

Long-chain hydrocarbon degraders from deep-sea

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Deep-sea life is featured by extreme environmental conditions, such as low temperature, limitation in nutrients and carbon sources, and high hydrostatic pressure (Jannasch, 1979). As a consequence, marine microbial communities have incredibly broadened their metabolic versatility to make efficient use of any resource available (Sibuet and Karine, 1998). In the present work, we collected deep-sea microbial communities (1km, equivalent to 100bars) and grew them using long-chain aliphatic hydrocarbons. Because of their solid nature at ambient temperature and pressure, such hydrocarbons have a higher chance to sink to the seafloor during spills with respect to gaseous or liquid, short-chained ones. Hence, our hypothesis was that the use of long-chain alkanes as unique carbon sources at high hydrostatic pressure would have resulted into microbial communities enriched in piezophiles, *i.e.*, bacteria growing better at high pressure rather than at atmospheric ones. Nevertheless, high hydrostatic pressure does not improve much the solubility of these compounds in water (Brunner, 1990), the bioavailability of which remains always extremely low. This condition is ideal to test the flexibility of piezophiles and address some of the basic questions regarding their metabolism. In particular, in these bacteria any biomolecule (enzymes, lipids, nucleic acids, etc.) must be adapted to properly function under high pressure. Hence, the range of reaction conditions suitable for biocatalysis in these microbes is supposed to be larger than for bacteria growing in surface waters.

Deep-sea samples (1km, 100bars) were incubated at three different pressure conditions (1, 100 and 200bars) using either icosane (C20) or triacontane (C30). In particular, these three pressure conditions were selected in order to separately enrich piezotolerant bacteria (growing well at both 1 and 100bars) from piezophiles (growing better at 100 and 200bars). High-pressure reactors were inoculated with the same initial environmental microbial community and run for 9 consecutive incubation periods, each of which lasted 10d. At the end of any incubation, aliquots of growing bacterial cells were transferred to a new reactor, for a total of 90d of enrichment. Experiments were followed according to pH, O₂ consumption, optical density, phosphates and sulphates consumption, hydrophobicity, cell number by flow cytometry and hydrocarbon solubility in the water phase. Furthermore, characterization of the microbial community shift was conducted through both DGGE and high throughput sequencing using Illumina platform.

In each culture condition, marine bacteria were able to grow by taking up C20 and C30. Respiration rates were always high, and production of surfactants occurred particularly at 1bar. This is probably due to the fact that a higher number of cells was noted at 1bar than at higher pressures, when filtering with 25µm. However, numerous bacterial populations smaller than 1.5µm were noted in all conditions. Hydrophobicity, *i.e.*, the capability to move towards oil droplets, was generally high in all conditions. Phosphates and sulphates consumption was statistically different according to the pressure applied and/or O₂ availability. Future work will be devoted to the understanding of specific enzymatic activity (through meta-proteomics) and to the characterization of the biomass features in terms of PLFAs, wax, esters and lipids.

References

- Brunner E. 1990. Fluid mixtures at high pressures. IX. Phase separation and critical phenomena in 23 (n-alkane + water) mixtures. *Journal of Chemical Thermodynamics* 22:335-353.
- Jannasch H.W. 1979. Microbial turnover of organic matter in the deep sea. *Bioscience* 29.4:228-232.
- Sibuet M. and O. Karine. 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. *Deep-Sea Research Part II* 45.1-3:517-567.