Goldschmidt 2000
September 3rd–8th, 2000
Oxford, UK.

Journal of Conference Abstracts
Volume 5(2), 144

Novel Hyper-thermophiles from Well-known and Previously Unexplored Shallow Marine Hydrothermal Sites

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To date, members of more than two dozen hyper-thermophilic genera have been isolated from marine hydrothermal systems, heated sediments, continental solfataras, and hot springs (Amend and Shock, 2000). The thermal vents around the Baia di Levante on Vulcano (Aeolian Islands) in southern Italy have yielded more culturable hyper-thermophiles than any other site and represent the 'type locality' for species of *Aquifex, Thermotoga, Archaeoglobus, Ferroglobus, Pyrococcus, Thermococcus, Pyrodictium, Staphylothermus*, and *Thermodiscus*. I used heterotrophic, anaerobic growth media to isolate several hyper-thermophiles from shallow submarine hydrothermal vents, hot springs, seeps, and geothermal wells on Vulcano. Based on 16S ribosomal RNA sequence data, one of these isolates (PI1), obtained from a geothermal well (Pozzo Istmo) near the Baia di Levante, is related to *Thermococcus* and *Pyrococcus*, but plots most closely on the phylogenetic tree to the yet unpublished new archaeal genus *Paleococcus*. PI1 is an obligate chemo-heterotroph that grows optimally in the lab near 80ºC on complex organic substrates such as yeast extract and peptone in the presence of elemental sulphur; CO_2 and H_2S are produced as metabolic reaction products. PI1 represents a hyper-thermophilic genus heretofore unknown in the thermal environment of Vulcano. I propose to name the new isolate *Paleococcus helgesonii* for H.C. Helgeson who has been instrumental in bridging geochemistry, microbiology, and organic chemistry in hydrothermal systems.

The temperature of the thermal water from P. Istmo is 56.6 C with an *in situ* pH of 4.70. Relative to waters from several other geothermal wells on Vulcano (representative values given in parentheses) those from P. Istmo are characterized by high conductivity = 97 mS (~7 mS); high concentrations of major ions: Na^+ = 12,170 ppm (323-563), Mg^{2+} = 1520 ppm (3-340), Ca^{2+} = 710 ppm (6-353), Cl^- = 21,450 ppm (118-258), NO_3^- = 10.4 ppm (0.1-0.5); high concentrations of certain trace elements including: Sr = 8960 ppb (20-826), Mn = 1857 ppb (3-186); and low concentrations of others, such as W = 14 ppb (96-379). The tolerance of *P. helgesonii* to ranges in temperature, pH, E_h, and chemical composition of the growth medium have yet to be fully investigated.

Approximately 25km to the north-east of Vulcano, off the coast of Panarea Island, are >20 known shallow submarine hydrothermal vents that reach temperatures up to ~100 C (Italiano and Nuccio, 1991). Until very recently, these sites were unexplored with respect to microbiology. We (Gugliandolo et al., 1999) reported the first isolation of a micro-organism (albeit a mesophile) from the Panarea vent fluids, a rod-shaped, chemo-autotrophic sulphur oxidizer that resembles *Thiobacillus*. Since then, I sampled submarine vents (depth ~20m) by SCUBA and isolated from these fluids anaerobic, heterotrophic, hyper-thermophilic Archaea at 80 C. Based on 16S ribosomal RNA sequences, two of the isolates (CS1 and LC1) are closely related to *Thermococcus peptonophilus, T. profundus, T. barossii*, and other *Thermococci* I isolated from Loihi Seamount near Hawaii. CS1 and LC1 represent the first hyper-thermophiles isolated from the largely unexplored, shallow marine hydrothermal sites near Panarea.

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