### 4.4.14

### Thermophiles and vent geochemistry at Vulcano (Italy) and Ambitle (Papua New Guinea)

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Marine hydrothermal vents can serve as windows to the deep biosphere. Much of the attention has been on deep-sea 'black smokers', but recent studies have also focused on shallow marine systems, including those offshore Ambitle Island (Papua New Guinea) and near the Aeolian Islands of Vulcano and Panarea (Italy). Owing to their accessibility and chemical diversity, the thermal fluids and the sediments through which they flow permit highly detailed studies of the high temperature microbiology-geochemistry interface.

In Tutum Bay, Ambitle Island, fluids at temperatures up to 100°C and enriched in arsenic (as high as ~1000ppb) emanate from discrete orifices and as diffuse discharge through sediment. Geochemical data were used to design site-specific growth media for culturing *in situ* chemotrophic microbial communities. These media targeted fermentation, aerobic and anaerobic As(III) oxidation, and As(V) reduction in which H<sub>2</sub> and organic matter served as electron donors. Aqueous media were inoculated with sediment slurries and incubated at temperatures up to 80°C. Results from culturing experiments will be presented and the relationship to the geochemical composition and microbial community structure will be explored. Preliminary findings indicate that the different modes of As redox are common metabolisms used by mesophiles and thermophiles at Ambitle.

In the Vulcano hydrothermal system, both acidic and circumneutral saline fluids are emitted from the subsurface in sediment seeps, geothermal wells, and submarine vents. Many of the known hyperthermophilic (T<sub>opt</sub>≥80°C) genera thrive at Vulcano, including the first of the Thermococcales (Palaeococcus helgesonii) to grow in the presence of O<sub>2</sub>. Analytical data on DOC, amino acids, carbohydrates, carboxylic acids, major ions, and trace elements were used to design media; enrichments were observed in more than a dozen distinct media types. These data were also used to calculate in situ values of  $\Delta G_r$  for ~100 possible metabolic reactions. The energetics indicate redox that chemolithoautotrophy with O2, Fe(III), or NO3- as terminal electron acceptor is strongly exergonic (70-125 kJ/mol e<sup>-</sup>), but the energy-yield from  $S^0$  and  $SO_4^{2-}$  reduction is low (2-20) kJ/mol e<sup>-</sup>). Nevertheless, high rates of microbial sulfate reduction have been documented in 90°C Vulcano sediments, and molecular studies (FISH, gene surveys) are beginning to bring the connection between geochemistry, reaction energetics, and thermophilic communities into focus.

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## Geochemical niches and novel Archaea in the hydrothermal systems of Vulcano, Italy

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The diversity of chemotrophs inhabiting the subsurface biosphere is intimately linked to the energetics of geochemical reactions. In hydrothermal systems fluid mixing leads to chemical disequilibria that can yield energy to chemotrophic thermophiles. Shallow marine hydrothermal systems are an ideal location to study the microbe/energy interface and the effect of geochemical energetics on productivity in the deep biosphere. There, chemical gradients are steep as reducing hydrothermal fluids mix with oxidized seawater in contact with the atmosphere. Vulcano Island (Italy) is one of the beststudied shallow marine hydrothermal systems and the vents, seeps and wells encompass a full spectrum of geochemical diversity. Recently, in situ geochemical energetics of >90 autotrophic and heterotrophic reactions were reported [1,2]. One of the most striking conclusions of this work is that large variations in the free energy of metabolic reactions are due to differences in geochemical composition and not temperature.

To determine whether the varying geochemical compositions dictate microbial diversity, we have conducted ecological surveys (16S rRNA gene sequencing) at various hydrothermal sites on Vulcano. A previous study of microbial diversity at acidic sites on Vulcano revealed a relatively low diversity [3]. Here we report an extensive community of Archaea at a thermal well, Pozzo Istmo, where the temperature is 56.4°C and the pH is 5.84. The Archaeal diversity was determined by cloning and sequencing 16S rRNA gene fragments amplified from genomic DNA that was extracted from thermal fluid. Approximately 40 unique archaeal phylotypes have been identified. These phylotypes have less than 87% identity with any cultivated organism. They are most closely related to archaeal sequences from Yellowstone National Park, Guaymas Basin and a deep-sea sulfide chimney. These phylotypes represent a novel group of deeply branching Crenarchaeota. The microbial diversity at Pozzo Istmo is compared to that at other hydrothermal sites on Vulcano and the unique geochemical niche of the novel group of Crenarchaeota is inferred from the energetics of metabolic reactions. We contend that the variability in the microbial communities is controlled by the energetics of chemotrophic reactions.

#### References

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