Archaea – Exploration of Thermophilic Adaptations

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- Historically, living organisms were categorized into five kingdoms
  - Plantae, Animalia, Fungi, Protista and Monera (Eubacteria)
- 1977 – Carl Woese identifies third form of life
  - The Archaea (prokaryotic)
  - Primary evidence lies in rRNA sequence patterns
  - Resulted in elimination of Monera Kingdom

- Archaea are generally categorized into three functional groups
  - Methanogens
    - Unique form of carbon fixation (reduction) utilizing CO or CO_2 to produce methane
  - Halophiles
  - Thermophiles
- May be extremely misleading

- They are fairly ubiquitous
  - Mesophilic inhabitants of soil and water
    - Form biofilms with bacterial species
  - Symbionts in the digestive tract of animals
    - Comprise 4% of human skin microbiota
    - None have been identified as pathogens
  - Also include pschrophiles, barophiles and acidophiles

- Hyperthermophiles are commonly associated with hot springs, geysers and volcanic vents (including hydrothermal vents)
  - Unique features of these environments:
    - Reduced available minerals due to precipitation
    - Low oxygen availability (most are anaerobes)
    - Steep temperature gradients
    - Extreme acidity
    - Extreme pressure

- Hyperthermophiles are adapted to grow optimally in temperatures ranging from 80^0C-106^0C
  - Highest recorded critical temperature for an Archaeal species is 121^0C
- The most extreme hyperthermophiles are also barophiles and/or acidophiles associated with hydrothermal vents
  - Steam (400^0C) meets sea water and rapidly cools
    - Results in precipitation of minerals (Black Smokers)
    - High pressure allows water to remain liquid at extremely high temperatures

Distinctly Unique Features of Archaea

- Isoprenoid membranes
  - Glycerol isomer with ether linkage
  - Branched isopronoid hydrocarbon chains
    - May cyclize to form ring structures or cross-link to form rigid tetraether monolayers
  - Thermal stability and reduced H+ permeability
    - H+ permeability is vital to bioenergetics
    - Reduced membrane permeability reduces energy stress

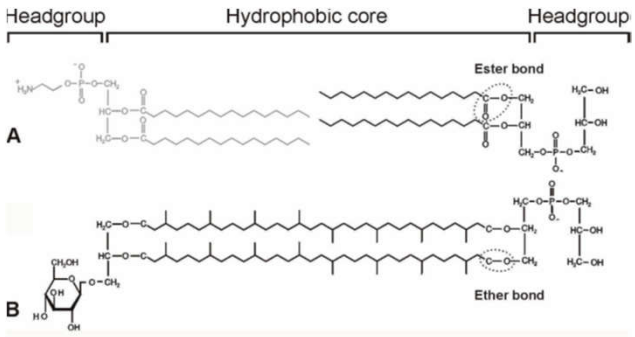


Figure 1. Lipids from archaea and bacteria. A: bilayer forming lipids in bacteria: Phosphatidylglycerol (PG) from *Escherichia coli*. The acyl chain is straight (not in all cases: some Bacterial lipids have a methyl branch, or a cyclohexyl group, at the end of the acyl chain, other lipids have one or more unsaturated bonds). The connection of the acyl chain with the headgroup is an ester. B: Monolayer forming lipids in thermoacidophilic archaea: Main glycophospholipid (MPL) of *Thermoplasma acidophilum*. The phytanyl chain contains isoprenoid-like branches. The connection of the phytanyl chain with the headgroup is an ether linkage. Archaeal membranes also contain bilayer forming diether lipids. Some acidophilic tetraethers contain cyclopentane lipids.

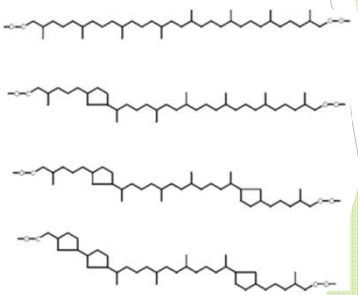
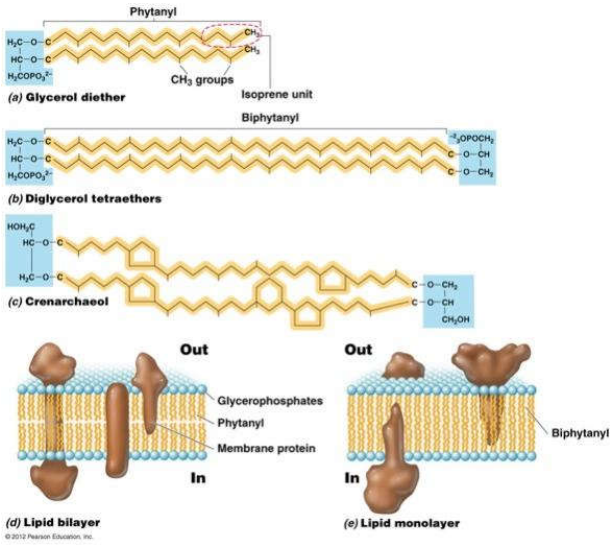


Figure 2. Cyclization of the phytanyl chains of the *S. solfataricus* tetraether lipids. Only one of phytanyl chains is shown. The degree of cyclization increases from top to bottom.

Albers, Van de Vossenberg, Driessen, and Konings, 2000



- Cell Wall or Envelope Structure
  - Many archaea lack a cell wall (atypical of prokaryotes)
  - If cell wall is present, distinctly different composition than bacteria
    - Resistant to compounds that degrade bacterial cell walls
  - May have an envelope created by fusion of S-layer of glocoproteins to cell wall or membrane
    - Highly protective to environmental stresses but very flexible

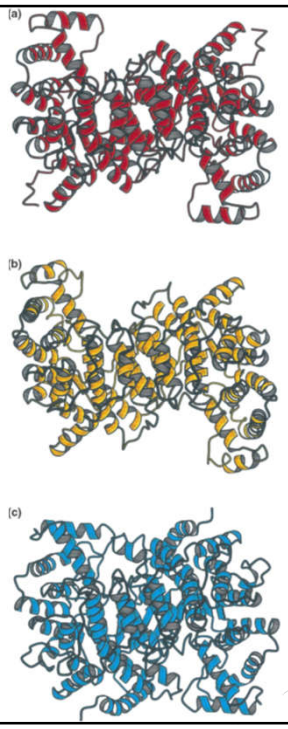
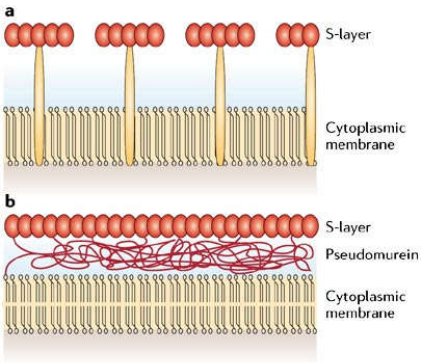
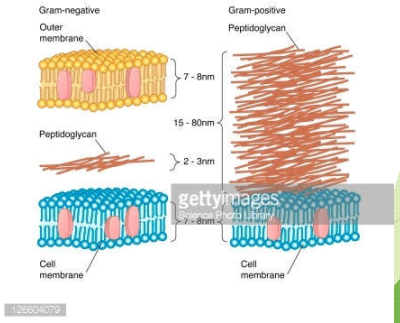


Fig. 2. Citrate synthase atomic structures. Schematic representation of the dimeric citrate synthases from (a) Pyrococcus furiosus, (b) Thermoplasma acidophilum and (c) pig. Each view is down the twofold axis

Danson and Hough, 1998



Archaea



Bacteria

Sulfolobus (Crenarchaota)

- Requires both extreme heat (80-90^{0}C) and extreme acidity (pH of 2-3)
- Tetraether mono-layer membrane with cyclic isopronid hydrocarbon chains
- Extensive S-layer protein envelope
- Autotrophic species oxidize H_2S
- Heterotrophic species use ferrodxin as an electron carrier
  - More stable at high temperatures than NAD

Pyrodictium (Crenarchaota)

- Anaerobes reducing precipitating sulfur to form H_2S
- Tetraether monolayer membrane
- S-layer protein envelope coated with zinc sulfides precipitating at the vent
- Produce a heat shock ATPase protein

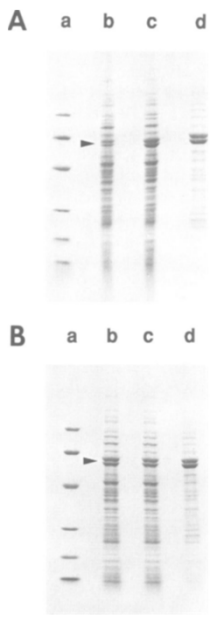


Fig. 9. Selective accumulation of *Pyrodictium occultum* ATPase complex in response to heat shock.

A) Steady state growth: SDS-PAGE of membrane-free lysates of *P. occultum* cells grown to exponential phase at different temperatures. Lane b, 90°C. Lane c, 100°C. Lane d,108°C.

(B) Heat shock: SDS-PAGE of membrane-free lysates of P. occultum cells. Lane b, cells grown to exponential phase at 102°C. Lane c, cells kept at 102°C and incubated for 4 h. Lane d, cells shifted to 108°C and incubated for 4 h.

Phipps, Hoffmann, Stetter and Baumeister, 1991

Thermococcus and *Pyrococcus* (Euryarchaeota)

- Found around submarine volcanic vents
- Temperatures are above 90^{0}C (up to 105^{0}C)
- "Vent Polymerases" are replacing Taq Polymerase for PCR
  - Limited stability above 95^{0}C

- Unique Genetic Composition
  - Size and gene number similar to bacteria, under operon control
  - Polymerase structure and action similar to eyukarya
    - Have introns (interruptive sequences) and histones (DNA packaging proteins)
  - 2/3 of genes in archaea have no homologue in bacteria or eukarya
  - Reverse gyrase enzymes resulting in positive supercoiling of DNA
    - Only present in hyperthermophiles
    - Prevents unnecessary unwinding and separation of DNA strands at high temperatures

Table 1. Distribution of reverse gyrase in completely sequenced genomes from Archaea and hyperthermophilic Bacteria

	Optimal growth temperature (°C)	Presence of genes encoding reverse gyrase
Pyrobaculum aerophilum	100	Yes (2)
Pyrococcus furiosus	100	Yes
Pyrococcus horikoshii	98	Yes
Methanopyrus kandleri	98	Yes
Pyrococcus abyssi	98	Yes
Aeropyrum pernix	90-95	Yes (2)
Aquifex aeolicus (B)	85	Yes (2)
Methanocaldococcus jannashii	85	Yes
Archaeoglobus fulgidus	85	Yes
Sulfolobus tokodaii	85	Yes (2)
Sulfolobus solfataricus	85	Yes (2)
Thermotoga maritima (B)	80	Yes
Methanothermobacter thermoautotrophicus	60-65	No
Thermoplasma volcanium	55-60	No
Thermoplasma acidophilum	55-60	No
Ferroplasma acidarmanus	55-60	No
Halobacterium NRC1	50	No
All mesophiles (Archaea; Bacteria, Eukarya)	Under 50	No

Bacteria are denoted by (B).
Two reverse gyrase genes are present in species marked (2).

Forterre, 2002

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