Diversity of alkane oxidizing bacteria and alkane hydroxylase genes in deep-sea hydrothermal vents

Ramaydalis Cruz and Costantino Vetriani*

Hydrothermal fluids at deep-sea vents are enriched in hydrocarbons of biogenic and abiogenic origin (1-3). At 13°N on the East Pacific Rise (EPR), the concentration of *n*-alkanes was found to be 250 times higher in hydrothermal fluids than in reference seawater (1). Despite the presence of high concentrations of hydrocarbons in hydrothermal fluids, the occurrence of hydrocarbon-oxidizing organisms at deep-sea vents has been poorly investigated. Therefore, we have initiated enrichment cultures for hydrocarbon oxidizing microorganisms from fluid samples and biomass collected from experimental microcolonizers that were deployed on diffuse flow vents on the EPR at 9°N. These enrichments led to the successful isolations of pure cultures of aerobic, mesophilic organisms capable of using *n*-alkanes (*e.g*., dodecane) as their sole carbon sources. A total of twenty-five pure cultures were isolated and identified by 16S rRNA sequencing. Our isolates were, for the most part, *Gammaproteobacteria* of the genus *Acinetobacter* and *Alcanivorax*. We also amplified by PCR a fragment of the *Alk*B gene from these isolates, and we carried out phylogenetic analyses of this gene. The *Alk*B gene encodes for the alkane hydroxylase, an enzyme that catalyzes the first reaction in the stepwise oxidation of *n*-alkanes. Furthermore, we detected the *Alk*B transcripts in two model organisms from the laboratory culture collection, *Alcanivorax* sp. strain EPR 7 and *Acinetobacter* sp. strain EPR 111. This experiment was carried out by growing the two isolates in rich media (Artificial Sea Water supplemented with peptone and yeast extract) and on minimal media supplemented with dodecane as the sole carbon source. Qualitative Reverse Transcription PCR (RT-PCR) experiments showed that *alk*B transcripts could be detected in both conditions, suggesting that in these strains the *alk*B gene is constitutively expressed. Finally, we carried out a functional gene survey of AlkB genes in vent natural microbial populations.

**Presenting Author: Rutgers University, IMCS Rutgers University, 71 Dudley Road New Brunswick, NJ 08901, USA*