MICROBIAL EVOLUTION, TAXONOMY & BIODIVERITY







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 AND EUKARYOTES
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MICROBIAL EVOLUTION

Microbial evolution refers to the genetically driven changes that occur in microorganisms and that are retained over time. Some microbial changes can be in response to a selective pressure.

The best examples of this are the various changes that can occur in bacteria in response to the presence of antibiotics

EVOLUTION

- Evolution states that living things change gradually through million years and these evolvements result in various types of heritable structural and functional changes through many generations
- Evolution concept:
 - 1. All new species originate from pre-existing species
 - 2. Closely related organisms have similar features because they evolved from common ancestral forms

The first discovery of microbial fossils

- First prokaryotes probably arose at least 3.5 to 3.8 billion years ago
 - ✓ cyanobacteria
- Fossilized microbial formation called stromatolites is found in sedimentary rock – evidence of life on earth
- Stromatolites layered rocks formed by incorporation of mineral sediments into microbial mats of photosynthetic bacteria
- Evolutionary scientist believe that the layers of material were formed when mats of cyanobacteria are layered on top of each other, became mineralized



Evolution of prokaryotes

- The evolutionary history was studied primarily through fossil examination
 - Paleontology the study of the history of life on Earth as based on fossils.
- This approach is useful to understand the evolution of plants and animals
- But, fossil cannot be used to explain microbial evolution
 - Microorganisms do not leaves behind fossils (majority)
 - They do not share any morphological traits with plants & animals
- The evolution of microorganism was explained after the structure of DNA was discovered and the evolutionary history is recorded in DNA sequences
 - Genealogy the study of families, family history, and the tracing of their lineages.



Evolution of eukaryotes

- Eukaryotes arose from prokaryotes through fusion of ancient bacteria and archaea ~ 1.4 billion years ago
 - endosymbiotic hypothesis
- Organelles
 - chloroplasts arose from free-living phototrophic bacterium that entered symbiotic relationships with primitive eukaryotes
 - mitochondria arose by similar mechanism

the chloroplast



The ENDOSYMBIOTIC THEORY



Modern heterotrophic eukaryote

Taxonomy

- science of biological classification
- consists of three separate but interrelated parts
 - classification arrangement of organisms into groups (taxa; s.,taxon)
 - nomenclature the process of assigning names to the various taxonomic rankings of each microbial species
 - identification determination of taxon to which an isolate belongs



Importance of Taxonomy

- allows scientists to organize huge amounts of knowledge
- allows scientists to make predictions and frame hypotheses about organisms
- In places organisms into meaningful, useful groups, with precise names, thus facilitating scientific communication
- essential for accurate identification of organisms

Classification system

Old classification system

- Natural classification based on anatomical characteristics
- Arranges organisms into groups whose members share many characteristics

e.g. human is classified as mammals because they have hair, self-regulating body temperature, and milk-producing mammary gland

- Most desirable system because it reflects biological nature of organisms
- However, it is only suitable for higher organisms (animals, plants)

Current practice

- Polyphasic taxonomy is used to determine the genus and species of newly isolated microbes.
- This approach includes these features
 - Phenetic
 - Phylogenetic
 - Genotypic

Classification system

Phenetic classification

- phenotypes is the observable characteristics of organism
- organisms are classified based on mutual similarity of phenotypes. E.g. flagella involve in motility of certain microorganism
- can reveal possible evolutionary relationships, but it is not enough

Phylogenetic / phyletic classification

- To compare the organisms based on the basis of evolutionary relationship
- phylogeny refers to the evolutionary development of a species
- usually based on direct comparison of genetic material and gene products
- Current theories based largely on characterization of rRNA sequences

Genotypic classification

- Compare genetic similarity between organisms
- Individual or whole genome can be compared
- Bacteria that have 70% homologous genome belong to the same species

Levels of Classification

- Taxon is a group or "level" of classification
- Hierarchical; broad divisions are divided up into smaller divisions:
 - Domain
 - Kingdom
 - Phylum (Called "Division" by botanists)
 - Class
 - Order
 - Family
 - Genus (plural: Genera)
 - Species (Both singular & plural)





Figure 19.1 Hierarchical Arrangement in Taxonomy. In this example, members of the genus *Shigella* are placed within higher taxonomic ranks. Not all classification possibilities are given for each rank to simplify the diagram. Note that *-ales* denotes order and *-ceae* indicates family.

Binomial system of nomenclature

- Carolus Linnaeus is the father of taxonomy
- The method of assigning the scientific name of different organisms.
- The scientific name is always a combination of the generic (genus) name followed by the species name
- each organism has two names
 - 1. genus name italicized and capitalized (e.g. Escherichia)
 - 2. species epithet italicized but not capitalized (e.g. *coli*)
- Can be abbreviated after first use (e.g., E. coli)



Definition of "Species"

- The "basic unit" of taxonomy, representing a specific, recognized type of organism
- For sexually reproducing organisms (e.g.plants/animals), a "species" is defined as a group of organisms, in which any two individuals of the appropriate sexes/ mating types can produce fertile offspring
- However, this definition fails for many microbial species (including bacteria), because they do not reproduce sexually



How to define prokaryotic species?

Possible definitions:

- collection of strains that share many stable properties and differ significantly from other groups of strains
- ► collection of strains with similar G + C composition and ≥ 70% sequence similarity
- collection of organisms that share the same sequences in their core housekeeping genes (genes that are required for the maintenance of basic cellular function, e.g ribosomes)



Definition of "Species"

- Definition of "**species**" in microbiology:
 - Classic definition: A collection of microbial strains that share many properties and differ significantly from other groups of strains
 - Species are identified by comparison with known "type strains": well-characterized pure cultures; references for the identification of unknowns
 - There are several collections of type strains, including the American Type Culture Collection (ATCC)



TABLE 13.3 Some international microbial culture collections

Collection	Name	Location	Web address
ATCC	American Type Culture Collection	Manassas, Virginia	http://www.atcc.org
BCCM/LMG	Belgian Coordinated Collection of Microorganisms	Ghent, Belgium	http://bccm.belspo.be
CIP	Collection of the Institut Pasteur	Paris, France	http://www.pasteur.fr
CBS	Centraalbureau voor Schimmelcultures	Utrecht, The Netherlands	http://www.cbs.knaw.nl
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen	Braunschweig, Germany	http://www.dsmz.de
JCM	Japan Collection of Microorganisms	Saitama, Japan	http://jcm.brc.riken.jp/
NCCB	Netherlands Culture Collection of Bacteria	Utrecht, The Netherlands	http://www.cbs.knaw.nl
NCIMB	National Collection of Industrial, Marine and Food Bacteria	Aberdeen, Scotland	http://www.ncimb.com
NRRL	United States Department of Agriculture, Agricultural Research Service Culture Collection	Peoria, Illinois	http://nrrl.ncaur.usda.gov

What is a "strain"?

- A population of microbes descended from a single individual or pure culture
- It is a subgroup of a species with one or more characteristics that distinguish it other subgroups of the same species
- Different strains represent genetic variability within a species
- **Biovars:** Strains that differ in biochemical or physiological differences
- Morphovars: Strains that vary in morphology
- **Serovars:** Strains that vary in their antigenic properties
- Each strain is identified by a name, number, letter, etc. (E.g. Lactobacillus acidophilus LA-5)



Major characteristics used in taxonomy

Different approaches are used in classifying and identifying new microorganism that have isolated and grown in pure culture

▶ 2 major characteristics used in taxonomy:

- 1. Classical characteristics The basis of phenetic classification, useful in identification & may provide phylogenetic information.
- 2. Molecular characteristics important for microbial identification & evolution



Table 19.2Some Morphological Features Used
in Classification and Identification

Feature	Microbial Groups
Cell shape	All major groups ¹
Cell size	All major groups
Colonial morphology	All major groups
Ultrastructural characteristics	All major groups
Staining behavior	Bacteria, some fungi
Cilia and flagella	All major groups
Mechanism of motility	Gliding bacteria, spirochetes, protists
Endospore shape and location	Some Gram-positive bacteria
Spore morphology and location	Bacteria, protists, fungi
Cellular inclusions	All major groups
Colony color	All major groups

1 Used in classifying and identifying at least some bacteria, archaea, fungi, and protists.

Table 19.3	Some Physiological and Metabolic Characteristics Used in Classification and Identification				
Carbon and nit	rogen sources				
Cell wall constituents					
Energy source	S				
Fermentation products					
General nutritional type					
Growth tempe	rature optimum and range				
Motility					
Osmotic tolerance					
Oxygen relationships					
pH optimum and growth range					
Photosynthetic pigments					
Salt requireme	nts and tolerance				
Secondary metabolites formed					
Storage inclusions					



bacterial sources. A methyl ester contains a methyl group (CH_3) in place of the proton on the carboxylic acid group (COOH) of the fatty acid. (b) Procedure. Each peak from the gas chromatograph is due to one particular fatty acid methyl ester, and the peak height is proportional to the amount.



Nucleic acid base composition

G + C content

Mol% (G+C) =
$$\frac{G+C}{G+C+A+T} \times 100\%$$

- Estimated by determining the melting temperature (T_m) of the DNA
- Higher G + C gives a higher melting temperature



- G + C content of strains within a particular species is constant & varies very little within a genus.
- Two microorganism are not closelyrelated if their G + C content differ by more than 10%

Nucleic acid hybridization

Nucleic acid hybridization

- By mixing ssDNA from two different species and determining the percentage of the DNA that can form dsDNA hybrids
- The greater the percent hybridization, the closer the species
- Two strains are considered as the same species if their DNA show at least 70% relatedness under optimal hybridization condition,



Hybridization experiment:



Figure 13.22 Genomic hybridization as a taxonomic tool. (*a*) Genomic DNA is isolated from the organisms to be compared and then sheared and denatured. Probe DNA is prepared from organism 1 by shearing, denaturing, and labeling the DNA (shown here as radioactive phosphate). (*b*) Sheared single-stranded target DNA from each genome is immobilized on a membrane and then hybridized with the labeled probe DNA from organism 1. Radioactivity in the hybridized DNA is measured. (*c*) Radioactivity in the control (organism 1 DNA hybridizing to itself) is taken as the 100% hybridization value.

Nucleic acid sequencing

- ▶ The nucleic acid sequence for the complete genome of several species is now available
- 16S rRNA (ribosomal RNA) sequences; comparison of these sequences has been extensively used to determine the phylogenetic relationships of microbial groups
- Polymerase chain reaction (PCR) is used to amplify regions of rRNA genes from the genome of organisms and sequence the DNA using automated sequencing technology.



Comparison of proteins

- Serological Tests
- Use group specific antiserum isolated from the plasma of animals that have been sensitized to the organism
 - The antiserum contains antibody proteins that react with antigens on the unknown organism.
 - The reaction can be detected by examining agglutination or by using sera labeled with colorimetric or fluorescent labels

Advantages:

- Highly specific
- Does not usually require the organism to be isolated into pure culture
- Can be used to identify organisms
 that can't be grown on medium

Bergey's Manual of Systematic Bacteriology

- Bergey's Manual of Determinative Bacteriology
 - published by David Bergey and 4 collegues in 1923.
 - Grouped bacteria into phenetic groups
 - Used as a reference guide for bacterial identification based on physiological and morphological traits
- Bergey's Manual of Systematic Bacteriology (1st ed)
 - Published in 1984
 - Contained description of all bacterial and archaeal species

- Bergey's Manual of Systematic Bacteriology (2nd ed)
 - Consist of 5 volumes, Published over a number of years starting in 2005,
 - Provides the morphology, physiology, growth condition, ecology
 - gives the most up-to-date phylogenic classification of prokaryotic organisms
- The classification in Bergey's Manual is accepted by most microbiologists as the best consensus for prokaryotic taxonomy.

10 min break



THE MAJOR DIVISIONS OF LIFE



BACTERIA

EUKARYA



(b)

Figure 1.36 Evolutionary relationships and the phylogenetic tree of life. (*a*) The technology behind ribosomal RNA gene phylogenies. 1. DNA is extracted from cells. 2. Copies of the gene encoding rRNA are made by the polymerase chain reaction (PCR; CP Section 12.1). 3, 4. The gene is sequenced and the sequence aligned with sequences from other organisms. A computer algorithm makes pairwise comparisons at each base and generates a phylogenetic tree, 5, that depicts evolutionary relationships. In the example shown, the sequence differences are highlighted in yellow and are as follows: organism 1 versus organism 2, three differences; 1 versus 3, two differences; 2 versus 3, four differences. Thus organisms 1 and 3 are closer relatives than are 2 and 3 or 1 and 2. (b) The phylogenetic tree of life. The tree shows the three domains of organisms and a few representative groups in each domain.

Tree of Life

- Carl Woese (1928–2012) realized that the sequence of ribosomal RNA (rRNA) molecules and the genes that encode them could be used to infer evolutionary relationship between organisms.
- Woese is famous for defining the Archaea in 1977
- Current theories based largely on characterization of rRNA sequences
- 16S rRNA gene provides a species specific signature sequence which is useful for bacterial identification process



Professor in the University of Illinois (USA)



Ribosomes: Large (red) and small (blue) subunit fit together

- Genes encoding rRNAs are excellent for phylogenetic analysis because
 - 1. Universally distributed
 - 2. Functionally constant
 - 3. Highly conserved (slowly changing)
 - 4. Adequate length to provide a deep view of evolutionary relationship

► 16S rRNA

- A component of 30S small subunit of ribosomes
- The genes coding for it are referred to as 16S rRNA gene
- The region is conserved slow rates of evolution
- Used in constructing phylogenies

Domain Archaea

Overview of the Archaea

- Composed of 5 phyla
 - Euryarchaeota, Crenarchaeota, Thaumarchaeota, Korarchaeota, and Nanoarchaeota.
- Multiplication by binary fission, budding, fragmentation
- Inhabit extreme environments (extremophiles)
 - very high/low temp./pH, concentrated salts, completely anoxic
- Diverse physiology
 - Aerobic, facultative anaerobic, strictly anaerobic
- Diverse morphology
 - Spherical, rod-shape, spiral, cuboidal, irregular, etc.
- None of them are pathogenic

Microbial phylogeny in Domain Archaea



Figure 17.1 Schematic representation of the phylogeny of the major taxonomic orders within the domain *Archaea*. Each of the five archaeal phyla and their major orders are indicated in a different color.

Phylum Crenarchaeota

- Metabolically diverse thermophiles
- Contain thermophylic and hyperthermophilic sulfur-metabolizing archaea
- Most of the thermophiles are sulfur dependent
 - Sulfur as electron acceptor in anaerobic respiration or electron donor by litothrophs.
 - Grow in geothermal heated water that contain sulfur
 - Optimum growth temp. 105°C



Figure 20.8 Habitat for Thermophilic Archaea. The Sulfur Cauldron in Yellowstone National Park. The water is at its boiling point and very rich in sulfur. *Sulfolobus* spp. grow well in such habitats.

Table 20.3Phylum Crenarchaeota; Class Thermoprotei

Order	Family or Families	Typical Morphology	Optimal Growth Temp Range	Growth Characteristics	Representative Genera
Thermoproteales	Thermoproteaceae	Cocci, clubs, rods	75 to >100°C	Facultatively anaerobic or anaerobic, chemolithoautotropic reduction of S ⁰ with H ₂ or chemoorganotrophic	Thermoproteus Pyrobaculum Thermocladium
	Thermofilaceae	Thin rods, filamentous	80 to 90°C	Obligately anaerobic, acidophilic, sulfur respiration	Thermofilum
Desulfurococcales	Desulfurococcaceae Pyrodictiaceae	Cocci, disc-shaped Cocci, disc-shaped	85 to 95°C 108 to 113°C	Most anaerobic, few aerobic; chemolithoautotrophic or heterotrophic growth with sulfur reduction or fermentation Facultatively anaerobic, or	Desulfurococcus Ignicoccus Thermodiscus Staphylothermus Pyrodictium
				anaerobic; chemolithoautotrophic reduction of S ⁰ or fermentative	Pyrolobus Hyperthermus
Sulfolabales	Sulfolobaceae	Cocci, irregular	75 to 85°C	Aerobic, facultatively anaerobic, or anaerobic; Chemolithoautotrophic or chemoorganotrophic; Acidiphilic	Sulfolobus, Metallosphaera Acidianus Sulfurisphaera Sulfurococcus
Caldisphaerales	Caldisphaeraceae	Cocci	70 to 75°C	Anaerobic, chemoorganotrophic, acidophilic	Caldisphaera

Phylum Euryarchaeota

- Contains primarily methanogenic archaea, halophilic archaea, and thermophilic, sulfurreducing archaea
- Methanogens are the largest group of Archea, strictly anaerobes (5 orders)
 - Generate methane from H₂ and CO₂ or organic molecule such as formate, acetate, and methanol
- Haloarcheae are extreme halophiles
 - Grow only in high-salinity habitats such as Dead sea in Jordan and Great Salt Lake in Utah.



The pond is extremely high in salt and mineral content. Haloarchea dominate the area and produce brilliant red pigments

Table 20.4 Selected Characteristics of Representative Methanogens

Order and Genera	Morphology	Wall Composition	Motility	Methanogenic Substrates Used	Capable of N ₂ Fixation
Order Methanobacteriales Methanobacterium	Long rods or filaments	Pseudomurein	_	$H_2 + CO_2$, formate	Yes
Methanothermus	Straight to slightly curved rods	Pseudomurein with an outer protein S-layer	+	$H_2 + CO_2$	No
Order Methanococcales Methanococcus	Irregular cocci	Protein	+	$H_2 + CO_2$, formate	Yes
Order Methanomicrobiales Methanomicrobium	Short curved rods	Protein	+	$H_2 + CO_2$, formate	No
Methanogenium	Irregular cocci	Protein or glycoprotein	-	$H_2 + CO_2$, formate	No
Methanospirillum	Curved rods or spirilla	Protein	+	$H_2 + CO_2$, formate	Yes
Order Methanosarcinales Methanosarcina	Irregular cocci, packets	Protein sometimes with polysaccharide	-	$H_2 + CO_2$, methanol, methylamines, acetate	Yes

DOMAIN BACTERIA

Overview of domain Bacteria

- There are over 80 phyla in Bacteria based on the analyses of 16S rRNA gene sequences from natural environments.
- Major phyla of bacteria that have cultivated species
 - Proteobacteria
 - Firmicutes
 - Actinobacteria
 - Bacteroidetes



Phylum Proteobacteria

- The largest group of gram-negative bacteria
- Extremely complex group, with over 400 genera and 1300 named species
- Proteobacteria constitute the majority of known bacteria of medical, industrial, and agricultural significance.
- All major nutritional types are represented: phototrophy, heterotrophy, and several types of chemolithotrophy
- Sometimes called the "purple bacteria," although very few are purple; the term refers to a hypothetical purple photosynthetic bacterium from which the group is believed to have evolved

Phylum Proteobacteria

Divided into 5 classes:

- > Alphaproteobacteria (α)
 - Purple nonsulfur bacteria, Rickettsia, Rhizobium,
- Betaproteobacteria (β)
- Gammaproteobacteria (γ)
 - Purple sulfur bacteria
- Deltaproteobacteria (δ)
 - Sulfate or sulfur-reducing bacteria
- Epsilonproteobacteria (ε)
 - Include genera: Camplylobacter and Helicobacter



Phylum Proteobacteria

Significant groups and genera include:

- Photosynthetic genera such as *Rhodospirillum* (a purple non-sulfur bacterium) and *Chromatium* (a purple sulfur bacterium)
- Sulfur chemolithotrophs, genera Thiobacillus and Beggiatoa
- Nitrogen chemolithotrophs (nitrifying bacteria), genera Nitrobacter and Nitrosomonas
- Other chemolithotrophs, genera Alcaligenes, Methylobacillus, Burkholderia
- The family Enterobacteriaceae, the "Gram-negative enteric bacteria," which includes genera Escherichia, Proteus, Enterobacter, Klebsiella, Salmonella, Shigella, Serratia, and others
- The family Pseudomonadaceae, which includes genus Pseudomonas and related genera
- Other medically important Proteobacteria include genera Haemophilus, Vibrio, Campylobacter, Helicobacter, Rickessia, Brucella

Phylum Firmicutes

- ▶ Latin: firmus, strong + cutis, skin (as in reference to cell wall)
- Gram +ve cell wall structure
- "Low G + C gram-positive" bacteria
- Divided into 3 classes
 - Class I Clostridia; includes genera Clostridium and Desulfotomaculatum, and others
 - Class II Mollicutes; bacteria in this class cannot make peptidoglycan and lack cell walls; includes genera Mycoplasma, Ureaplasma, and others
 - Class III Bacilli; includes genera Bacillus, Lactobacillus, Streptococcus, Lactococcus, Geobacillus, Enterococcus, Listeria, Staphylococcus, and others

Phylum Aquificae

- Hyperthermophiles bacteria
- Represent the earliest "deepest" branch of the Bacteria
- Contains genera Aquifex and Hydrogenobacter that can obtain energy from hydrogen via chemolithotrophic pathways
- Aquifex pyrophilus
 - Extreme thermophiles
 - 85°C-95°C
 - Gram-negative rod
- Grow in active geothermal area



Phylum Cyanobacteria

- Cyanobacteria / blue-green algae are the largest and most diverse group of photosynthetic bacteria
- Cyano "Colour of the bacteria" Greek "Blue"
- Obtain energy through photosynthesis
- Photosynthetic prokaryotes produce O₂
- The photosynthetic apparatus closely resemble that of eukaryotes
- Diverse morphology
- Reproduce by binary fission, budding, fragmentation







Chroococcus turgidus



Phylum Chlorobi

- Anaerobic photosynthetic bacteria
- ▶ The "green sulfur bacteria"
- Obligate anaerobic photolithotrophs that use hydrogen sulfide, sulfur, and hydrogen as electron source
- Found in sulfide-rich muds at the bottom of lakes and ponds
- Green-grass or chocolate brown in colour
- Includes genera Chlorobium





Phylum Spirochaetes

- Greek: spira a coil; chaete hair
- Morphologically unique long bacteria with a flexible & helical shape.
- Cell moves in corkscrew like movement
- Gram negative, chemoorganotrophic bacteria
- Important pathogenic genera include Treponema, Borrelia, and Leptospira
- Often grow in anoxic and sulfide-rich freshwater and marine environment



(b)

Figure 21.19 Spirochete Morphology. (a1) A surface view of spirochete structure as interpreted from electron micrographs. (a2) A longitudinal view of *Treponema pallidum*. (b) A cross section of a typical spirochete showing morphological details.

Phylum Actinobacteria

- "High G + C gram-positive" bacteria
- Includes genera Actinomyces, Streptomyces, Corynebacterium, Micrococcus, Mycobacterium, Propionibacterium

DOMAIN EUKARYA

- The domain Eucarya is divided into four kingdoms by most biologists:
 - Kingdom Protista, including the protozoa and algae
 - Kingdom Fungi, the fungi (molds, yeast, and fleshy fungi)
 - Kingdom Animalia, the multicellular animals
 - Kingdom Plantae, the multicellular plants





"Animal Like" Paramecium aurelia





"Fungus Like" Fuligo septic

Colonial Algae Volvox carteri





THANK YOU