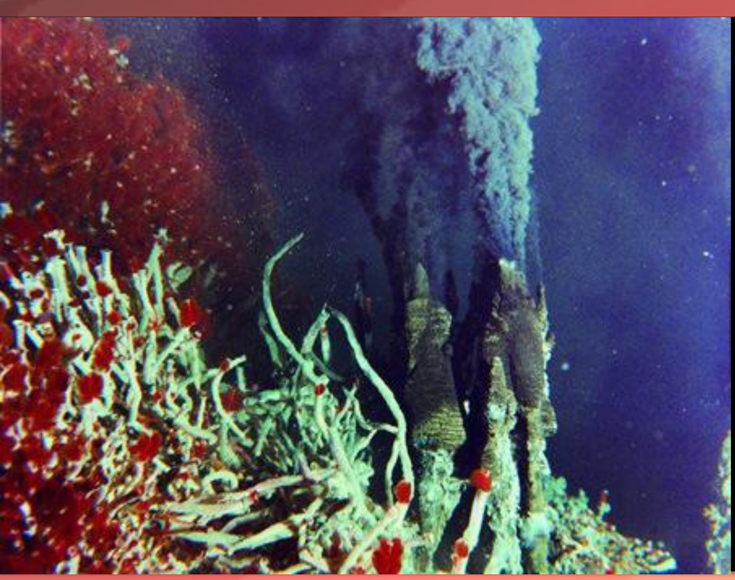

Life in Submarine Hell

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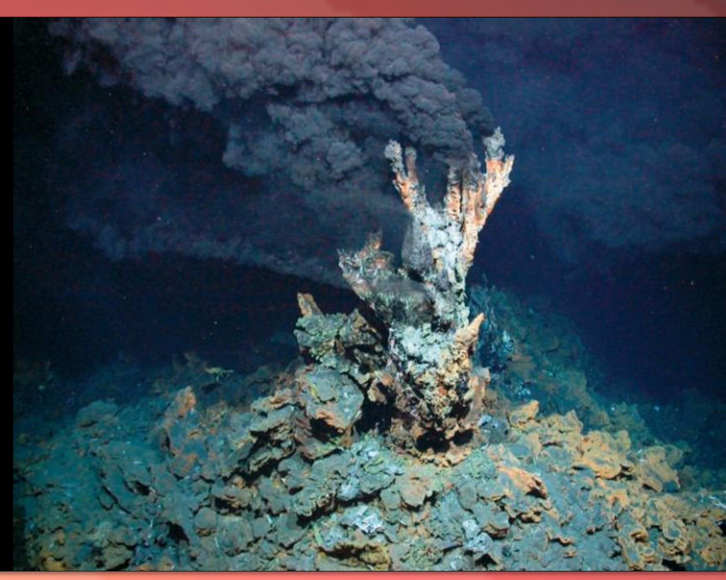
Treball Fi de Grau 2013 – Grau de Microbiologia



INTRODUCTION

Deep-sea hydrothermal vents were first discovered in 1977. They are geophysical and geochemical phenomena from volcanic and tectonic processes that occur at seafloor spreading centers (Fig.1). The vent fluid are hot (350ºC), anoxic and contain high concentrations of hydrogen sulfide and other compounds. An extrem environment given by the pressure (260 atm), high temperature, chemical toxicity of the fluids and total lack of photosynthetic production for animal nutrition. Its communities characterized by large clams, mussels, shrimps and vestimentiferian worms thrive on chemosynthetic microbial production. Microorganisms form the base of the ecosystem's food chain which are primary producers and also live symbiotically with larger fauna.

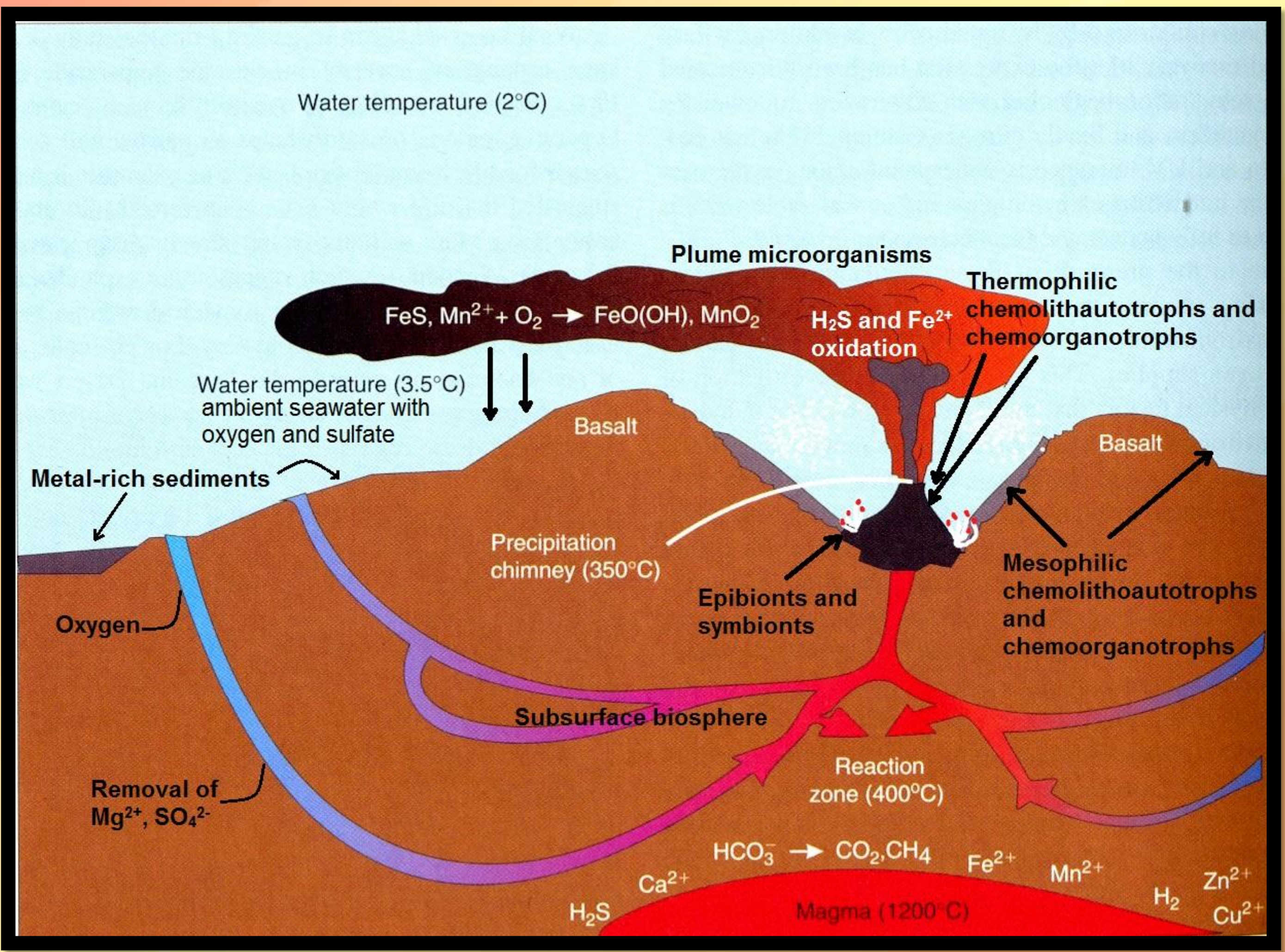


Fig.1 Formation and a sketch of a hydrothermal vent.

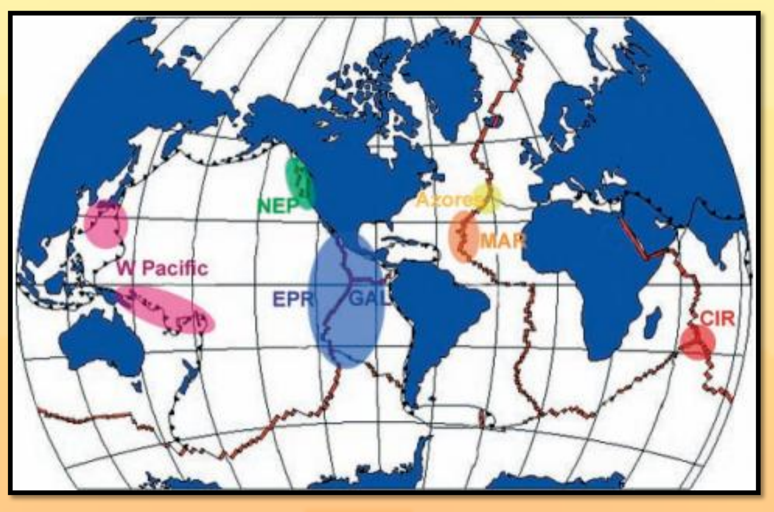


Fig.2 Locations of the submarine hydrothermal vents.

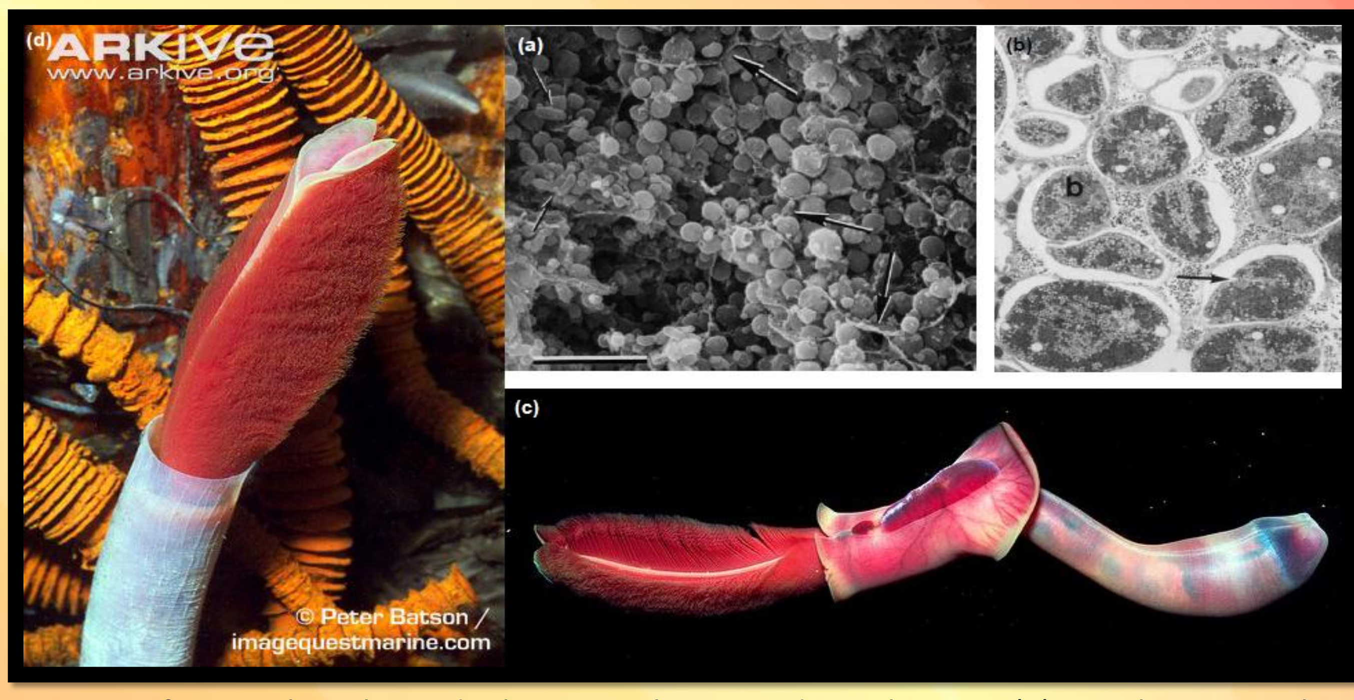


Fig.3 *Riftia pachyptila* and chemosynthetic endosymbionts. (a) Symbionts within trophosome, symbionts occur as both spherical and rod-shaped cells. (b) Portion of trophosome lobule. (c) Giant tube worm specimen. (d) *Tevnia jerichonana* in background.

RESULTS

Most of vent fauna depends on H_2S to survive. Symbiotic relationships:

- *Riftia pachyptila* → giant tube worm without mouth and digestive system. Chemoautotrophic bacterial symbionts are inside his trophosome (Fig.3).
- Large clams (*Calyptogena*) and mussels (*Bathymodiolus*) have bacterial endosymbionts too → majority are gammaproteobacteria.
- The pompeii worms (*Alvinella pompejana*) and shrimps (*Rimicaris*) have epibiotic bacteria → majority are epsilonproteobacteria.

There is a considerable diversity of bacteria in which epsilonproteobacteria are the most abundant (Fig.4).

Large part of found archaea are methanogenics or marine groups Crenarchaeota and Euryarchaeota representatives.

Taxonomic level Phylum	Taxonomic level Class	% of prokaryotic SSU 454 sequence reads
Proteobacteria	*Gammaproteobacteria*	0.2
Proteobacteria	*Betaproteobacteria*	0.1
Proteobacteria	***Epsilonproteobacteria***	**36.1**
Proteobacteria	*Deltaproteobacteria*	0.1
Firmicutes	*Clostridia*	0.1
Deferribacteres	*Deferribacterales*	0.1
Thermotogae	*Thermotogae*	0.6
Aquificae	***Aquificae***	**26.1**
Thermodesulfobacteria	*Thermodesulfobacteria*	0.8
Candidate division SR1	–	0.8
Chloroflexi	*Dehalococcoides*	0.1
Crenarchaeota	*Thermoprotei*	0.9
Euryarchaeota	*Thermoplasmata*	0.3
Euryarchaeota	***Methanococci***	**2.8**
Euryarchaeota	***Archaeoglobi***	**1.8**
Euryarchaeota	***Thermococci***	**28.4**
Thaumarchaeota	*Marine Group I*	0.1

Bold font indicates taxonomic groups represented by more than 1% of the total 16S rRNA gene pool.

Fig.4 Taxonomic affiliation, abundances and 16S rRNA gene numbers of microbial populations of a hydrothermal vent at the Arctic Mid-Ocean Ridge.

The most significant microbial process taking place is **chemosynthesis**: biosynthesis of organic carbon compounds from CO_2 with the source of energy being chemical oxidations.

Dominant autotrophic metabolisms according to the groups of microorganisms:

- **Mesophiles** (2-40ºC) as gamma- and epsilonproteobacteria → H_2S/S^0 and H_2 oxidation with O_2 or NO_3^{2-}.
- **Thermophiles** (40-80ºC) as epsilonproteobacteria, Aquificales, Desulfurobacteriaceae and methanogenic archaea → same as above but H_2 oxidation coupled to SO_4^{2-} and S^0 reduction and methanogenesis.
- **Hyperthermophiles** (80-125ºC) as Desulfurobacteriaceae, methanogenic archaea, Thermococcales and crenarchaeota → methanogenesis, S^0 reduction (Fig.5) and acetogenesis?.

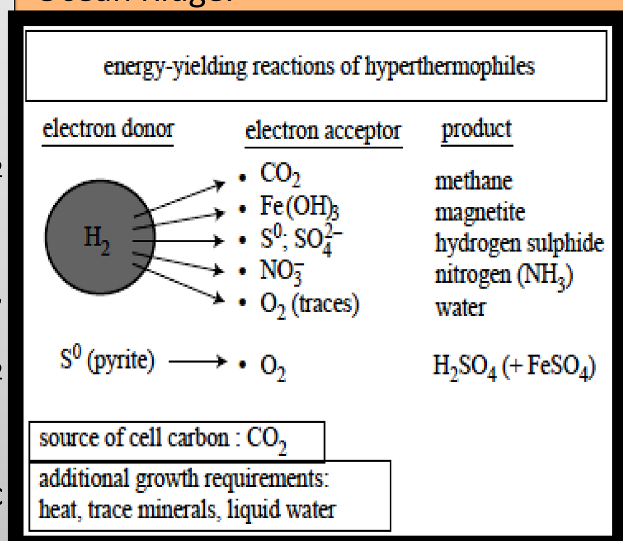


Fig.5 Schematic of main energy-yielding reactions in chemolithoautotrophic hyperthermophiles.

Hyperthermophiles

- Bacteria
  - *Thermotoga* 80ºC
  - *Aquifex* 85ºC (most thermophile bacteria)
- Archaea
  - Phylum Euryarchaeota
    - Thermococcales: *Thermococcus* 90ºC; *Pyrococcus* 100ºC
    - Methanococcales: *Methanocaldococcus* 85ºC
    - Methanopyrales: *Methanopyrus* 100ºC
    - *Nanoarchaeum* 90ºC: obligate symbiotic of *Ignicoccus*
    - Arqueoglobales: *Archaeoglobus* 83ºC; *Ferroglobus* 85ºC
  - Phylum Crenarchaeota
    - Thermoproteales: *Pyrobaculum* 100ºC
    - Desulfurococcales: *Pyrodictium* 105ºC; *Pyrolobus* 105ºC; Strain 121 106ºC; *Desulfurococcus* 85ºC; *Ignicoccus* 90ºC; *Staphylothermus* 92ºC

Nanoarchaeum equitans is a member of a novel group of hyperthermophilic virus-sized archaea. It has the smallest and the most compact microbial genome known to date. Cells grow attached to the surface of a specific crenarchaeal host, a member of the genus *Ignicoccus*.[4]

Archaeal strain 121 has upper temperature limit of 121ºC, which is the highest upper temperature limit reported.

The membrane lipids of thermophiles contain more saturated and straight chain fatty acids than mesophiles. This allows to grow at higher temperatures by providing the right degree of fluidity needed for membrane function. Many archaeal species contain a paracrystalline surface layer (S-layer) with protein or glycoprotein and this is likely as an external protective barrier.

Histone-like proteins identified in hyperthermophiles protect their DNA (Fig.7). Reverse gyrase, a type 1 DNA topoisomerase causes positive supercoiling and stabilize the DNA. Heat shock proteins, chaperones, are likely to play a role in stabilizing and refolding proteins as they begin to denature.[4]

STATE OF THE ART

- Biotechnology interest[4,3]. Wide industrial and medical applications:
  - Useful to combat industrial pollution (H_2S) and clean up sites contaminated with Cu, Cd, Hg,...
  - Source of new heat resistant industrial chemicals and new drugs to combat germs now resistant to plant and soil based drugs.
  - Renewable source of natural gas.
  - Proteins and enzymes of hyperthermophiles with high thermal stabilities: longer shelf life, produce less waste and lower risk of contamination.
  - Paper and detergent industries.
  - DNA polymerases for DNA amplifications by PCR (*Thermococcus littoralis*)
- Inactive sulfide chimneys represent a biogeochemically active microbial ecosystem. Communities on inactive sulfides are different from those on active chimneys.
- Interest in origin of life studies.

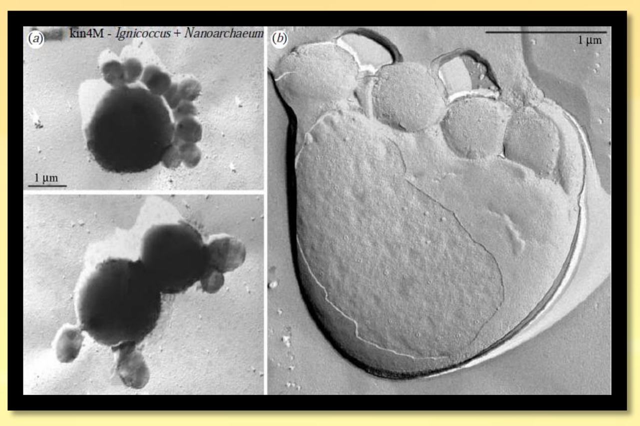


Fig.6 Cells of *N. equitans* (small) attached to *Ignicoccus.*

Fig.7 Histone like proteins protecting DNA.

CONCLUSIONS

- Microbial primary producers have an important role to keep alive these ecosystems.
- The findings of recent years revealed significant metabolic and phylogenetic diversity of cultivated thermophilic microorganisms.
- The discovery of *Nanoarchaeota* suggests that further major groups of microbes may still be unrecognized, and are waiting for their isolation to tell us more about the origin and evolution of life.
- The potential biotechnological applications associated with these microbes and their products has driven extensive and intensive research efforts on deep-sea hydrothermal vent microorganisms.

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