenotrophic, Methylotrophic,	Wetlands, rice paddies,freshwater sediments	*Methanomicrobium Methanoplanus, Methanoculleus*	Mesophilic to thermophilic	Zhu et al. (2012), Brablcová et al. (2015)
Methanosarcinales Hydrogenotrophic Methylotrophic	Diverse anaerobic habitats (animal guts, etc)	*Methanosarcinan, Methanosaeta, Methanococcoides*	Mesophilic to thermophilic	Jablonski et al. (2015), Söllinger et al. (2016)
Methanopyrales Hydrogenotrophic	Hydrothermal vents, deep-sea environments	*Methanopyrus*	Hyperthermophilic	Takai et al. (1999), Slesarev et al. (2002)

6.1.2.-Halophilic group

The halophilic group are frequently referred to as haloarchaea, represents an interesting group of extremophiles that grow in various environments with very high salt levels, including salt lakes, estuaries, and salt deserts, are home to these organisms. One of the most important characteristics of haloarchaea is its capacity to grow in environments containing containing 2 to 6 M NaCl, indicating their exceptional resistance to osmotic stress (Oren, 2014; Baquero & Moreno-Paz, 2020). The most important orders of the halophilic group and their characterizations are presented in Table 36.

Table 37: characteristics and comparative overview of halophilic archaeal groups within the phylum of *Euryarchaeota*

Halobacterial Group	***Halobacteriales***	***Haloferacales***	***Natrialbales***
Most Genera	*Halobacterium, Halorubrum, Haloarcula*	*Haloferax, Halogeometricum, Haloquadratum*	*Natronobacterium, Natronococcus, Natrialba*
Salinity Tolerance	Up to 5 M NaCl	Up to saturation (> 5 M NaCl)	Hypersaline, alkaline environments
Adaptations	Accumulation of compatible solutes (glycerol, trehalose) for osmotic balance Production of pigments like carotenoids for UVprotection	- Unique membrane lipid compositions for maintaining membrane integrity - Sophisticated mechanisms for pH homeostasis	Accumulation of compatible solutes for osmotic balance - Na+/H+ antiporters for adaptation to high-pH conditions
Ecological Significance	- Prominent members of hypersaline environments - Play crucial roles in biogeochemical cycling	- Diverse metabolic capabilities - Highly adapted to extreme hypersaline conditions	- Less abundant in some hypersaline environments - utilise a variety of organic compounds
Biotechnological Potential	- Production of biofuels - Bioremediation- Development of novel enzymes and metabolic systems	- Production of biofuels- Bioremediation - Development of novel enzymes	- Production of biofuels - Bioremediation - Development of novel enzymes
References	Oren (2014), Kundu et al. (2019), Ghai et al. (2011), Ventosa et al. (2015)	Oren (2014), Boujelben et al. (2012), Borrel et al. (2014),	Mesbah & Wiegel (2012), Sorokin et al. (2015), Oren (2014

Additionally, *Haloarchaea* are metabolically different and their bioactive substances and distinctive enzymatic features, such as halotolerance and thermostability, contribute significantly to their importance in biotechnological applications. Their carotenoidal pigements have antioxidant, anti-inflammatory activies. Furthermore, haloarchaea have showed the ability to produce polyhydroxyalkanoate (PHA), biodegradable bioplastics with potential applications. In addition, haloarchaea have been explored for their capacity to produce nanostructures and their promise for bioremediation during the detoxification of toxic metals (Hou et al., 2020; Guo et al., 2021; Farhadian et al., 202).

6.1.3.- Group *Thermoplasmata*

The members of this group are classified as *Thermococci*, pleomorphic, which are strictly anaerobic. Some member are chemoorganoheterotrophs using a variety of carbon substrates (peptides and carbohydrates, ete), and reduce sulfur to sulfide, and other members are chemolithotrophic oxidising pyrite (FeS) present in these masses into sulfuric acid (Golyshina,2011; Cumming et al. 2018). They are responsible for their motility, and grow at 45 to 65 °C, but their ideal growing temperatures range from 88° to 100°C. Despite the absence of cell walls, these microorganisms possess lipid-containing polysaccharides, glycoproteins, and caldarchaeol, a diglycerol tetraether lipid, which protect their plasma membranes. However, their growth is significantly inhibited at pH levels below 3.5, with an optimal of 0.7 (Golyshina et al. 2009 ; Kozubal et al. 2012).

The most characterizations of members of this group are presented in Tab 38 (see page 85).

6.1.4. Hyperthermophile and sulfate reducer groups

Hyperthermophiles include some very interesting species that can survive in extreme temperatures, specifically above 80°C. The microbial community is primarily composed of archaea, with only a limited number of bacteria, including *Geothermobacterium ferrireducens*, possessing the ability to survive in such high temperatures (Bertoldo and. Antranikian, 2006. Particular metabolic pathways are observed in these hyperthermophiles, including a modified Embden-Meyerhof pathway, which is not frequently encountered in bacteria and eucarya (Sakuraba et al., 2020). Furthermore, it has been observed that hyperthermophiles are found in various high-temperature environments, including hot springs, where they are essential for the decomposition of polymers, including glucose, cellulose, and keratin (Sakuraba et al., 2020). Due

to their remarkable resistance to high temperatures and adaptability in metabolism, hyperthermophiles have become attractive subjects for fundamental investigations and biotechnological treatments. The potential for biotechnological advancements is suggested by the metabolic adaptations observed in hyperthermophiles, including the use of unconventional pathways for sucrose oxidation and energy conservation (Huber and Stetter, 2001). These adaptations provide valuable insights into the earliest phases of evolution

Table 38: characteristics and comparative overview of Thermoplasma orders within *Euryarchaeota*

Characteristic	***Thermoplasmatales***	***Ferroplasmales***
Genera	*Thermoplasma, Picrophilus,*	*Ferroplasma, Acidiplasma*
Temperature Range	Thermophilic (45-65°C)	Thermoacidophilic (45-55°C)
pH Range	Extremely acidic (pH 0-3)	Extremely acidic (pH 0-2)
Metabolism	Chemoorganoheterotrophic (a variety of organic compounds)	Chemolithoautotrophic (oxidation of ferrous iron (Fe2+))
Cell wall Structure	Lacking a cell wall	Lacking a cell wall
Adaptations	- Production of heat-shock proteins and other molecular chaperones -Acidophilic enzymes and proton-pumping mechanisms - Accumulation of compatible solutes like trehalose.	- Extremely acidophilic and thermoacidophilic enzymes - Iron-oxidation pathways - Unique membrane lipid compositions
Ecological Significance	- Play a role in the cycling of carbon and other elements in acidic, high-temperature environments.	- Play a role in the biogeochemical cycling of iron in acidic, high-temperature environments - Involved in the formation of acid mine drainage
Biotechnological Potential	- Extremozymes for industrial applications: bioremediation of metal-contaminated environments	- Extremozymes for industrial applications: bioremediation of metal-contaminated environments
References	Golyshina (2011), Kozubal et al. (2012)	Dopson & Lindström (2004), Golyshina et al. (2009), Cumming et al. (2018)

The Order *Thermococcales* is one of the well-studied hyperthermophilic taxa within the *Euryarchaeota*. Genera such as *Thermococcus* and *Pyrococcus* are commonly found in high-temperature environments, including submarine hot springs, marine hydrothermal vents, and other similar settings (Marteinsson et al., 1999; Atomi et al., 2011)

Other orders such as *Archeoglobales* and *Geoglobales*, have attracted significant attention due to their metabolic features and intended uses in different fields. *Archeoglobales* are a well-investigated group of metabolically competent anaerobic archaea that thrive in hot, extremely anoxic environments found in deep-sea hydrothermal and geothermal vents (Oran et al., 2018; Slobodkina et al., 2016). This group is also well-known for their heterosulfate reduction metabolism: they can reduce sulfate as the last electron acceptor, with the aim of making hydrogen sulfide in the process (Jahn et al., 2018). This metabolic is one of the most prominent features of *Archeoglobales* that perform intensive cycle functioning of various elements like sulfur in their surrounding ecosystems. In contrast, geoglobales are a group of anaerobic archaea that are widespread that can be found in soil, sediment, and subsurface environments. These bacteria can perform fermentation, an anaerobic process resulting in the conversion of organic matter into carbohydrates, lactate, and other organic acids (Bond et al. 2017; Liu et al., 2018) (Table 39).

Table 39: Characteristics and comparison between Orders of *Thermococcales*, *Archaeoglobales*, and *Geoglobales*

Orders / Characteristic	***Thermococcales***	***Archaeoglobales***	***Geoglobales***
Genera	*Thermococcus, Pyrococcus*	*Archaeoglobus, Geoglobus, Ferroglobus*	*Geoglobus*
Morphology	Cocci or irregular cocci, 0.8-2 µm in diameter, motile	Cocci or irregular, 0.5-2 µm in diameter, non-motile	Cocci or irregular, 0.5-2 µm in diameter, non-motile
Temperature Range (°C)	60 - 110	60 - 95	60 - 90
Metabolism	Anaerobic, heterotrophic, using carbohydrates and peptides	Anaerobic, heterotrophic, some capable of sulfate reduction	Anaerobic, heterotrophic, iron-reducing
Habitat	Marine hydrothermal vents, submarine hot springs	High-temperature, anoxic environments (e.g., hydrothermal vents, geothermal springs, subsurface oil reservoirs)	High-temperature, anoxic environments (e.g., hydrothermal vents)
Ecological Role	Involved in the cycling of carbon and other elements	Involved in the cycling of sulfur, carbon, and other elements	Involved in the cycling of iron and other elements
	Atomi et al., 2011; Kawaichi et al., 2020 ; Nguyen et al., 2021)	Slobodkina et al., 2016 ; Oran et al., 2018; Mardanov et al., 2021	Kashefi & Lovley, 2003; Slobodkina et al., 2016)

6.2.-The phylum *Crenarchaeota*

Crenarchaeota is the second diverse phylum of *Archaea*, anaerobic and hyperthermophilic, with ideal temperatures for growth between 80°C and 113°C. Its members exhibit a variety of morphologies, including cocci, rods, and irregular shapes. Its most members have been found in specific environments, such as deep-sea hydrothermal vents, volcanic areas, and hot springs (Huber et al., 2006; Wemheuer et al., 2019). They play significant parts in the cycling of carbon, nitrogen, and sulfur in various ecosystems. Several species are chemolithoautotrophs, obtaining their energy as well as carbon from inorganic compounds such as sulphur, carbon dioxide, and hydrogen (Nakagawa & Takai, 2006; Offre et al., 2013).

Additionally, the phylum is classified into four orders: *Desulfurococcales, Sulfolobales, Thermoproteales and Acidilobales* (Spang et al., 2017; Wemheuer et al., 2019). This last order, *Acidilobales*, is a recently proposed order within the *Archaea*, including the genera *Acidilobus* and *Caldisphaera*. These genera are distinguished by their physiological properties and phylogenetic position, derived from acidophilic and hyperthermophilic anaerobic cocci isolated from hot springs ((Prokofeva et al., 2009). Certain of the well-known species and genera within the *Crenarchaeota* phylum are listed in the table 40:

Table 40: characteristics and comparison between four orders of the phylum *Crenarchaeota*

Order / Characteristic	***Thermoproteales***	***Desulfurococcales***	***Sulfolobales***	***Acidilobales***
Representative Genera	*Thermofilum, Thermoproteus, Pyrobaculum*	*Desulfurococcus, Aeropyrum, Ignicoccus*	*Sulfolobus, Acidianus*	*Acidilobus Caldisphaera*
T Range (°C)	80 - 105	80 - 100	65 - 90	70 - 95
Metabolism	Chemolitho-autotrophic, hydrogen-oxidizing, sulfur-reducing	Chemolitho-autotrophic, sulfur-oxidizing, hydrogen-oxidizing	Chemolitho-autotrophic, sulfur-oxidizing	Heterotrophic, anaerobic, acidophilic
Habitat	Terrestrial hot springs, deep-sea hydrothermal vents	Deep-sea hydrothermal vents, terrestrial hot springs	Terrestrial volcanic and geothermal areas	Acidic, high-temperature environments
Ecological role	Carbon, nitrogen, and sulfur cycling	Sulfur cycling, hydrogen oxidation	Sulfur cycling, carbon fixation	Carbon and energy cycling in acidic, high-temperature environments

Order / Characteristic	*Thermoproteales*	*Desulfurococcales*	*Sulfolobales*	*Acidilobales*
References	Casanueva et al., 2008; Breuker et al., 2013 ;	Nakagawa & Takai, 2006; Jahn	Huber et al., 2006	Prokofeva et al., 2009

6.3.- Other phyla of *Archaea*

6.3.1-Phylum *Thaumarchaeota*

Members of this phylum are aerobic, chemolithoautotrophic organisms that can oxidize ammonia as source of energy; therefore, they are highly significant in nitrification processes, and can be colonize all environments, from marine to terrestrial ecosystems, and adapted to a very wide range of temperatures and pH values (Tourna et al., 2011; Kerou et al., 2016). These archaea are reported to be distributed globally and play an important role in nitrogen and carbon global biogeochemical cycling (Alves et al., 2018). Other characteristics are reported in the table 41.

6.3.2.- Phylum *Nanoarchaeota*

This phylum *Nanoarchaeota* consists of extremely small archaea that reside as ectosymbionts on the surfaces of various archaeal hosts. It has been discovered in various extreme environments, including hypersaline ecosystems and deep-sea hydrothermal vents (Reva et al., 2023). This phylum, characterized by its limited biosynthetic capacities, manifests a symbiotic lifestyle. These entities are distinguished by their complex cellular defense mechanisms, which include CRISPR/Cas systems used to defend against viruses and the presence of enormous surface proteins (Straub et al., 2018 ; John et al., 2022). Other characteristics are reported in the table 41.

6.3.3.- Phylum *Korarchaeota*

The phylum *Korarchaeota* is characterized by small cell size, slow growth, and a phylogenetic position with some unique features that suggest this group is a possible representative of an ancient *Archaea*. It has found in many anaerobic, high-temperature ecosystems, such as acidic hot springs, or even in the zone of deep-sea hydrothermal vents, though they can be successfully cultivated under very restricted conditions (Auchtung et al., 2006; Elkins et al., 2008). Other characteristics are reported in the table 41.

Table 41: Characteristics and comparison of *Thaumarchaeota, Nanoarchaeota*, and *Korarchaeota*:

Phylum / Characteristic	*Thaumarchaeota*	*Nanoarchaeota*	*Korarchaeota*
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 (°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
References	Offre et al., 2013 ; Kerou et al., 2016)	Jahn et al., 2008; Podar et al., 2013	Auchtung et al., 2006; Elkins et al., 2008

7.-Importance and Applications

Archaea possess a wide variety of significance and practical applications, from biotechnology and bioremediation to understanding the evolution of cellular processes and the limits of life. They are now well-known for life in extreme environments, such as high temperature, low pH, high salinity, and anoxic settings. This makes it of high value as models of the limits to life and mechanisms of organisms to adapt to environmental conditions (Oren, 2013; Zeldovich et al., 2007). Example: The potential applications include biofuel production with thermophilic archaea

and development of thermostable enzymes from *Pyrococcus furiosus* (Blumer-Schuette et al., 2008).

Archaea possess many biotechnological applications due to their specific metabolic versatility, such as in the decomposition of recalcitrant compounds and synthesis into valuable biomolecules (Goyal et al., 2022). Example: *Halobacterium salinarum* and other members of halophilic archaea have been tapped for their bioremediation profile against heavy metals, along with synthesis of biofuels and bioplastics (Margesin and Schinner, 2001).

Biogeochemical cycling: The Archaea also conduct all essential biogeochemical cycles on earth in regards to their broad array of metabolic activities in carbon, nitrogen, and sulfur cycling (Offre et al., 2013; Kato et al., 2021). Example: Ammonia-oxidizing archaea are recently discovered *Thaumarchaeota* phylum members and have been described as playing key roles in nitrification in environments (Könneke et al., 2005)

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