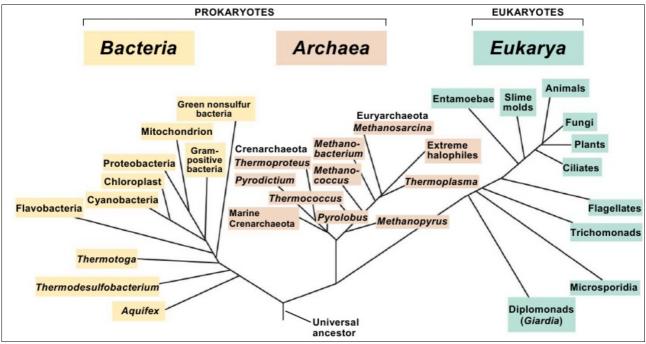
# **COMPENDIUM – TBT4110 MIKROBIOLOGI**

Phylogenetic map of the three domains:



Classification: Domain - Kingdom - Phylum - Class - Order - Family - Genus - Species - Strains

#### Classification of bacteria

1. How the organism obtains carbon for synthesizing cell mass:

- **autotrophic** carbon is obtained from carbon dioxide (CO2)
- **heterotrophic** carbon is obtained from organic compounds
- **mixotrophic** carbon is obtained from both organic compounds and by fixing carbon dioxide

2. How the organism obtains **reducing equivalents** used either in energy conservation or in biosynthetic reactions:

- lithotrophic reducing equivalents are obtained from inorganic compounds
- organotrophic reducing equivalents are obtained from organic compounds

3. How the organism obtains energy for living and growing:

- **chemotrophic** energy is obtained from external chemical compounds
- phototrophic energy is obtained from light

Respiratory organisms use chemical compounds as a source of energy by taking electrons from the reduced substrate and transferring them to a terminal electron acceptor in a redox reaction. This reaction releases energy that can be used to synthesize ATP and drive metabolism. In aerobic organisms, oxygen is used as the electron acceptor. In anaerobic organisms other inorganic compounds, such as nitrate (NO<sub>3</sub>-), sulfate (SO<sub>4</sub><sup>2-</sup>) or carbonate (CO<sub>3</sub><sup>2-</sup>) are used as electron acceptors. This leads to the ecologically important processes of denitrification, sulfate reduction and acetogenesis, respectively.

Another way of life of chemotrophs in the absence of possible electron acceptors is fermentation, where the electrons taken from the reduced substrates are transferred to oxidized intermediates to generate reduced fermentation products (e.g., lactate, ethanol, hydrogen, butyric acid). Fermentation is possible, because the energy content of the substrates is higher than that of the products, which allows the organisms to synthesize ATP and drive their metabolism.

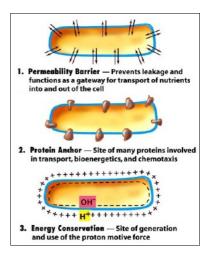
Obligate anaerobes may use fermentation or anaerobic respiration.

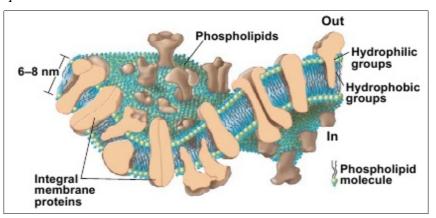
**Stickland fermentation** or *The Strickland Reaction* is the name for a chemical reaction that involves the coupled oxidation and reduction of amino acids to organic acids. The electron donor amino acid is oxidized to a volatile carboxylic acid one carbon atom shorter than the original amino acid. For example, alanine with a three carbon chain is converted to acetate with two carbons. The electron acceptor amino acid is reduced to a volatile carboxylic acid the same length as the original amino acid. For example, glycine with two carbons is converted to acetate. In this way, amino acid fermenting microbes can avoid using hydrogen ions as electron acceptors to produce hydrogen gas. Amino acids can be Stickland acceptors, Stickland donors, or act as both donor and acceptor. Only histidine cannot be fermented by Stickland reactions, and is oxidized.

#### The bacterial cytoplasmic cell membrane

The cell membrane of bacterias are used as a barrier, to store energy, and to organize proteins.

The cell membrane of bacterias is a thin structure surrounding the cell. It's the **barrier** separating the inside of the cell from the environment with selective permeability. Functioning as a barrier for protons and hydroxyl ions, bacteria can **store energy** as a chemical potential called the proton motive force. Both respiratory and phototrophic bacteria store energy using the proton motive force/potential, and this is used to generate ATP, in contrast with fermentative bacteria, where substrate-level phosphorylation occurs. The general structure is a phospholipid bilayer, 6-8 nm wide. **Membrane proteins** are either *integral (transmembrane or anchored)*, or *peripheral*.





One major difference between eukaryotes and prokaryotes in cytoplasmic membrane composition is that eukaryotes have *sterols* in their membranes (methanotrophic bacteria and mycoplasmas are exceptions). The presence of sterols strengthens and stabilizes the membrane, making it less flexible. *Hopanoids* are similar molecules, present in many Bacteria.

## Active transport across bacterial cell membranes

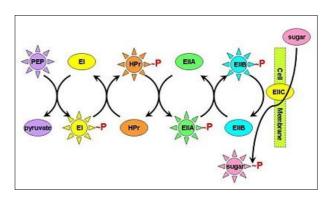
The transport systems have common evolutionary roots, all systems have 12 transmembrane  $\alpha$ -helix domains, forming a channel.

- Simple transport
- Group translocation
- ATP-binding cassette

Simple transport: uniporters, symporters and antiporters.

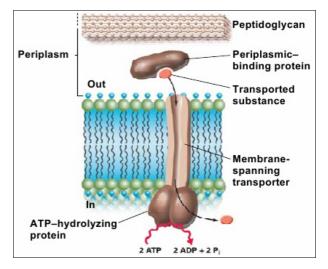
**Group translocation:** The phosphotransferase system. The phosphotransferase system is involved in transporting many sugars into bacteria, including glucose, mannose, fructose and cellobiose. The phosphate group on phosphoenolpyruvate (PEP) is eventually transferred to the imported sugar via several proteins. All the proteins have the phosphate group transferred to a conserved histidine residue.

In glucose transport, PEP transfers its phosphate to a histidine residue on Enzyme I. Enzyme I in turn transfers the phosphate to histidine protein (HPr). From HPr the phosphate is transferred to Enzyme II A. Enzyme II A is specific for glucose and it further transfers the phosphate to a juxtamembrane Enzyme II B and transmembrane Enzyme II C. Enzyme II C phosphorylates glucose as it passes through the plasma membrane, forming glucose-6-phosphate. The benefit of transforming glucose into glucose-6-



phosphate is that it will not leak out of the cell, therefore providing a one-way concentration gradient of glucose. The HPr is common to the phosphotransferase systems of the other substrates mentioned earlier, as is the upstream Enzyme I.

ATP-binding cassette transporters (ABCtransporter) are members of a protein superfamily that is one of the largest and most ancient families with representatives in all extant phyla from prokaryotes to humans. ABC transporters are transmembrane proteins that utilize the energy of adenosine triphosphate (ATP) hydrolysis to carry out certain biological processes including translocation of various substrates across membranes and non-transport-related processes such as translation of RNA and DNA repair. They transport a wide variety of substrates across extra- and intracellular membranes, including metabolic products, lipids and sterols, and drugs. Proteins are classified as ABC transporters based

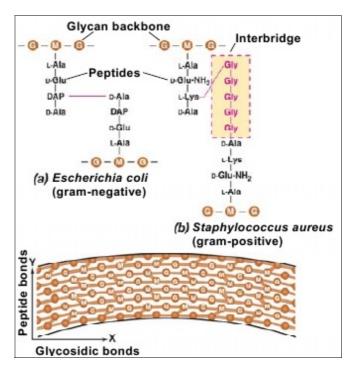


on the sequence and organization of their ATP-binding cassette (ABC) domain(s).

## The bacterial cell wall

Gram-positive bacteria have a thick layer of peptidoglycan, constituting the cell wall, which gives

rigidity to the cell and prevents lysis when encountering osmotic gradients. Peptidoglycan is a polysaccharide composed of two sugar derivatives: Nacetylglucosamine N-acetylmuramic and acid, together with a few amino acids (Dalanin, L-alanin, D-glutamic acid and lysine or diaminopimelic acid (DPAP)). The peptidoglycan are long chains of alternative N-acetylglucosamine and N-acetylmuramic acid, connected through  $\beta$ -1,4-glycosidic linkages. The specific wall structure differs from organism to organism, with variations the on following scheme: The Nacetylmuramic acids have side chains of (Lala) - (D-Glu) - (L-lys or DAP) - (D-ala), with cross-links between DAP and D-ala (Gram-negative bacteria), and interbridges (5x Gly in s. aureus) between L-lys and D-ala (Gram-positive bacteria). 90% of the Grampositive bacterial cell wall is peptidoglycan. constituents teichoic Other are acids

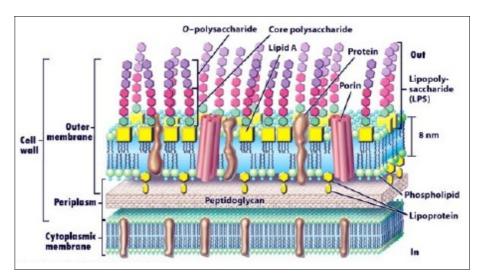


(contains glycerophosphate or ribositol phosphate residues), which are embedded in the cell wall and covalently bound to N-acetylmuramic acids. Teichoic acids bind  $Ca^{2+}$  and  $Mg^{2+}$  for transport into the cell.

Lysozyme breaks the  $\beta$ -1,4-glycosidic linkages in the peptidoglycan, creating a *protoplast* under isosmotic conditions.

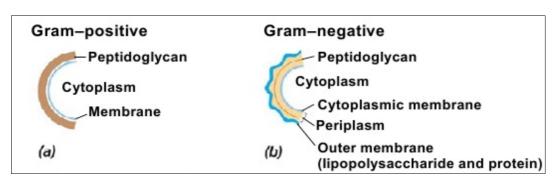
**Gram-negative bacteria** has a thin layer of peptidoglycan located in the periplasmic space, and an outer membrane of lipopolysaccharide (LPS). The peptidoglycan of Gram-negative bacteria contains no interbridges, only crosslinks. The LPS is a second lipid bilayer with lipid-polysaccharide complexes in addition to phospholipids and proteins. The major function of the LPS is structural, but one important biological property is its toxicity to animals. The toxic portion of

the LPS layer is often termed the endotoxin. The outer membrane in Gram-negative bacteria is relatively permeable to small molecules, due to the presence of *porins*. Several different porins exist, both specific and non-specific. Thev transmembrane are structures consisting of three identical subunits, forming three channels (and one small between



the three subunits).

The Gram staining procedure utilizes the difference in the thickness of the peptidoglycan layer of Gram negative vs Gram positive bacteria. An insoluble crystal violet-iodine complex forms inside the cell. Alcohol extracts this from Gram-negative bacteria, but not Gram positive bacteria, due to dehydration of the pores in the thick peptidoglycan layer of Gram positive bacteria.



#### Gram-negative vs Gram-positive cell wall

## The archeal cytoplasmic cell membrane

The membrane lipids of *Archea* differ from those of *Bacteria* and *Eukarya*. The lipids of *Archea* use ether linkages instead of ester linkages, and repeating units of isoprene (5 C) instead of fatty acids. The glycerol unit is the same however. The major lipids are phytanyl (20 C) in glycerol diethers (bilayer membrane), or biphytanyl (40 C) in diacylglycerol tetraethers (monolayer membrane).

## The archeal cell wall

Archea does not contain peptidoglycan (hence the resistance to lysozyme and penicillins), and usually not an outer membrane.

**Pseudomurein** is found in some Archea (e.g. methanogenes), which is a polysaccharide very similar to peptidoglycan, consisting of alternating N-acetylglucosamine and N-acetyltalosaminuronic acid, connected by  $\beta$ -1,3-glycosidic linkages. The amino acids are all of the L stereoisomer.

**S-layer** is the most common cell wall of Archea, which is a paracrystalline surface layer. S-layer consists of protein or glycoprotein in an ordered appearance with various symmetries (hexagonal, tetragonal, trimeric). S-layers probably functions as a sieve, excluding large molecules and structures.

## **Bacterial cell division**

Fts proteins (filamentous temperature sensitive) are universally distributed among prokaryotes, including *Archea*, and are related to tubulin. The Fts proteins interact to form the cell division apparatus called the divisome. In rod-shaped cells, formation of the divisome begins with the attachment of molecules of FtsZ in a ring precisely around the center of the cell. FtsZ proteins polymerize to form a ring, and the ring then attracts other divisome proteins (FtsA and ZipA). ZipA anchors the FtsZ ring to the cytoplasmic membrane. FtsI is required for peptidoglycan synthesis, and is one of the penicillin binding proteins.

The Min proteins control where the FtsZ ring polymerizes. The Min proteins oscillates from pole to pole, and inhibits polymerization of FtsZ proteins. They spend least time at the center, and this is where the divisome forms.

## **Characteristics of bacteria**

#### Enteric bacteria (Gammaproteobacteria)

- <u>Gram-negative straight rods</u>
- motile by peritrichous flagella or nonmotile
- nonsporulating
- <u>facultative aerobes</u>
- producing acid from glucose
- sodium neither required nor stimulatory
- <u>catalase positive</u>
- <u>oxidase negative</u>
- usually reduce nitrate to nitrite (not to N<sub>2</sub>)
- 16S rRNA gene of Gammaproteobacteria
- <u>ferment sugars</u>

Fermentation patterns in enteric bacteria - anaerobic fermentation of glucose:

- $CO_2:H_2 = 1 \rightarrow Mixed-acid fermentation: acetic, lactic, succinic acid + ethanol, <math>CO_2$  and  $H_2$ . Equal amounts of  $CO_2$  and  $H_2$  since these are produced only from formic acid.
- $CO_2:H_2 > 1 \rightarrow 2,3$ -butanediol fermentation:  $CO_2 + H_2$  (some acids). More  $CO_2$  than  $H_2$  since  $CO_2$  is produced from formic acid and during formation of butanediol.

#### Mixed acid fermenters

**Escherichia** are almost universal inhabitants of the intestinal tract of warm-blooded animals. Synthesizes vitamin K. Facultative aerobe  $\rightarrow$  ensures anoxic intestines.

Salmonella are closely related to Escherichia, but usually pathogenic.

Shigellas are also genetically close to Escherichia. Commonly pathogenic.

Proteus are motile and produces *urease*.

#### *Butanediol fermenters* Enterobacter, Klebsiella and Serratia

### Pseudomans and the Pseudomonads (Proteobacteria ( $\alpha$ , $\beta$ and $\gamma$ ))

- <u>Gram-negative</u>
- straight or curved rods, not vibroid
- no spores
- polar flagella, motile
- respiratory metabolism, aerobic
- non-fermentative
- <u>oxidase positive</u> (except enterics)
- <u>catalase positive</u>
- absence of gas formation from glucose

Zymomonas are used for fermentation of sugars to ethanol in Asia, Africa and South America, and is distinguished from psuedomonas by its fermentative metabolism. Microaerophilic or anaerobic.

## Acetic Acid Bacteria (Alphaproteobacteria)

• <u>Gram negative</u>

- obligate aerobic
- motile
- produces acetic acid from ethanol
- flagellated (polarly: Gluconobacter, peritrichously: Acetobacter)
- <u>dissolves CaCO<sub>3</sub></u> on agar plates containing ethanol

Acetic acid bacteria are used for the production of vinegar from ethanol, and ascorbic acid from sorbose/sorbitol.

### Vibrio, Aliivibrio, and Photobacterium

- Gram-negative
- facultatively aerobic
- rods or curved rods
- fermentative
- oxidase positive
- bioluminescence using luciferase, quorum sensing

#### Staphylococcus and Micrococcus

- Gram-positive cocci
- catalase positive
- Staphylococcus and Micrococcus separated by O/F (Oxidation/Fermentation) test: Micrococcus is an obligate aerobe, Staphylococcus is facultative aerobic, producing acid from glucose under both aerobic and anaerobic conditions.

### Lactic acid bacteria

- Gram positive rods and cocci
- produce lactic acid (major or sole fermentation product)
- fermenting
- aerotolerant anaerobic

Homofermentative lactic acid bacteria produce a single fermentation product: lactic acid. Heterofermenative lactic acid bacteria produce ethanol and  $CO_2$  in addition to lactate.

**Streptococcus** are divided in *Lactococcus* (diary significance) and *Enterococcus* (fecal origin, can be pathogenic). Streptococci are divided into two groups based on hemolysis:  $\alpha$ -hemolysis (green discoloration) or  $\beta$ -hemolysis (complete lysis of red blood cells). Streptococci are also divided into immunological groups called Lancefield groups based on specific antigens present.

Lactobacillus are rod-shaped, and are common in dairy produces. They are usually acidotolerant.

Listeria are coccobacilli, growing in chains of three to five cells. L. monocytogenes causes listeriosis.

### Bacillus

- <u>Gram positive</u>
- <u>aerobic</u>
- rod-shaped

- produce antibiotics (bacitracin, polymyxin)
- <u>spore-forming</u> (spores used as biological insecticies)

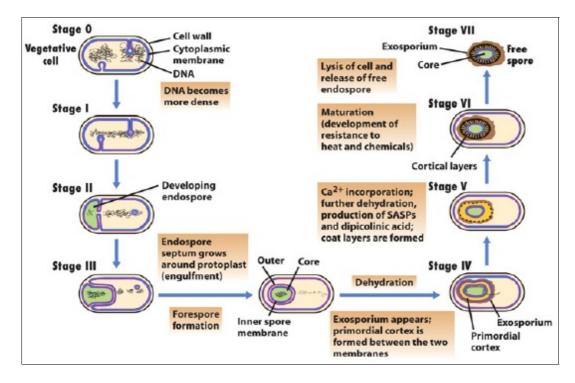
**Bacillus thuringiensis** (or Bt) is a Gram-positive, soil-dwelling bacterium, commonly used as a biological alternative to a pesticide; alternatively, the Cry toxin may be extracted and used as a pesticide. Additionally, B. thuringiensis also occurs naturally in the gut of caterpillars of various types of moths and butterflies, as well as on the dark surface of plants. Spores and crystalline insecticidal proteins produced by B. thuringiensis have been used to control insect pests since the 1920s. They are now used as specific insecticides under trade names such as Dipel and Thuricide. Because of their specificity, these pesticides are regarded as environmentally friendly, with little or no effect on humans, wildlife, pollinators, and most other beneficial insects. The Belgian company Plant Genetic Systems was the first company (in 1985) to develop genetically engineered (tobacco) plants with insect tolerance by expressing cry genes from B. thuringiensis.

#### Clostridium

- <u>Gram positive</u>
- <u>anaerobic</u>
- fermentative, producing butyric acid as a major end product
- rod-shaped
- <u>spore-forming</u>
- some uses the Stickland reaction
- soil main habitat

The clostridia (eg c. botulinum, c. tetani, c. perfringens) lack a respiratory chain, and obtain their ATP only by substrate-level phosphorylation. Several clostridia ferment sugars, producing butyric acid as a major end product.

#### **Spore formation**



Endospores are highly resistant to harsh environments, and endospore formers are easily isolated by heating a sample to 80  $^{\circ}$ C for 10 min.

### Corynebacteria

- Gram-positive
- non-motile
- aerobic
- rod-shaped
- V-shaped cell arrangement during growth

Corynebacterium diphteriae

### Propionic Acid Bacteria

- Gram-positive
- anaerobic
- ferment lactic acid
- produces propionic acid, acetic acid and CO<sub>2</sub>
- used in cheese production (Swiss Emmentaler)

### Mycobacterium

- acid-fast
- Ziehl-Neelsen stain (fuchsin + phenol) gives red bacteria, others are blue
- mycolic acid covalently linked to peptidoglycan
- Gram stain not effective due to high surface-lipid content
- M. tuberculosis isolated on Lowenstein-Jensen medium

#### Streptomyces

- Gram-positive
- filamentous growth
- spore-forming: sporophores  $\rightarrow$  conidia
- geosmines gives "earthy" odor
- 50% of streptomyces produce antibiotics (>60 in human use)

#### Cyanobacteria

- Gram-negative, but distantly related to Gram-positive
- oxygenic phototrophic
- chlorophyll *a*, and phycobilins as accessory pigments
- gas vesicles
- heterocysts for nitrogen fixation
- stores nitrogen in cyanophycin structures (copolymer of aspartic acid and arginine)
- gliding motilty phototaxis and chemotaxis
- produces neurotoxins

### Prochlorophytes

• Resemble cyanobacteria, but have clorophyll b instead of phycobilins, in addition to chlorphyll a

## Phototrophy

Light is used in the conversion of CO<sub>2</sub> to cell material (photoautotrophy) or organic carbon to cell material (photoheterotrophy). Organisms using H<sub>2</sub>O as electron donor produce O<sub>2</sub> as a by-product, and these organisms are termed *oxygenic*. Organisms using other electron donors (H<sub>2</sub>S, S<sup>0</sup>, S<sub>2</sub>O<sub>3</sub><sup>2-</sup> or H<sub>2</sub>) are termed *anoxygenic*. To perform photosynthesis the organisms need chlorophyll (oxygenic phototrophs) or bacteriochlorophyll (anoxygenic phototrophs). Unlike cytochromes, chlorophylls contain magnesium instead of iron at the center of the porphyrin ring. Chlorophyll *a* is green because it absorbs red and blue light preferentially and transmits green light. Each chlorophyll and bacteriochlorophyll is characterized by its unique absorption spectrum. Among prokaryotes, cyanobacteria produce chlorophyll *a*, whereas anoxygenic phototrophs (eg purple and green bacteria) can have a number of bacteriochlorophylls. Since different pigments have different absorption spectrums, different organisms can coexist in the same illuminated habitat.

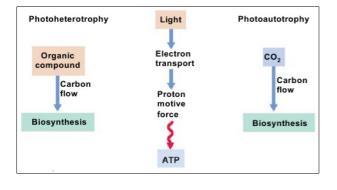
The chlorophylls are located within special membrane systems, *the photosynthetic membranes*. In eukaryotes, photosynthesis happens in the chloroplasts' *thylakoids*. In prokaryotes photosynthetic pigments are integrated into internal membrane systems, arising from 1) invaginations of the cytoplasmic membrane (purple bacteria), 2) the cytoplasmic membrane itself (heliobacteria), 3 in both the cytoplasmic membrane and membrane enclosed structures called chlorosomes (green bacteria), or 4) in thylakoid membranes (cyanobacteria).

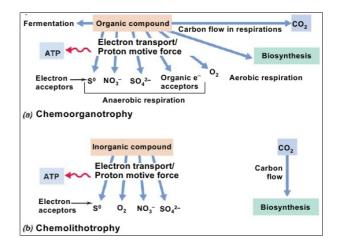
The light harvesting protein complexes has a *reaction center* where light is converted to ATP, and *antenna pigments* around the reaction center that funnels energy to the reaction center. Phycobilins are pigment proteins that function in light-harvesting in cyanobacteria.

**Carotenoids** and other accessory pigments protect the organism from photoinduced damage. Their energy can be transferred to reaction centers.

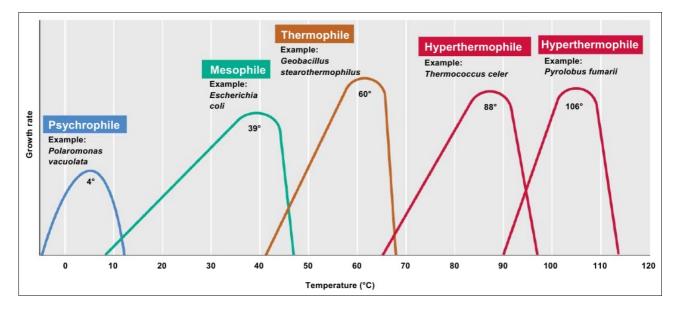
**Bacteriorhodopsin** function in light-driven synthesis of ATP that is not photosynthesis (ie without chlorophyll pigments). Halobacterium has bacteriorhodopsin in their cytoplasmic membranes, conjugated to retinal, which absorbs light. Light absorption induces a *trans* to *cis* configuration change, and the decay back to trans configuration is coupled with the transport of a proton from the cytoplasm to the outside. The proton motive force is then used to generate ATP. Other rhodopsins than bacteriorhodopsin also exists.

### Phototrohpy vs chemotrophy





## **Cardinal temperatures**



## Extremophiles

**Extreme halophiles** (e.g. the *Archeae* Halobacterium) require large amounts of sodium for growth, at least 1,5 M (9%). To withstand osmotic forces organisms must use solutes intracellularly to match the osmolarity of the environment. These are called compatible solutes, since they don't interfere with biochemical processes inside the cell, and are usually organic. Halobacterium, however, pumps large amounts of K<sup>+</sup> intracellularly, with  $[K^+_{inside}] > [Na^+_{outside}]$ . The Halobacterium cell wall is composed of glycoproteins and stabilized by Na<sup>+</sup>. When insufficient amounts of sodium is present, the cell lyses. The cell wall has a high content of the acidic (negatively charged) amino acids aspartate and glutamate, and the negative charges are shielded by sodium ions. When the concentration of sodium is too low these negative amino acids repel each other. Intracellular potassium serves a similar role as sodium extracellularly: to shield the cytoplasmic acidic proteins. Some halophilic members of Bacteria also use potassium intracellularly.

**Hyperthermophiles** are found both among *Archea* and *Bacteria*. Of the *Archea* some species show growth-temperature optima > 100°C, whereas no known *Bacteria* grow at temperatures > 95°C. To increase heat stability of proteins only minor changes are required. Membrane stability is ensured by lipids rich in saturated fatty acids. Hyperthermophiles of the *Archea* contain  $C_{40}$  hydrocarbons instead of fatty acids, and therefore have a cytoplasmic membrane which is a lipid monolayer instead of a lipid bilayer.

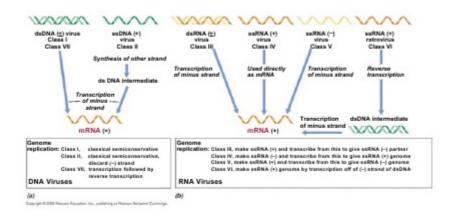
Acidophiles (eg Acidithiobacillus (*Bacteria*), Thermoplasma (*Archea*)) grow optimally at pH < 6. A critical factor is membrane stability. Alkaliphiles (eg Bacillus sp) are microorganisms with a pH optimum > 9. Some extremely alkaliphilic bacteria also halophilic, and most of these are *Archea*. Some enzymes from alkaliphilic organisms (proteases and lipases) are used as supplements for laundry detergents. For both acidophiles and alkaliphiles the internal pH can not be too far from neutrality (range 4.6-9.5 for different organisms). This is because DNA is acid-labile, and RNA is alkaline-labile, and the integrity of these molecules is mandatory. To limit pH deviations, buffers are used. Around neutral pH KH<sub>2</sub>PO<sub>4</sub> and CaCO<sub>3</sub> are good buffers.

### Viruses

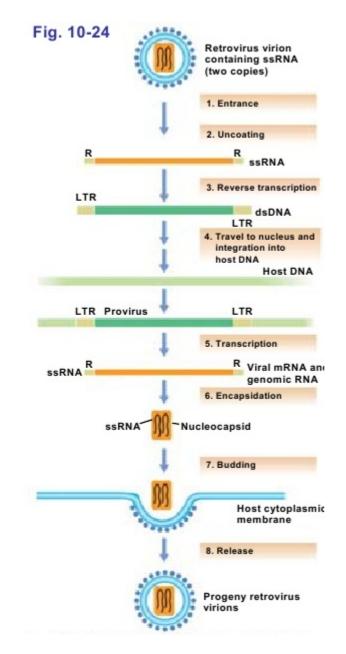
The Baltimore classification system of viruses, based on the relationship of the viral genome to its mRNA.

		Examples	
Class	Description of genome and replication strategy	Bacterial viruses	Animal viruses
í .	Double-stranded DNA genome	Lambda, T4	Herpesvirus, pox virus
11	Single-stranded DNA genome	фX174	Chicken anemia virus
Ш	Double-stranded RNA genome	ф6	Reoviruses ( Section 19.10)
IV	Single-stranded RNA genome of plus sense	MS2	Poliovirus
V	Single-stranded RNA genome of minus sense		Influenza virus, rabies virus
VI	Single-stranded RNA genome that replicates with DNA intermediate		Retroviruses
VII	Double-stranded DNA genome that replicates with RNA intermediate		Hepatitis B virus

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### Retroviruses



## Eukarya

#### Protists

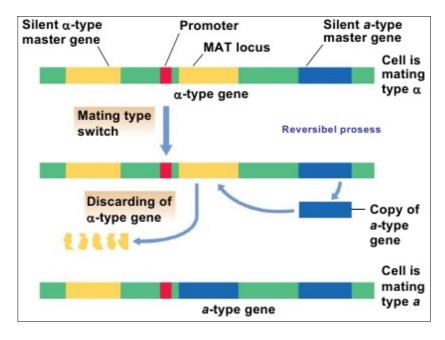
- **Diplomonads and Parabasalids:** Unicellular, flagellated protists that lack chloroplasts, live in anoxic habitats, using fermentation. *Diplomonads* (e.g. Giardia lamblia) contain two nuclei. *Parabasalids* (e.g. Trichomonas vaginalis): Contain a parabasalid body, giving structural support to the Golgi complex.
- <u>Euglenozoans</u>: Unicellular, flagellated, distinguished from other protists by the presence of crystalline rod in their flagella. Parasitic, or free-living phototrophs or chemorganotrophs. *Kinetoplastids* (e.g. Trypanosoma brucei) contain kinetoplasts, a mass of DNA in their single, large mitochondrion. *Euglenids* (e.g. Euglena) are nonpathogenic and phototrophic, living in aquatic habitats.
- <u>Alveolates:</u> Characterized by the presence of alveoli. *Ciliates* (e.g. Paramecium) possesses cilia at some stage in their life cycle. Contains two nuclei with different functions. *Dinoflagellates* (e.g. Gonyaulax) have flagella encircling the cell imparting movements that give dinoflagellates their name (dino = gr. whirling). *Apicomplexans* (e.g. Plasmodium, Toxoplasma) are obligate parasites of animals.
- <u>Stramenopiles:</u> Include chemoorganotrophic and phototrophic organisms. Flagellated. *Oomycetes* ("egg fungi") resemble fungi with multinucleate hyphae. *Diatoms* have a crushresistant cell wall made of silica, to which protein and polysaccharide are added. *Golden Algae* are golden brown, and are phototrophs.
- <u>Cercozoans and Radiolarians:</u> Distinguished from other protists by threadlike pseudopodia that help them move and feed.
- <u>Amoebozoa:</u> Terrestrial and aquatic protists, that uses lobe-shaped pseudopodia for movement and feeding. *Gymnamoebas* (e.g. Amoeba) are free-living protists in aquatic and soil environments. Uses amoeboid movement to move (streaming of cytoplasm). *Entamoebas* (e.g. Entamoeba histolytica) are parasites of vertebrates and invertebrates, usually intestines. *(Plasmodial) Slime Molds* (e.g. Physarum) have fruiting bodies with spores. *Cellular Slime molds* are usually haploid, in contrast to plasmodial slime molds who are diploid.

#### Fungi

- Large group of organisms: the molds, mushrooms and yeasts. Closely related to animals. Diverse habitats, including water, however most are terrestrial.
- Fungi are chemoorganotrophic, and most are aerobes. They feed by secreting extracellular enzymes that digest complex organic materials, such as polysaccharides or proteins.
- Most fungi are multicellular, forming a network of filaments called hyphae. Hyphae are tubular cell walls that surround the cytoplasmic membrane, and are often septate. Hyphae grow in tufts, know as mycelium (mold). On hyphal branches that reach up into the air asexual spores called conidia are formed. Conidia function in the dispersal of the fungus to new habitats. Some fungi produce reproductive structures called fruiting bodies.
- Fungal cell walls resemble plant cell walls in basic structure, but not in chemistry, and most fungi contain chitin (polymer of the glucose derivative N-acetylglucosamine). 80-90% of fungal cell walls are usually polysaccharides.
- Most plants live in symbiosis with fungi to facilitate uptake of minerals from soil.
- Most fungi reproduce asexually: by growth and spread of hyphal filaments, by asexual production of spores, and by simple cell division (budding yeasts).
- Some fungi produce spores as a result of sexual reproduction. The spores develop from the fusion of either unicellular gametes or specialized hyphae called *gametangia*. Alternatively,

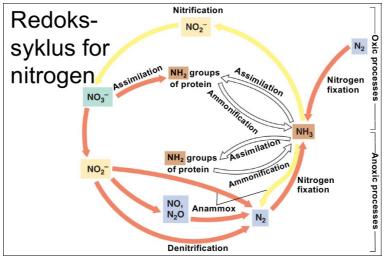
sexual spores can originate from the fusion of two haploid cells to yield a diploid cell, then undergoing meiosis and mitosis to yield individual haploid spores. Spores formed within an enclosed sac (ascus) are called *ascospores* (e.g. Saccharomyces cerevisiae).

- <u>Ascomycetes</u> (e.g. Saccharomyces cerevisiae, Candida albicans): Large and highly diverse group of fungi. Uses ascospores for sexual reproduction, or conidia from conidiophores for asexual reproduction. Important decomposers. *Saccharomyces* are single-celled, typically spherical, oval, or cylindrical. Cell division by budding (new cell forms as small outgrowth of the old cell).
- Some yeasts exhibit sexual reproduction by a process called *mating*, i which two yeast cells fuse. S. cerevisiae can exist in a haploid state, but when two haploid yeast cells fuse (mate) a diploid cell forms. Cells of opposite mating types a and a form a diploid cell. The MAT (mating type) locus can carry either gene a or a, which determines 'sex'. Elsewhere in the genome are both gene a and a, but these are not expressed. The silent copies can be inserted in the MAT locus. Both a and a are regulatory genes.



## Cycles

## The nitrogen cycle



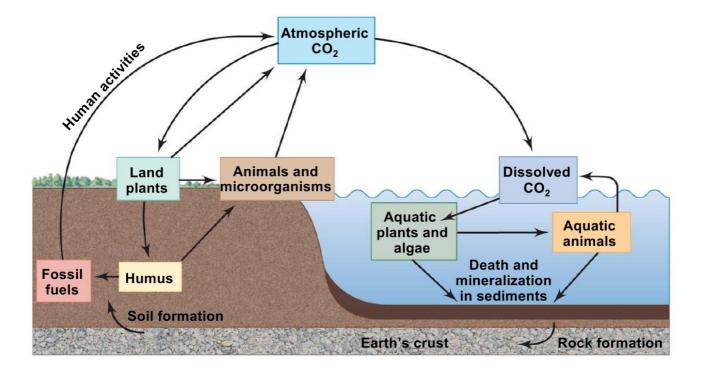
#### Bacteria

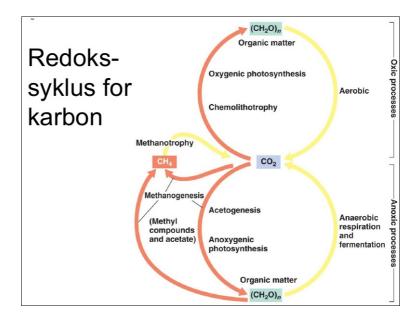
Nitrification:	Nitrosomonas + nitrobacter (easily isolated by growing in mineral salts media containing nitrite or ammonia as electron donor and bicarbonate as sole source of carbon)
Nitrogen fixation:	Azotobacter (aerobic)
	Clostridium (anaerobic)
Nitrate reduction:	E. coli
Denitrification:	Pseudomonas
Demumication.	
Anammox:	Brocadia

#### **Redox reactions**

Nitrification:  $NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O$ Anammox:  $NH_4^+ + NO_2^- \rightarrow N_2 + H_2O$ 

#### The carbon cycle



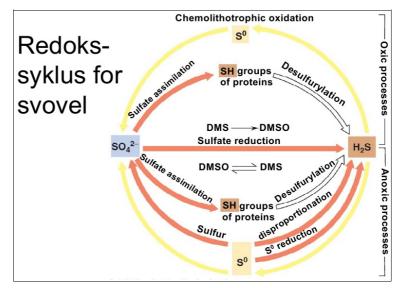


#### The sulfur cycle

Common oxidation states of sulfur in nature: Sulfhydryl and sulfide (-2), elemental sulfur (0) and sulfate  $SO_4^{2-}$  (+6).

Sulfide is produced from sulfate reduction:  $SO_4^{2-} + 8H^+ \rightarrow H_2S + 2$  $H_2O + 2$  OH<sup>-</sup>. Sulfate reducing bacteria are widespread in nature. Sulfide oxidizes spontaneously under oxic conditions at neutral pH, a process catalyzed by sulfur-oxidizing chemolithotrophic bacteria.

Elemental sulfur is chemically stable, but is readily oxidized by sulfur-



oxidizing chemolithotrophic bacteria such as *Thiobacillus* and *Acidithiobacillus*. Elemental sulfur can also be oxidized under anaerobic respiration, common among hyperthermophilic *Archea*.

#### Bacteria

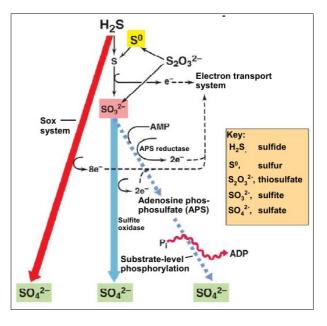
Bucceria	
Sulfide/sulfur oxidation:	Thiobacillus (aerobic)
	Purple and green phototrophic bacteria (anaerboic)
Sulfate reduction:	Desulfovibrio (anaerobic)
Sulfur reduction:	Many hyperthermophilic Archea (anaerobic)
Sulfur disproportionation:	Desulfovibrio (produces both hydrogen sulfide and sulfate from S <sup>0</sup>
Desulfurylation:	Many organisms

Colorless sulfur bacteria uses reduced sulfur compounds as electron donors (chemolithotrophy).

The most common sulfur compounds used as electron donors are hydrogen sulfide, elemental sulfur and thiosulfate  $(S_2O_3^{2-})$ . In most cases the final product of sulfur oxidation is sulfate.

Hydrogen sulfide oxidation occurs in stages, with the first oxidation step yielding elemental sulfur, which in some bacteria is stored intracellularly in deposits for use when external supply of sulfide is scarce. Sulfur oxidation produces protons, lowering the pH of the surroundings.

There are several different oxidation systems among sulfur chemolithotrophs. In two of the systems a common intermediate product is sulfite  $(SO_3^{2-})$ , which is oxidized to sulfate by two different systems: 1) Sulfite oxidase oxidizes sulfite to sulfate, directly transferring electrons from sulfite to cytochrome c, with subsequent



ATP production. 2) Sulfite is oxidizes to sulfate via a reversal of adenosine phosphosulfate (APS) reductase, converting one AMP to ADP.

Sox (e.g. Paracoccus pantotrophus) is functionally distinct, oxidizing sulfide to sulfate with a yield of 8 electrons.

**Sulfate-reducing bacteria** uses sulfate as an electron acceptor, reducing sulfate to hydrogen sulfide. Electron donors can be hydrogen gas, lactate, ethanol, acetate etc.

**Purple sulfur bacteria** (anaerobic photoautotrophic) utilize hydrogen sulfide as an electron donor for  $CO_2$  reduction in photosynthesis. Oxidized sulfide is stored in globules inside the cells, until it is oxidized to sulfate. Purple sulfur bacteria live in anoxic zones of lakes. Sulfide from sulfate reduction diffuses upward where purple sulfur bacteria reside, often together with green sulfur bacteria.

**Purple non-sulfur bacteria** (facultative aerobic photoheterotrophic) also uses hydrogen sulfide, but in lower concentrations.

**Green sulfur bacteria** also utilize hydrogen sulfide as an electron donor. The sulfur produced by green sulfur bacteria is stored extracellularly, in contrast with purple sulfur bacteria. Some organic compounds can be assimilated by light – photoheterotrophy. In addition to bacteriochlorophyll a the green sulfur bacteria has bacteriochlorophylls c, d or e located inside unique structures: chlorosomes. Chlorosomes are highly efficient, reducing the need for light. Green sulfur bacteria can live in a *consortium* with a chemoorganotrophic bacterium.

**Green non-sulfur bacteria** (Chloroflexus) also uses hydrogen sulfide. Grows by photoheterotrophy or photoautotrophy.

Together, the purple sulfur bacteria, purple non-sulfur bacteria, green sulfur bacteria and green non-sulfur bacteria, form the four groups of *anaerobic phototrophic bacteria*.