EXPERIMENTAL STRATEGIES RESPECTING DEEP-SEA CONDITIONS: TOWARDS A RELIABLE MEASUREMENT OF THE IN SITU ACTIVITY OF DEEP-SEA PROKARYOTES

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Abstract

High-pressure and low temperature conditions are the main characteristic of deep-sea environments. So, we developed special gears to collect and incubate samples without decompression. Data from deep-seawater (1) as well as from deep hypersaline anoxic basins show that each step involved in the organic matter degradation is carried out by prokaryotes adapted to the ambient conditions. Conversely decompression of near-bottom water provokes an overestimation relative to the rates measured *in situ* using a benthic lander (2). Such an opposite behavior facing pressure conditions is likely due to different origins of prokaryotes in the water column and at the water-sediment interface.

Keywords: Microbial ecology of mesopelagic and bathypelagic zones; High-Pressure conditions; deep-sea prokaryotes

In deep-sea, prokaryotes are submitted to external factors (low availability of nutrients, low temperature, high-pressure), all able to limit their metabolism (4-9). Some environments, as the deep hypersaline anoxic basins (DHABs) of the eastern Mediterranean, offer even more severe conditions for life: hypersalinity (up to 300), anoxia, extreme concentrations for Mg, SH_2, and high pressure (~35 MPa). Therefore it is important to measure microbial activities in the deep-sea as closely as possible to the *in situ* conditions. So, we developed special gears to collect and incubate samples without decompression (Fig. 1).

Station	Month	Bacteria (cells ml^{-1})	Bacteria (µg C l^{-1})	Temp (°C)	Salinity (g ‰)	phytoplankton (cells l^{-1})
Sulina	April	47000	0.94	9.75	16.17	88320
Sulina	July	160000	32	28.18	12.75	2880100
Sulina	September	160000	32	22.25	7.42	3017200
Sf. Gheorghe	April	1600000	32	8.92	15.99	1127000
Sf. Gheorghe	July	1600000	32	27.6	15.5	2117720
Sf. Gheorghe	September	1600000	32	22.03	2.47	255350
Portita	April	3500000	70	9.97	14.61	642550
Portita	July	1600000	32	nd	nd	1075000
Portita	September	nd	nd	nd	nd	2872600
Constanta	April	920000	18.4	12.45	9.95	1091820
Constanta	July	1600000	32	25.38	14.25	21350
Constanta	September	1600000	32	23.47	14.1	6025380
Constanta	October	2400000	48	18.32	17.07	nd
Mangalia	April	92000000	1840	11.82	11.96	484660
Mangalia	July	38000	0.76	25.73	15.25	262800
Mangalia	September	nd	nd	nd	nd	496440
Mangalia	October	16000000	320	17.53	16.38	nd

Fig. 1. Diagrammatic representation of the high-pressure bottles (HPBs) in configuration of samples filling

When the filling valve is opened, the natural hydrostatic pressure pushes down the floating piston and the seawater enters into the upper chamber of 2 HPBs. The distilled water is flushed out from the lower chamber of the syringes to the exhaust tanks, through a nozzle that acts as an hydraulic brake. During retrieval, hydrostatic pressure is maintained thanks to a check valve that avoids any decompression within the 'high pressure' HPBs, in contrast to the 'decompressed' one without check valve (8).

Compiled data from stratified water columns show that each step involved in the organic matter degradation is carried out by prokaryotes adapted to the ambient pressure condition. Metabolic rates measured on decompressed samples are underestimated by a factor equal to 3.6 ± 4.3 (mean ± S.D.; n=99). Because the pressure effect is highly variable, a single factor cannot be used to correct rates measured on decompressed samples (1).

During the EU program BIODEEP (contract n° EVK3-2000-00042), we measured diverse metabolic rates maintaining all the characteristics of DAHBs during sample retrieval and incubation. All the measured rates (peptidase, phosphatase, assimilation and respiration of glutamate; bacterial biomass production) were higher under ambient conditions (×12.5 ± 23.6; mean ± S.D., n = 6) than those obtained on the decompressed samples. Hence, we demonstrated that DAHBs' prokaryotic populations are adapted to extreme ambient conditions and may actively participate in the biogeochemical cycles in these basins. Several strains of potential industrial interest have been cultivated, some of which exhibiting very unusual morphological and physiological features. Some newly described bacterial catabolic genes with potential application in bioremediation have also been retrieved and they are under investigation.

Although decompression of deep-sea water and DHABs samples leads to an underestimation of microbial activities, decompression of near-bottom water samples provokes an overestimation (by 1 order of magnitude) relative to the actual rates measured *in situ* using a benthic lander (2). This apparent contradiction can be due to the difference in origin for deep-sea water and benthic water microbial populations.

A large fraction of the bacterial consortia are transported into the deep-sea by settling particles (9).

Since attached bacteria plays an important role in the mineralization of particles (8, 10, 11) and in the recycling of biogenic elements (silicate and carbonate), we did an experiment to simulate the fall of particles through the whole water column. This experiment demonstrates that metabolic rates of bacteria attached to the sinking phytoplankton aggregates are slowed down by increasing pressure. The gear we developed for this experiment will allow to precize calculations for mineralization and dissolution rates of particles sinking throughout the water column.

The experimental approaches we used to study deep-sea waters, sinking particles and benthic waters, respecting the main conditions of these deep-sea environments, and so permit to study the quantitative and qualitative evolution of the chemical composition of the organic matter, concomitantly with the evolution of microbial diversity, from the sea surface to the bottom.

Reference

1 - Bianchi, A., Tamburini, C. and Garcin, J., submitted. Effect of pressure conditions on microbial activity measurements in deep-sea water: a review. *Deep-Sea Research I* .

2 - Tamburini, C., De Bovée, F. and Garcin, J. Microbial activities at the deep-sea water-sediment interface of the NW Mediterranean: in situ versus on board measurements. *Journal of Geophysical Research.*

3 - Turley, C. and Lochte, K., 1990. Microbial response to the input of fresh detritus to the deep-sea bed. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 89: 3-23.

4 - Poremba, K., 1994. Impact of pressure on bacterial activity in water columns situated at the european continental margin. *Netherlands Journal of Sea Research,* 33: 29-35.

5 - Yayanos, A.A., 1995. Microbiology to 10,500 meters in the deep-sea. *Ann. Rev. Microbiol,.* 49: 777-805.

6 - Tholosan, O., Garcin, J. and Bianchi, A., 1999. Effects of hydrostatic pressure on microbial activity through a 2000 m deep water column in the NW Mediterranean Sea. *Marine Ecology Progress Series*, 183: 49-57.

7 - Tamburini, C., Garcin, J., Ragot, M. and Bianchi, A., 2002. Biopolymer hydrolysis and bacterial production under ambient hydrostatic pressure through a 2000 m water column in the NW Mediterranean. *Deep-Sea Research II,* 49: 2109-2123.

8 - Tamburini, C., Garcin, J. and Bianchi, A., 2003. Role of deep-sea bacteria in organic matter mineralization and adaptation to hydrostatic pressure conditions in the NW Mediterranean Sea. *Aquatic Microbial Ecology,* 32: 209-218.

9 - Turley, C.M. and Mackie, P.J., 1995. Bacterial and cyanobacterial flux to the deep NE Atlantic on sedimenting particles. *Deep Sea Research I,* 42: 1453-1474.

10 - Cho, B.C. and Azam, F., 1988. Major role of bacteria in biogeochemichal fluxes in the ocean's interior. *Nature,* 332: 441-443.

11 - Smith, D. C., Simon, M., Alldredge, A. L. and Azam, F., 1992. Intense hydrolytic enzyme activity on marine aggrgates and implications for rapid particle dissolution. *Nature,* 359: 139-142.