

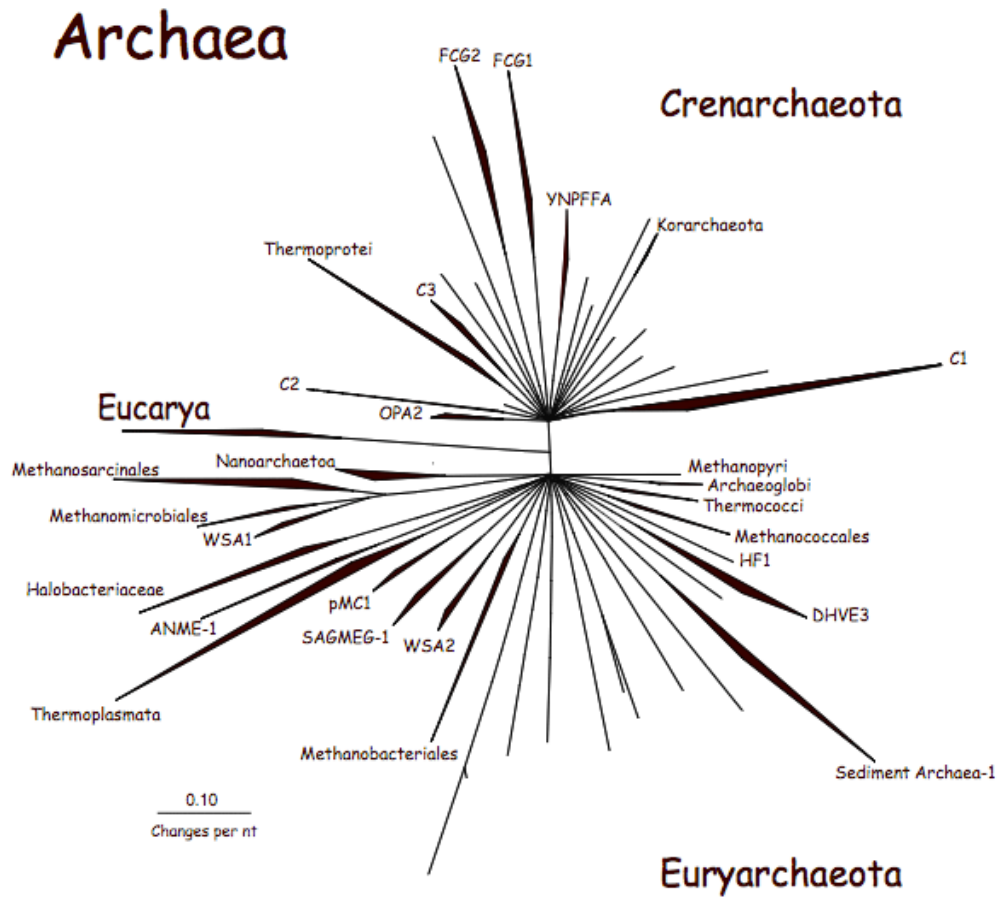
Lecture 10: a Bit about Archaea

Text: Chapter 17, pp. 487-513 (Ch. 13 in 11th.)

Garrett et al., "Archaea: Evolution, Physiology and Molecular Biology".
Blackwell, 2007.

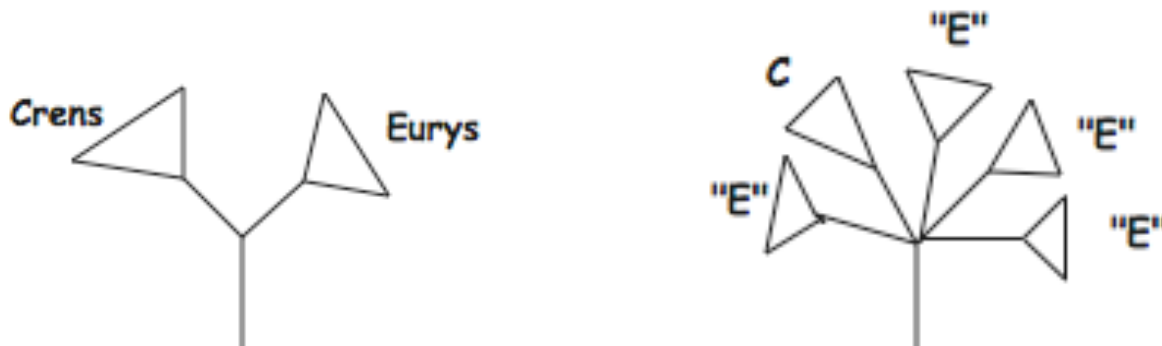
Cavicchioli, "Archaea: Molecular and Cellular Biology," ASM Press, 2007

1. Archaea were recognized as a novel line of descent in 1977 by Carl Woese, using rRNA sequence comparisons. This also was the first view of the Big Tree. The phylogenetic clarity explained the "oddness" of some disparate organisms, e.g. *Sulfolobus* spp. (high temp, acid), methanogens (e.g. Methano"bacterium" spp., extreme anaerobes), and extreme halophiles (e.g. *Halobacterium*, now *Haloferax* spp.).
 - A. Although bacteria and archaea (mostly not eucs) share the lifestyle of very small size, their biochemistry, particularly nucleic acid metabolism, often more resembles eucaryotes. Although the popular press beats on the "extremophilic" quality of archaea, they are everywhere.
2. Archaea (note text pictures)
 - A. Recall the archaeal tree: This is the conventional picture, two main clades, Crenarchaeota and Euryarchaeota.



B. **But**, phylogenetic results with environmental sequences are breaking down the monophyly of Euryarchaeota: Crenarchaeota remains “coherent” but Eurys become “polyphyletic”.

Vs.



Which large-scale topology is correct? What to do to resolve?

Some Factoids on Archaea, mostly from limited culture results:

3. Euryarchaeota ("group with diverse properties")- methanogens
halophiles, extremely thermophilic heterotrophs (e.g. *Thermococcus*,
Pyrococcus spp.)

A. Methanogens:

1. Hard-core anaerobes, so not discovered until fairly late
(1950s). In a review article in the mid-70's only 13 species
(cultures) were known!

2. Metabolically relatively uniform: chemolithotrophic (H₂/CO₂
or H₂/acetate, making methane).

3. Typically simple genomes: 1500-3000 genes (1738 ORFs in
Methanococcus jannaschii)

4. Commonly occur as symbionts, sucking up to H₂:

a. with bacteria (e.g. *Syntrophus*).

b. with anaerobic protists (e.g. *Pelomyxa*). **Why does
this happen – what are the methanogens after?**

c. with animals (e.g. cows. We have but probably don't need).

How do these symbioses differ? What is the commonality?

B. Halophilic Archaea:

1. Usually thought-of in the context of brines: Dead Sea (high mono and divalent cations); Salted fish - was an early source for *Halobacterium* spp.; floor of Red Sea??
 2. But note - many microenvironments may also be high salt - e.g. drying soil surfaces; e.g. a drying armpit or a used sneaker.
 3. Note common occurrence in environment; e.g. evaporation ponds in south S.F. Bay, flying over Great Salt Lake.
- Invented (maybe - no longer clear) (bacterio)rhodopsin; serves to generate membrane potential using light, but supposedly can't make enough ATP to grow by photosynthesis. Most cultivated archaeal extreme halophiles are considered chemoheterotrophs. Some can do methanogenesis.
- Homologous "rhodopsin" genes recently have been seen in proteobacterial genomic fragments from marine metagenomic libraries! Now shown to occur widely, even in "common" bacteria such as *Vibrio comma*, a common water-borne organism.

4. Intracellular salt concentrations typically high even in methanogens that do not live in brines. Note that much subsurface "geological" water occurs as brines; European ocean is probably a brine. (Soluble salts are leached out of rock matrix by groundwater circulation.)

6. Halophiles seem to be derived from a particular lineage of methanogens (the *Methanosarcina* / *Methanospirillum* group). There is a rudimentary genetic system in some *Methanosarcina* spp.

Some names:

Methanococcus, *Methanopyrus* - methanogens (eury)

Thermococcus, *Pyrococcus* - non-methanogenic Euryarchaeota, anaerobic heterotrophs with sulfur, most commonly cultivated organisms at 90°-100°C (and really smell-up the lab!)

Archaeoglobis - H₂-driven sulfate-reducers (relationship between sulfate and CO₂ metabolism (to CH₄)??)

4. Crenarchaeota

A. All **cultivated** instances (except now one) are hydrogen/sulfur-metabolizing and thermophilic. However, molecular analyses show that **uncultivated** low-temp. crenarchaeota are abundant in all/most [?] environments. Indeed, most of crenarchaeal diversity is uncultured types, detected only by rRNA sequences.

B. Based on cultivars (only a few dozen, all save one high-temp.), metabolic diversity in the Crenarchaeota group is pretty limited: H_2/S^0 is common (e.g. *Pyrodictium*, *Pyrobaculum*, probably *Sulfolobus*). Some will also oxidize organics, using about anything as electron acceptor. *Sulfolobus* will even breath molybdate!

1. *Sulfolobus* has a developing genetic system. Numerous phages have been identified, some capable of integration. Note there may be a technical difficulty of frequent (!) gene rearrangements in the genome.

C. *Pyrodictium*, *Pyrolobus* - growth at the highest temps in culture, 110-120°C. Metabolism by H_2/S^0 , growth on surfaces of sulfur globs in a mineral salts mixture under a few At. of H_2/CO_2 . Hard country to live-in!

D. A pretty inconspicuously boring bunch based on cultivars -- What are all those Crenarchaeota out in the soils and sediments and in the open ocean-column that have been detected only by sequence doing?

E. A sponge sponge symbiont:

1. The first "tractable" experimental system for a low-temp crenarchaeon was "*Cenarchaeum symbiosum*", which lives in the interspicule space of the marine sponge *Axinella*. Sponges maintained in the lab for a few years still have the cren, and dividing cells can be visualized microscopically, so they must be dividing. What are they making a living on??

2. The genome of *C. symbiosum* is just finished using the "chromosome walking" approach. Among other things so far encountered is a DNA polymerase gene that, upon expression in *E. coli*, turned out to have a T_{opt} of about 20°C - mesophilic. This was the first solid evidence, beyond occurrence in low temperature environments, that crenarchaeotes can be other than thermophilic.

3. Korarchaeota - uncultivated, so far detected only in anoxic high-temp. settings around the world. With rRNA-based probes, morphotypes are boring, very thin rods forming filaments (one of a few most-common of life's favorite morphologies). Mentioned in text as third "kingdom" of archaea, but new results say that they are solidly Crenarchaeote.

4. The so-called, deeply branching, slowly evolving Archaea are often touted as the modern organisms that are "most closely related" (shortest line segments in trees) to the last common ancestor of all life, in terms of rRNA sequence-divergence – does this mean that they have primitive character (phenotype)?

a. Note that in early (pre-now) trees, low-temp lines spin-off shorter, high-temp lines in trees. It is argued by some that the short lines (high-temp organisms) are the ancestral type. It is possible, however, that the longer lines of mesophiles reflect more "sequence space" in rRNA available to those organisms.

5. Are the low-temp Crens simply volcanic exudate spit-out of the Mid-Ocean Ridge? No, e.g. the occurrence of sponge symbionts. Moreover,

genes cloned from naturally occurring cren genes (in “metagenomic” gene libraries) express mesophilic enzymes.

6. The greatest Cren diversity (rRNA-wise) at this time is represented by environmental sequences. E.g. in the ocean: Karner et al. Nature 409, 507-510, 2001. Using fluorescent (“FISH”) probes:

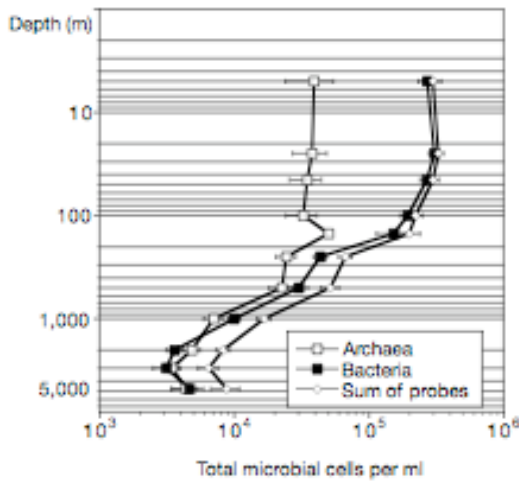


Figure 3 Mean annual depth profiles of microbial domains in the North Pacific subtropical gyre. Numbers are total cell abundances of bacteria and archaea (pelagic crenarchaeota and euryarchaeota combined). Bacteria and archaea were enumerated using whole-cell rRNA targeted fluorescent in situ hybridization with fluorescein-labelled polynucleotide probes. Data are averages of up to 14 roughly monthly samplings over a 1-yr period at the Hawaii Ocean Time-series station, ALOHA. See also Supplementary information.

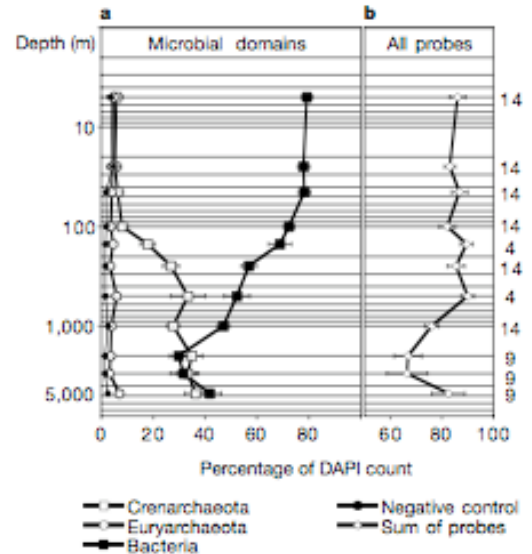


Figure 2 Mean annual depth profiles of microbial domains in the North Pacific subtropical gyre. Numbers are percentages of bacteria and archaea as compared to total microbial

Wha??? That’s a lot of stuff out there in the deep blue. What are they doing for a living? Where do they get their carbon? Autotrophic or heterotrophic? (What means?)

7. Ingalls et al., PNAS 103:5442-7, 2006:

- A. Sampled from depth using a “Niskin bottle,” extracted ether-linked lipids, analyze by gas chromatography and mass spectrometer. Determine isotopic compositions of C^{12} , C^{13} , C^{14} .

B. δC^{13} indicates 83% autotrophic / 17% heterotrophic; C14 (bomb carbon) indicates the CO₂ is old.

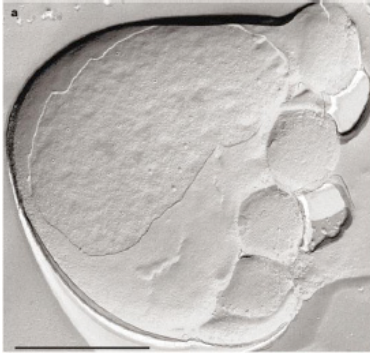
C. Hmmm. Where are they getting energy??

8. At this time the Common Wisdom thought is that they (at least can) oxidize ammonia, present in the oceans at concentrations of tens of nM in the deep sea to micromolar offshore.

- A. Putative crenarchaeal ammonia monooxygenase (AMO) genes (and transcripts) are abundant where crenarchaeotes are abundant, e.g. in soils.
- AMO carries out the reaction: $NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O$, the first step in ammonia oxidation. (Then hydroxylamine oxidoreductase does $NH_2OH \rightarrow NO_2^- + 4e^-$. These enzymes are membrane bound). [Are these putative AMO genes really orthologs of known AMA genes, or are they MethaneMO genes?

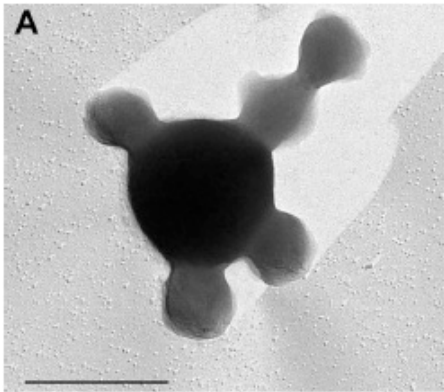
9. Note in passing: *Nanoarchaeum*: Small (0.05-0.1 micron diameter!), parasitic on *Igneococcus* spp. (high temp crenarchaeotes), very small genome (only 0.5mbp!).

A. Lacks genes for most metabolic functions, e.g. amino acids, nucleotides, coenzymes, catabolic enzymes.



(Freeze-fracture EM)

Note e.g. of "S-layer" in b



B. Note that there are bacterial parasitic species, e.g. *Bdellovibrio*, *Vampyrella*. These organisms blast into the periplasm of host bacteria and replicate there, killing the host. (There is a lot of interesting molecular biology there.)

10. A few archaeal specific qualities:

1. Ether-linked lipids (as opposed to bacterial and eucaryotic ester linkages). Archaeal lipids don't seem to undergo phase changes with temperature. (!!!) The structural reason is unknown.
2. Transcription and translation machineries are more like eucaryotes than bacteria. Bets off on other genes.
3. They seem to flourish where the going nutritionally is very poor.
4. There are no archaeal pathogens. Why is that??