

Isolation and partial characterization of aerobic chemosynthetic thiosulfate oxidizing bacteria from diffuse flow hydrothermal vents from the East Pacific Rise

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Deep-sea hydrothermal vents are located at spreading centers along mid-oceanic ridge systems, and are characterized by either low temperature diffuse flows or high temperature focused flows. Diffuse flow vents are sites of rapid biomass production sustained by the activity of chemosynthetic microorganisms that obtain energy mostly by the oxidation of sulfur compounds, considered the most efficient energy source in this environment. Due to the important role that sulfur oxidizers have in diffuse flow vents, we initiated a study to: 1) determine their abundance in hydrothermal fluids, 2) isolate, identify and characterize representative aerobic thiosulfate oxidizing bacteria from hydrothermal fluid samples obtained from diffuse flow vents on the East Pacific Rise (EPR) at “9°50’N, 104°17’W”, and 3) to assess the composition of natural microbial communities in diffuse flow vent fluids. For (1) we used the Most Probable Number (MPN) technique employing growth medium specific for chemosynthetic thiosulfate oxidizing autotrophic microorganisms. MPN counts were compared to total microscopic direct counts. For (2) microbial isolations were done from primary enrichments and from the highest MPN dilutions that showed growth. Isolates were identified by DNA extraction, followed by amplification and sequencing of the 16S rRNA gene. Our data show that counts of chemosynthetic thiosulfate oxidizers ranged from 1.1×10^4 to 2.4×10^7 cells/ml and represented 0.01% to 14.0% of the total cell counts. Gamma and Alpha-*Proteobacteria* related to the genera: *Thiomicrospira*, *Halothiobacillus*, *Hydrogenovibrio*, *Thioclava*, *Thalassospira*, *Salipiger*, *Halomonas*, *Marinobacter*, *Vibrio* and *Pseudomonas* were identified, as well as one isolate, strain EPR 70, a possible novel specie, related to the genus *Salinisphaera* (95 % sequence similarity). This strain is currently being characterized in detail. In order to compare cultivation-dependent and independent data (3), the diversity of the natural bacterial communities in diffuse flow vents is being analyzed via the construction, screening and sequencing of 16S rRNA gene clone libraries. Preliminary results of the environmental survey on three different hydrothermal fluids samples, indicate that Gamma-*Proteobacteria* are abundant in all libraries, and that the abundance of Epsilon-*Proteobacteria* is related to the temperature fluctuations in the fluid samples.

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