Hyperthermophilic Bacterial Communities within Terrestrial and Marine Hydrothermal Areas

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Keywords: thermophilic, hyperthermophiles, hydrothermal, solfataras, archaebacteria

INTRODUCTION

During the last years, members of communities of hyperthermophilic bacteria growing at temperatures between 80 and 110°C have been isolated (1 - 3). As a rule, they are so well adapted to the high temperatures that they do not grow below 60°C. Hyperthermophilic bacteria mainly belong to the archaebacterial kingdom (4). Some of them are also present within the eubacteria (5, 6). The communities consist of primary producers and consumers of organic matter. Due to their existence within phylogenetically divergent groups, the lack of closely related mesophiles and their biotopes having existed since the archaean age, hyperthermophiles may have adapted to the hot environment billions of years ago.

I. BIOTOPES

Communities of hyperthermophilic bacteria are known to exist within submarine hydrothermal areas and continental solfataras. The surfaces of the solfataric fields are usually rich in sulfate and exhibit an acidic pH value (0.5 to 6). At depth, solfataras are less acidic (pH 5 to 7). Sometimes, acidic solfataric fields may also harbour a few weakly alkaline hot springs (pH 7 to 9). Submarine hydrothermal systems are slightly acidic to alkaline (pH 5 to 8.5) and normally contain the high amounts of NaCl and SO_4^{2-} present within sea water. Due to the low solubility of oxygen at high temperatures and the presence of reducing gases, most biotopes of hyperthermophiles are anaerobic. Within solfataric fields, oxygen is present only within the upper (acidic) layer which appears ochre-colored due to the presence of ferric iron (7).

Hyperthermophiles may be able to survive for years at temperatures below 60°C, although not growing under these conditions. Some of the anaerobic hyperthermophiles tolerate oxygen much better at the low non-growth temperatures than at the growth temperatures. This property may be important for dissemination of these organisms through oxygen-rich low temperature areas.

II. COMMUNITIES OF HYPERTHERMOPHILES WITHIN SOLFATARIC FIELDS

A. Terrestrial Solfataras

The strongly acidic upper layer within the solfataric fields contains communities of aerobic and facultatively anaerobic acidophiles which grow only at low ionic strength and are therefore non-marine organisms. They are extreme acidophiles (opt pH3). Phylogenetically, they belong to the archaebacterial genera *Sulfolobus*, *Metallospharea*, *Acidianus* and *Desulfurolobus* which all consist of coccoid cells (1, 8-11). The moderately thermophilic, facultatively anaerobic, pleomorphic archaebacterium *Thermoplasma* is also an extreme acidophile occurring within terrestrial solfataric fields (12). Members of the genus *Sulfolobus* are strict aerobes growing autotrophically by oxidation of S° and S^{2-}, forming sulfuric acid.

Many *Sulfolobus* isolates are facultative heterotrophs and are therefore "opportunistic" consumers of organic matter within the biotope. Some members of the *Sulfolobales* like *Metallosphaera sedula* are able to grow by oxidation of sulfidic ores like pyrite, chalcopyrite, and sphalerite forming sulfuric acid and solubilizing the heavy metal ions (9). Sulfidic ores like pyrite are formed within solfataric fields (Stetter, unpublished). Similar to *Sulfolobus*, members of the genus *Acidianus* are able to grow by oxidation of S° (13). Some strains of *Acidianus* grow also on sulfidic ores, but less efficiently than *Metallosphaera* (Huber and Stetter, unpublished). In contrast to all other members of the *Sulfolobales*, *Acidianus* is able to grow anaerobically on H_2 and S° forming H_2S. *Desulfurolobus* is very similar to *Acidianus* in its physiological properties and by DNA/DNA hybridization (13). Together with the obligate thermoacidophiles, slightly acidophilic and neutrophilic hyperthermophiles are found within terrestrial solfataric fields. The latter are strict anaerobes and may therefore mainly occur within the deeper more neutral anaerobic zone within terrestrial solfataric fields. Members of the genera *Thermoproteus* and *Pyrobaculum*, which consist of rod-shaped cells (about 0.5 µm in width) are facultative or obligate autotrophs growing by formation of H_2S from H_2 and S° (2, 14). Alternatively, the facultative autotrophs (e.g. *Pyrobaculum islandium*) grow by sulfur respiration of organic matter (15). The thin (0.17 µm in width) filamentous *Thermofilum* and the coccoid *Desulfurococcus* are obligate sulfur respirers (2). From solfataras in the southwest of Iceland, rod-shaped lithoautotrophic methanogens growing at temperatures up to 97°C were isolated which are obviously primary producers of organic matter within the biotope. Two species are known: *Methanothermus fervidus* and *Methanothermus sociabilis* (16). Within neutral continental hot springs in Djibouti, Africa, was found the extremely thermophilic eubacterial species *Thermotoga thermarum* which grows only at low ionic strength (17). Like the marine members of the genus *Thermotoga*, *T. thermarum* is a strictly anaerobic heterotroph growing by fermentation of carbohydrates.

B. Submarine Hydrothermal Systems

Many hyperthermophiles are adapted to the marine thermal environments. They are represented by primary producers of organic matter like members of the genera *Pyrodictium*, *Archaeoglobus* and *Methanococcus* and by consumers like *Staphylothermus*, *Thermodiscus*, *Thermococcus*, *Pyrococcus* and *Thermotoga*. The organisms with the highest growth temperatures are members of *Pyrodictium*, growing up to 110°C (18). Cells of *Pyrodictium* are so well adapted to high temperatures that they do not even grow below 80°C. *Pyrodictium occultum* and *Pyrodictium brockii* are able to grow autotrophically, gaining energy by reduction of S° by H_2. Cultures of *Pyrodictium* grow in flocs, cells being disc-shaped and connected by a network of very thin hollow fibres. Many submarine hydrothermal systems contain coccoid-shaped archaebacterial sulfate reduces of the genus *Archaeoglobus*. *Archaeoglobus fulgidus* is a facultative autotroph gaining energy by reduction of sulfate or thiosulfate by H_2. It grows heterotrophically by sulfate respiration on various organic substances (19). *Archaeoglobus* members grow at temperatures up to 90°C. Similar to methanogens, cells of *Archaeoglobus* show a blue-green fluorescence in the UV light at 420 nm due to the possession of factor 420 (19). A further autotrophic marine hyperthermophile is *Methanococcus jannaschii* which grows at temperatures up to 86°C (20). Very recently, novel rod-shaped methanogens which grow at least at 110°C (Stetter, unpublished) were isolated from an abyssal hydrothermal system. The marine thermal environment contains also a variety of strictly heterotrophic hyperthermophiles. *Staphylothermus* and *Thermodiscus* are coccoid and disc-shaped sulfur respirers growing on various kinds of organic matter (21). The genera *Thermococcus* and *Pyrococcus* are coccoid cells which are widely distributed within marine hydrothermal systems (22, 23). *Thermococcus celer* utilizes tryptone, yeast extract and protein as carbon sources. Growth is stimulated by sucrose. In closed culture vessels, optimal growth is obtained in the presence of sulfur and about 1.5 moles of H_2S are formed per mole of CO_2 (22). *Thermococcus* and *Pyrococcus* can also grow without sulfur by an unknown type of fermantation. *Pyrococcus furiosus* grows at temperatures up to 103°C and shows a much lower GC-content than *Thermococcus celer* (23). At 100°C, the doubling

time is only 37 minutes. The mode of fermentation of *Pyrococcus* and *Thermococcus* is still unclear. Many submarine hydrothermal fields contain representatives of the eubacterial genus *Thermotoga* which thrive together with hyperthermophilic archaebacteria in the same environment. Members of *Thermotoga* can be easily identified by their rod-shape and outer sheath-like structure overballooning at the ends (5). *Thermotoga maritima* and *Thermotoga neapolitana* are fermentative hyperthermophiles growing at temperatures up to 90°C (5, 24). They use various carbohydrates as energy sources forming as end products, L-lactate, acetate, H_2 and CO_2 (5).

III. DISCUSSION

The isolation of different groups of autotrophic and heterotrophic bacteria from geothermally and hydrothermally heated environments shows an unexpected variety of organisms within these almost unexplored ecosystems. Within these, the primary production of organic matter and consumption proceeds at temperatures up to about 110°C. The energy-yielding reactions are based on oxidation or reduction of inorganic sulfur compounds by O_2 or H_2. In the case of methanogens, CO_2 is reduced by H_2. Aerobic hyperthermophilic autotrophs seem to occur only within acidic terrestrial solfataric fields. The anaerobic hyperthermophilic autotrophs use H_2, CO_2 and S° which are formed within the environment. These organisms are therefore completely independent of any sun. The consumers of organic matter are most likely using cell components of decaying primary producers. Most of them grow by sulfur respiration and fermentation. Many strains of hyperthermophilic autotrophs are facultatively heterotrophic. This property may be an "opportunistic" feature and may be important for successful competition within this extreme environment.

SUMMARY

Hyperthermophilic bacterial communities within terrestrial and marine thermal areas are very complex. They consist of chemol toihoautotrophic and heterotrophic bacteria, growing optimally between 80° and 110°C. Primary production and consumption of organic matter is going on at these high temperatures.

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