

## Summary - Bacterial Systematics

**Bacterial Phylogeny - Genotypic Characteristics** of Bacteria: Nucleic acids are universally distributed (RNA and chromosomal DNA) and they alone can be used as standards for wide-ranging comparisons.

**DNA-Analysis:** DNA is far more stable than RNA (RNAase rapidly decomposes RNA).

- **Base Composition (G-C Ratio):** Among prokaryotic DNA the ratio of the nucleotide bases adenine (A) plus thiamine (T) to guanine (G) and cytosine (C) varies within the range of 23-78 [mol%];

$\frac{[C+G]}{[A+T+G+C]} \cdot 100$  expressed in [mol%]; G+C ratios w/ differences >20-30% among species samples seem less closely related; determination of C-G ratio is required in order to establish new taxa.

Samples showing 10% - 20% differences should be assigned to the same genus, 5% is the maximum range permissible for same species. A >G+C ratio requires a > temperature to break the DNA-double helix into single strands; G+C is held by 3 H-bonds, whereas A+T has only 2 H-bonds (see genetics).

- **DNA-Fingerprinting:** Via **Restriction Fragment Length Polymerization** (requires little DNA), the genetic probe is broken by endonucleases into certain fragments. The number and locations of these units / cuts are unique for each genome (includes pattern / groups of these fragments of different size). The cleaved DNA-fragments are amplified via **Polymerase Chain Reaction**, purified, separated by gel-electrophoresis (larger units by **Pulse Field Gel Electrophoresis**), and compared with already existing patterns.

**ERIC-PCR:** selected primers are used to amplify specific DNA sequences.

**RAPD (Random Amplified Polymorphic DNA):** Randomly selected primers are used to specify for its existence, and if present, to amplify that particular sequence to be analyzed in gel-electrophoresis.

- **DNA Marker:** Certain radio-labeled markers do have complementary base sequences of the DNA-sample under investigation, therefore can bind at the proper complementary site of the strand.
- **DNA-Relatedness:** The more genetically closely related two organisms are, the more nucleotide base sequences they will have in common; such DNAs would be expected to hybridize to one another in proportion to the similarities of their sequence.

**Hybridization:** Artificial formation of a duplex nucleic acid molecule with strands derived from different sources by complementary base pairing. Radioactive labeling easily determines relatedness among related organisms (reannealing of parental strand and its related homologue).

**DNA:DNA H.:** Hybridization of entire DNA-contents of both organisms under investigation; reassociation of >70[mol%] of single DNA-strands of different organisms reveals a direct relationship at species level, whereas <70% is considered as scarcely related (detected by absorption spectra).

**DNA:rRNA H.:** Hybridization of DNA-content of organism A with rRNA of organism B (see also RNA-analysis).

**RNA-Analysis:** rRNAs are ancient molecules and represent only a small part ( $\approx 0.35\%$ ) of the genome, are functionally constant, universally distributed, and moderately well conserved across broad phylogenetic distances (molecular clock); similarities in rRNA sequences b/w two organisms indicates their evolutionary relatedness. This continuity implies that even distantly related species can have similar rRNA sequences, however, it should not be used as a single tool to determine relationships among species, rather in combination w/ e.g. DNA:DNA reassociation. Different RNA compounds are separated via gradient-centrifugation or electrophoresis; the S-rRNAs used in these analysis', are usually the small subunits (which, along with the larger unit form the ribosome complex needed for protein synthesis):

- **5S rRNA A.:** 120 nucleotide long molecule; although short, and limited in its information content, enabled the construction of phylogenetic trees and the recognition of major bacterial divisions.
- **16S rRNA A.:** 1500 nucleotide long molecule; sequencing of these molecules enhances resolution of phylogenetic traits because these are more manageable than 23S rRNA; similarities of >96% among 16S rRNA among species are considered to belong to the same species.
- **23S rRNA A.:** 2900 nucleotide long molecule;
- **DNA:RNA Hybridization:** Similar to DNA:DNA hybridization; single stranded DNA are used as templates for RNA synthesis; as single stranded RNA molecules do not pair with each other, rRNA relatedness is determined by hybridization with complementary (template strand) DNA. A radioactively labeled  $^{14}\text{C}$ -16S or 23S rRNA is used to hybridize with the DNA.
- **Oligo-Nucleotide Cataloging:** ONs (oligo-nucleotide) are short nucleic acid signature molecules unique to certain domains (Archaea, Bacteria, Eukarya); other signatures defining major taxa w/n each domain have been detected; these ONs are generally found in defined regions of the 16S rRNA.
- **Sequence Analysis:** Sequencing a DNA copy obtained from reverse transcriptase out of 16S rRNA. Can also be used to obtain the sense-strand of ATP-synthetase or chaperonin molecules as well.

**Bacterial Phylogeny - Phenotypic Characteristics** of Bacteria are rather unreliable, and therefore only limited in application, only experienced bacteriologists should consider them as valuable tools.

**Biochemical:** Include tests for specific enzymes and pathways for a particular bacterial group:

- Decomposition of simple carbohydrates: acid from glucose in an-/aerobic conditions (arabinose, fructose, lactose, xylose, etc.) ; gas from glucose;
- Metabolism of nitrogenous compounds: reduction of nitrate ( $\text{NO}_3^-$ ) to nitrite ( $\text{NO}_2^-$ ); detection of metabolic end- / byproducts including  $\text{H}_2\text{S}$ , HCN, keto acids, detection of urease, and decarboxylation of amino acids, etc.
- Decomposition of large molecules: like phospholipases (egg-yolk), lipases, proteases, amylase, cellulase, chitinase, DNase, etc.
- Terminal respiratory enzymes: catalase and cytochrome oxidase tests.
- Miscellaneous: coagulase, phosphatase, and haemolysis-tests.

**Cultural:** Colony based characters such as colony shape, margin, elevation, surface appearance, opacity, texture, pigmentation, odor, and appearance of growth.

**Inhibitory Agents:** Certain substances inhibit growth; e.g. selective media, antibiotics, dyes, toxins, etc.

**Morphological:** Cell based characters; this include cell shape, curvature, size and arrangement (single, chains, etc.), pleomorphism, formation of coccoid bodies, cysts, spores (shape, size, etc.), presence of flagella, capsules, metachromatic granules, staining reactions, etc.

**Motility:** Determination by phase-contrast microscopy on wet or gaseous preparations.

**Nutritional:** Bacteria show a great diversity in the spectrum as well as a very wide range of substances they can feed on, including carbohydrates, organic acids and amino acids; these can be tested as sole sources of carbon, energy and nitrogen.

**Physiological:** associated to life processes (biochemical), activities, and functions;

- acido- /alkalophile: Organisms favoring acidic conditions ( $\text{pH}<7$ ), or basic conditions ( $\text{pH}>7$ )
- an-/aerophil: Organisms which can/not survive without air; using solid (gelatinous) media and surrounded by a  $\text{CO}_2$ - or  $\text{O}_2$ -rich atmosphere, a gaseous gradient is will eventually develop to enable bacterial growth at which the preferred gas concentration is present.
- anorexo-/ throphophile: Organisms which feed on very little, or on a vast variety of sources.
- barophile: Organisms which require a high pressured environment to survive.
- halophile: Organisms favoring salty environment; growth at different salt-concentration in liquid media.
- meso-/thermophile: Organism favoring temperatures  $<90^\circ\text{C}$  /  $>90^\circ\text{C}$ ; max.- minimal temperatures permitting sustained growth; are reliable tests for in-liquid media incubations in water-baths; temperature tolerance is an easily determinable character.

**Bacterial Phylogenetic Trees:** Evolutionary distances ( $E_D$ ) needed to plot phylogenetic trees are obtained by using the Distance-Matrix-Method (DMM); two rRNA sequences are aligned and  $E_D$  is calculated by recording the number of positions in the sequence at which the two differ. The  $E_D$  separating any two organisms is directly proportional to the total length of branches separating them.

**Chemo-Taxonomic Classification:** Phenotypic analysis based on classification (identification and nomenclature) of bacterial cell wall constituents; Environmental conditions sometimes do interfere with bacterial metabolism; consequently, they may have altering effects upon its cellular constituents.

**Cell Wall:** Besides the classical plasma membrane bilayer, separating the cytoplasm from the environment, bacteria possess also a peptidoglycan and a LPS layer (see FA):

**Gram Test:** Test to verify the presence of an LPS-layer, possible in 3 different ways:

- (1) emerging strains into 3% KOH solution; causes coagulation of cytoplasmic DNA in G-neg. bacteria;
- (2) testing for amino-peptidase enzymes - do not occur in G-pos.;
- (3) using organic, cationic dyes to stain cells; (positively charged) compounds that combine with negatively charged (anionic) cellular constituents such as nucleic acid and acidic polysaccharides. G-pos. stain blue, G-neg. stain red providing an increased contrast for bright-field microscopy.

**G-Negative (G-neg.):** 3-unit cell wall; an internal cytoplasmic membrane, a relatively thin peptidoglycan (10%) layer, and a protein-dotted outer LPS layer (a thick complex lipid structure containing unusual sugars, i.e.: O-specific, and core-specific polysaccharides) and glucosamine-lipids).

**G-Positive (G-pos.):** A prokaryotic cell whose 2-unit cell wall consists chiefly of peptidoglycan (90%) and the internal cytoplasmic membrane (lack the outer LPS-membrane of Gram-negative cells).

#### Cell Wall Constituents:

- **Fatty Acids (FA):** The non-hydroxylated fatty acids (FA) with chain lengths of up to 20 C-atoms, found in the plasma- and lipo-polysaccharide membrane (LPS); these FAs can be grouped as:

**Branched FA:** anteiso, asymmetrically at different sites branched FA; iso, symmetrically branched FA; methy, H swapped w/ CH<sub>3</sub> group.

**Cyclopropane FA:** FA-chain is interrupted by a ring of C-atoms (C<sub>3</sub>H<sub>8</sub>).

**Hydroxy FA:** H swapped w/ OH group of some FA-C-atoms; e.g.: 2-OH, 3-OH; in Archaea periodically branched CH<sub>3</sub> side chains.

**Length of FA:** typical are lengths between 24-90 C-atoms;

**Saturated FA:** No double bond b/w any C of the FA-chain; all C-atoms are saturated w/ H-atoms;

**Poly-Unsaturated FA:** Have one or more positions along the FA-polymer chain where 2 adjacent C are linked by double bond (2 shared pairs of electrons); consequently, fewer Hs are bonded to the Cs.

FA - shorthand notation	sample
number of C-atoms : e.g. 16	16 :
number of double bonds (indicating saturated FA tail), e.g. at 1 <sup>st</sup> C	1
position of double bond ( $\omega$ , from the HC end) of the <i>cis</i> or <i>trans</i> isomer,	$\omega$ 9t
position of side-chain ( <i>cis</i> on the O-side of the CO head, <i>trans</i> opposite),	cis / trans
symmetrical side-chain <i>iso</i> , or unsymmetrical side-chains <i>anteiso</i> ,	iso / anteiso
position of C-ring – <i>cyclo</i> (from CO-head of FA tail) e.g. at positions 9-10	cyclo 9-10
position of <b>OH</b> side-chain (from CO-head of FA tail) e.g. at pos 2(3)	-2(3)OH

- **Lipids:** Water-soluble (polar) organic molecules that are important for the structure of the cytoplasmic membrane and the cell wall. Lipids are a useful non-genetic criteria to differentiate Archaea from Bacteria. Polar L.s are essential components of the plasma membrane and play an important role in its regulation and permeability (displayable by 2D-TLC chromatography). Some representative phosphoglycerides are:

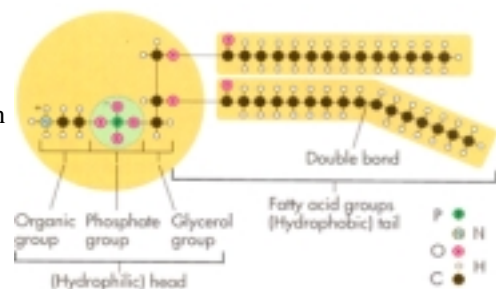
**Phospholipid (PL or P<sub>I</sub>):** Lipids containing a substituted P-group and 2 fatty acid chains on a glycerol backbone (see scan). Archaea and Bacteria are distinguished by the bond b/w the FA tail and the glycerol head (*ether*-bond in former, *ester*-bond in later domain)

**Phosphatyl-glycerol (PG or P<sub>V</sub>):** PL with an attached glycerol molecule CHOH(CH<sub>2</sub>OH)<sub>2</sub> to the P-group;

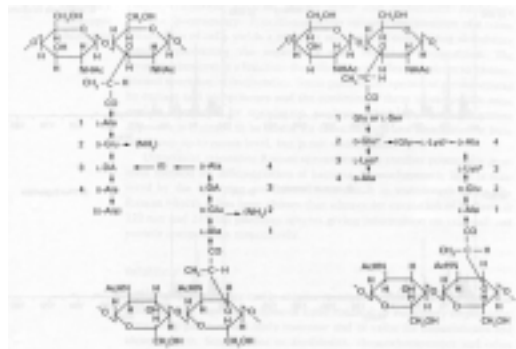
**Di-phosphatyl-glycerol (DPG or P<sub>IV</sub>):** =cardiolipin: two PLs bound together by a glycerol molecule CHOH(CH<sub>2</sub>OH)<sub>2</sub>

**Phosphatyl-ethanolamine (PE or P<sub>II</sub>):** PL w/ an extra ethanolamine molec. OH(CH<sub>2</sub>)<sub>2</sub>NH<sub>3</sub><sup>+</sup> to the P-group;

**Phosphatyl-choline (PC or P<sub>III</sub>):** PL with an attached choline molecule OH(CH<sub>2</sub>)<sub>2</sub>N(CH<sub>3</sub>)<sub>3</sub> to the P-group;



- Peptidoglycan** (= murein): A rigid permeability barrier, (cell wall-polymer) predominant in G-pos.-bacteria. Composed of N-acetyl-glucosamine, N-acetyl-muramic acid, and a few amino-acids (L-alanine, D-glutamate, L-diamino acid, D-alanine). In G-neg. bacteria, this layer is poorly developed; therefore, unsuitable to classify G-neg. bacteria. Archaea and Eukarya lack peptidoglycan completely. To stiffen the peptidoglycan layer, cross-links are formed between glycan chains of amino acids, characterized by 2 groups:



Shorthand notation	inter-peptide bridge type:	variation at position 3 (subclass):			
<b>Group A</b> peptidoglycan: Cross-links b/w position 3 of 1 <sup>st</sup> 4 of the 2 <sup>nd</sup> amino acids (AA) side-chain.	1, direct cross-linkage	$\alpha$ L-Lys	$\beta$ L-Orn	$\gamma$ m-Dpm	-
	2, polymerized peptide units	-	-	-	-
	3, monocarboxylic L-AA or gly or both	$\alpha$ L-Lys	$\beta$ L-Orn	$\gamma$ m-Dpm	-
<b>Group B</b> peptidoglycan: Cross-links b/w pos. 2 of 1 <sup>st</sup> and pos. 4 of the 2 <sup>nd</sup> AA	4, di-carboxylic-amino acid	$\alpha$ L-Lys	$\beta$ L-Orn	$\gamma$ L-Dpm	-
	1, L-di-amino acid	$\alpha$ L-Lys	$\beta$ L-Hsr	$\gamma$ L-Glu	$\delta$ L-Ala
	2, D-di-amino acid	$\alpha$ L-Orn	$\beta$ L-Hsr	$\gamma$ L-Dab	-

AA-Abbr.: **Alanine**, **Diamino-butric acid**, **Diamino pimelic acid**, **Glycine**, **H<sup>+</sup>s<sup>+</sup>r<sup>+</sup>?**, **Lysine**, **Ornithine**;

- Polyamine**: Polycationic compounds found in all bacterial cells that are primarily synthesized from amino acids and contain 2 or more amino groups. Although their function is not clear, they seem to play a role in DNA replication, tRNA stability, rRNA structure, and protein biosynthesis. Polyamid content in a living cell depends on the physiologic activity of the organism, i.e. growing cultures show distinct distributions of polyamines (**cadaverin** rises while **putrescine** falls; easily determinable by turbidity measurements in photo-spectrometry, and can be analyzed by **HPLC** chromatography).

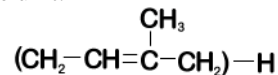
Common name (protonized)	Polyamines - stoichiometric notation	C-ratio
<b>1.3-Diamino-propan</b> DAP	$H_3N - (CH_2)_3 - NH_3^+$	$C_3$
<b>2-Hydroxy-putrescin</b> HPUT	$H_3N - CH_2 - CHO - (CH_2)_2 - NH_3^+$	$C:C_2$
<b>Cadaverin</b> CAD	$H_3N - (CH_2)_5 - NH_3^+$	$C_5$
<b>Putrescin</b> PUT	$H_3N - (CH_2)_4 - NH_3^+$	$C_4$
<b>Spermidine</b> SPD	$^+H_3N - (CH_2)_3 - NH - (CH_2)_4 - NH_3^+$	$C_3:C_4$
<b>Homo-spermidine</b> HSPD	$^+H_3N - (CH_2)_3 - NH - (CH_2)_3 - NH_3^+$	$C_3:C_3$
<b>Nor-spermidine</b> NSPD	$^+H_3N - (CH_2)_4 - NH - (CH_2)_4 - NH_3^+$	$C_4:C_4$
<b>Spermine</b> SPM (tetra-amine)	$^+H_3N - (CH_2)_3 - NH - (CH_2)_4 - NH - (CH_2)_3 - NH_3^+$	$C_3:C_4:C_3$

- Proteins**: Genetically coded cellular proteins represent a large part of the bacterial genome; closely related bacteria have similar protein contents. High resolution techniques (such as **2D-Poly Acrylic Gel Electrophoresis**) provide a perfect tool but only a few strains at a time can be analyzed.
- Quinones**: Respiratory coenzymes of the plasma membrane in bacteria are of essential importance for the electron transport chain and oxidative phosphorylation (ATP-generation as a result of the transfer of electrons from NADH or FADH<sub>2</sub> to O<sub>2</sub> by a series of electron carriers).

Quinone - shorthand notation	sample
type of quinone involved	Mk-
number of isoprenoid side-chains found in this particular quinone; e.g. n = 7	7
number of (C=C) present; each double bond releases 2H <sup>+</sup> ; even bond suffices only	(H <sub>2</sub> , 4, 6, etc.)



Isoprene unit:



The length (n) of these units vary between taxa. Therefore, a useful tool to group organisms according to their isoprene characteristics.

Certain quinones are unique to certain genera

**Standard Techniques** applied in the study of bacterial classification:

**Blot Analysis:** In honor of E.M. Southern who developed a technique to transfer genetic material.

Widely used for detecting the presence of specific macromolecules (proteins, mRNAs, or DNA sequences) in a mixture. A sample is first separated on an agarose or polyacrylamide gel usually under denaturing conditions; the separated components are transferred (blotting) to a nitrocellulose sheet, which is exposed to a radiolabeled molecule that specifically binds to the macromolecule of interest, and then subjected to autoradiography.

- **Northern B.:** Transfer of electrophoretically separated fragments of RNA from the gel to an absorbent sheet, which is then immersed in a labeled probe (DNA or RNA) that will bind to the RNA of interest.
- **Southern B.:** Transfer of electrophoretically separated fragments of DNA from the gel to an absorbent sheet (paper). This sheet is then immersed in a solution containing a labeled probe (RNA or DNA).
- **Western B.:** Involves a protein-antibody binding rather than nucleic acids;

**Chromatography:** A sensitive technique for separating and identifying components of a mixture; it relies on the different abilities of substances to stick to surfaces (stationary phase) while the carrier medium (mobile phase) pushes the components along the sticky surface;

- **GLC (Gas Liquid C.):** Vaporized sample is carried in a stream of He-gas through a narrow tube. The tube is coated or packed with alumina ( $Al_2O_3$ ) soaked in a liquid that does not vaporize readily. Some molecules of the sample are adsorbed on (stick to) the coating (the stationary phase) more rapidly than others and therefore emerge later, but eventually all pass through in the stream of carrier gas (typically He); less readily adsorbed component emerge first, followed by the more readily adsorbed.
- **HPLC (High Performance Liquid Chromatography):**
- **PC (Paper C.):** The components of a mixture are separated by washing them along a paper (stationary phase) with a solvent (mobile phase). As the solvent spreads out from the wet areas to the edges of the paper, it carries the components along. Heavier components, which stick more easily to the stationary phase will migrate slower towards the edges of the paper than the lighter ones.
- **TLC (Thin Layer C.):** A silicagel-coated glassplate (stationary phase) placed in a standing position of a sealable container slightly dipped into the mixture (mobile phase) containing the dissolved components. According to the adherence of the components, certain elements will migrate faster upwards along the plate as others.

**2D-TLC:** A probe is placed as a single dot of origin onto the plate;

separation of the components results in two steps: a one dimensional separation, followed by a second in which the stationary phase is turned by  $90^\circ$ .

**Fractionation:** The different sedimentation rates of various cellular components make it possible to separate them partially by centrifugation. Each soluble fraction can be further separated by density-gradient centrifugation, i.e. gradually increasing rotation to as much as  $300 E^3 g$ .

**Electrophoresis:** The procedure by which charged molecules are allowed to migrate along an electric field; the rate of migration being determined by the size, shape of the molecule, its molecular weight, their electric charge, and the pore size of the carrying medium.

- **Gel E.:** Carrying medium is a gel-like substance, usually poly-acrylamide or agarose which represent the complex network of fibrils; pore size is controlled by the way in which the gel is prepared.
- **PAGE (Poly-Acrylamide Gel Electrophoresis):**
- **PFGE (Pulse Field Gel Electrophoresis):** Larger molecules cannot be separated by simple gel-electrophoresis ( $>40E^3$  bp); involves sending short pulses of electricity (DC) to an array of electrodes surrounding the agarose gel ( $120^\circ$  apart in the plane in an alternating sequence). This procedure yields a "2D"-plot of the sample analyzed.

**PCR (Polymerase Chain Reaction):** A method used to amplify a specific DNA sequence *in vitro* by repeated cycles of synthesis using specific primers and DNA polymerase.

- **ERIC-PCR:** selected primers are used to amplify specific DNA sequences.

**Photo-Spectrometry:** A photocell measures incident light unscattered by cells in suspension and gives readings in optical density or photometer units; modern units split the beam of the light-source to obtain a reference and a sample beam, and allow spectrometric analysis as well (scanning from the IR-, through the visible- into the UV-spectrum).

**RFLP or REA (Restriction Fragment Length Polymerization or Restriction Endonuclease Analysis):** Simple and rapid tool to roughly sequence DNA. Added endonuclease cleaves DNA at particular sites; fragments amplified by PCR, purified, separated by gel-electrophoresis (larger units by PFGE), and analyzed.

- **RAPD (Random Amplified Polymorphic DNA):** Randomly selected primers are used to specify for its existence, and if present, to amplify that particular sequence to be analyzed in gel-electrophoresis.

**Taxa:** Determination of common properties of organisms enabling the gathering of various groups into taxa. A vastness of microbiological strains awaits classification (e.g. any intestinal strain of insects). According to 16S rRNA sequence comparison, the Domain **Bacteria** is grouped into:

Kingdom **Acidobacteria:**

Kingdom **Actinobacteria:**

Suborder **Propionibacterineae** - Family **Propionibacteriaceae** - Genus **Propionibacteria** with 2 species:

*P. propionis* (type A) in Swiss cheese, responsible for the taste and the bubbles of the *Emmentaler*;

*P. coryneforma* (type B) predominantly in skinfolds, responsible for the typical smell (axila, pimples);

Genus *Propioniferax*: anteiso- 15:0, 17:0 and iso- 15:0, 16:0 as a characteristic FA in their membrane lipids.

Kingdom **Aquificales** - Genus *Aquifex pyrophilus*: Marine, hyper-thermophilic (67-95°C), chemolithotrophic bacteria that oxidizes H<sub>2</sub>. Motile rod-shaped cells w/ peritrichous flagella, Gram-neg., G+C=41.5[mol%]

Kingdom **Rickettsias and Chlamydias** - Order **Chlamydiales** - Family **Chlamydiaceae:**

Small distinct division that just contains 4 species; all of them have an inner and outer LPS-membrane but lack a peptidoglycan layer; some Chlamydiaceae are thought of being responsible for artery-vascular diseases, even heart-attacks; the close relatives of

*Chlamydia psittaci*, pathogenic in animals e.g. in parrots) and

*C. trachomatis* in 3 biovars: (1) infections of genital tract, conjunctivitis; (2) *urethritis* and other sexually related diseases; (3) infections of the respiratory tract; and finally the more distantly related

*C. pneumoniae* (infections of the respiratory tract) and *C. pecorum*;

Genus **Aquificales:**

Kingdom **Copro-Thermobacter:**

Kingdom **Cyanobacteria:**

Kingdom **Gram-positive Cocci** - Order **Deinococcales** - Family **Deinococcaceae:** Gram-positive, although resemble Gram-negative in their micro structure; spherical cells occurring in clusters of pairs or tetrads, others are paired rods; non-motile; pink to deep red in color; aerob, mesophil, chemo-organotrophic, respirative metabolism, withstand  $\gamma$ -, and UV-rays (multiple copies of their genome); and desiccation. Mk-8; cell wall of L-ornithin-Gly-2-3 peptidoglycan; G+C=60-70[mol%];

Genus **Meiothermus:** Gram-negative, all with iso-2-OH-FA as their principle polar building unit as well as some anteiso-branched FA in their membrane lipids; Mk-8; ornithine as another characteristic peptidoglycan; rods in various lengths with short filaments; colony-color reddish to yellow; mesophilic (50-65°C) at slightly alkaline conditions (pH=8); G+C=59-70[mol%]; non-motile;

Genus **Thermus:** Gram-negative with a glyco-, and a phospholipid as a dominant pattern in their polar membrane lipids with mainly iso- and anteiso-branched FA; some have 3-OH-FA whereas 2-OH-FA are completely absent; Mk-8; ornithine as a characteristic peptidoglycan; rod-shaped in various lengths with short filaments; colorless to yellowish pigmented; optimal growth at mesophilic (65-75°C), and slightly alkaline conditions (pH=7.8); G+C=60-65[mol%]; non-motile;

Kingdom **Dictyoglomus Group:**

Kingdom **Fibrobacter:**

Kingdom **Flexibacter:**

Kingdom **Fusobacteria:**

Kingdom High **G+C-Gram-positive:**

Kingdom Low **G+C-Gram-positive:**

Kingdom **Green-S Bacteria:**

Kingdom **Green-non-S Bacteria:**

Kingdom **Nitrospira:**

Kingdom **Flexibacter:**

Kingdom **Planctomycetales:**

Kingdom **Spirochetes** - Order **Spirochaetales** splits into 2 families Leptospiraceae and Spirochaetaceae: Have distinct peri-plasmatic flagella, located b/w inner and outer membrane (lack LPS).

Family **Leptospiraceae:** with the following genera: *Serpulina*, *Leptonema*, *Leptospira*;

Family **Spirochaetaceae** with the following genera: *Spirochaeta* (anaerobic to facultative anaerobic bacteria; free living in waterbodies with a swampy bottom; G+C = 50-60[mol%], 10-20 $\mu$ m long, *S. plicatilis* up to 0.25mm),

*Borrelia* (arthropod-mediated pathogens, *B. burgdorferi* causes *M. encephalitis*),

*Rhizobia* as micro-aerophilic N-fixing bacteria; microaerophil, cell wall of ornithin and muramic acid peptidoglycan; G+C = 27-32[mol%], 8-30 $\mu$ m in length),

*Treponema* (some pathogenic forms, anaerob-microaerophil, G+C=25-54[mol%], 5-20µm in length, e.g. syphilis causing bacteria: *T.pallidum*);

Kingdom **Verruco-microbiales:**

Kingdom **Thermodesulfo-Bacterium Group:**

Kingdom **Thermotogales:**