Vol. 111: 137–141, 1994 | MARINE ECOLOGY PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published August 11

Bacterial response to hydrostatic pressure in seawater samples collected in mixed-water and stratified-water conditions

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ABSTRACT: Effects of hydrostatic pressure conditions on microbial incorporation of ^{14}C-glucose were studied in 3 h incubations of decompressed and undecompressed seawater samples collected at 1100 m depth in the northwestern Mediterranean Sea. Even in these relatively shallow environments, hydrostatic pressure conditions influence microbial activity measurement. Depending on hydrological conditions this parameter provokes stimulatory or adverse effects on microbial metabolism. During the stratified-water period, microbial activity in samples from 1100 m depth was reduced under decompressed conditions. On the other hand, during the mixed-water period, microbial activity was stimulated by decompression. Therefore measurements of microbial activity in the water column must be done taking *in situ* pressure conditions into account.

KEY WORDS: Deep-sea bacteria · Bacterial activity Pressure-retaining sampler · Pressure effect

INTRODUCTION

Referring to the global oceanic system, most of the photosynthetic primary production is recycled into the upper layers of the water column. Nevertheless, the geochemical cycling in the global ocean cannot tolerate an irreversible loss of this organic material flowing or sinking to the sea bottom. Decomposition of this material depends mostly on the microbial activity in the deep-sea.

The most characteristic features of the deep-sea are scarcity of nutrients, low temperature and high pressure. To act efficiently in deep environments, bacteria have to be adapted to these conditions. Particularly, indigenous deep-sea bacteria should be well-adapted to high-pressure conditions. On the other hand, allochthonous bacteria, coming from the upper part of the water column, would appear unadapted to high-pressure conditions (Yayanos et al. 1982). Depending on the hydrological conditions, the proportion of allochthonous and indigenous bacteria in deep-sea microflora can vary. This discrepancy could explain the contradictory results concerning the effects of hydrostatic pressure on the microbial activity of seawater samples collected in diverse areas (Jannasch & Wirsen 1973, Wada et al. 1975, Jannasch et al. 1976, Deming 1985, Deming & Colwell 1985, Cahet & Sibuet 1986, Cowen 1989, De Angelis et al. 1991).

Sampling northwestern Mediterranean waters at 1100 m depth in stratified conditions we have previously observed a reduction of microbial activity by decompression during retrieval (Bianchi & Garcin 1993). Here we present the comparison of microbial activity exerted in decompressed and undecompressed samples collected at the same depth, but in stratified- and mixed-water conditions.

MATERIAL AND METHODS

Study sites. We collected surface-water samples (20 m deep) and deep-water samples (1100 m) in 2 areas in the northwestern Mediterranean Sea where the sea bottom is 1700 m deep (Fig. 1). The first area, 30 miles southeast of Marseilles, was sampled in stratified-water conditions. The second area, 30 miles southeast of Nice, was sampled both during a mixed-water period and in stratified-water conditions. In this

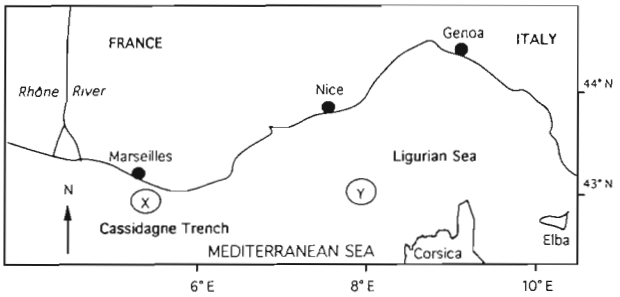

Fig. 1. Location of the sampling stations in the northwestern Mediterranean Sea. Stn X is in the Cassidagne Trench area (stratified waters), Stn Y is in the Ligurian Sea (mixed or stratified waters depending on sampling period)

part of the Mediterranean Sea, at 20 m depth, seawater temperature is ca 20 °C in summer, and 13 °C in winter. The 1100 m deep water is always ca 13 °C.

Sampling procedure. Surface water samples were collected from a depth of 20 m using a Niskin sampler. Before use, the Niskin sampler was acid-cleaned (10% HCl in distilled water), alcohol-sterilized (50/50: v/v) and rinsed with sterile distilled water. Deep-sea samples were collected at 1100 m depth with a pressure-retaining sampler (PRS) already described (Bianchi & Garcin 1993) and with a Niskin sampler fixed 1 m above the PRS, on the same hydrowire. The Niskin samples were transferred immediately into sterile polycarbonate vessels. These vessels and the PRS were transported to the land-laboratory in polystyrene-insulated containers. Under these conditions temperature change during transportation was limited to ±2 °C. This transport delayed sample processing by less than 4 h.

Microbial activity measurements. Decompressed samples were incubated in 2 l polycarbonate flasks kept in the dark under ambient atmosphere at *in situ* temperature ±0.5 °C. A fraction of the decompressed samples was transferred into six 150 ml stainless steel cultivating devices pre-chilled in a water bath at *in situ* temperature and then compressed to 11.14 MPa of hydrostatic pressure. We previously ensured that microbial activity measurements resulting from a 3 h incubation period in polycarbonate vessels or in these stainless steel vessels did not differ significantly (Bianchi & Garcin 1993).

Undecompressed samples were distributed without decompression into six 150 ml cultivating devices (Bianchi & Garcin 1993) pre-chilled in a water bath at *in situ* temperature. We used D-(U-^{14}C)-glucose, specific activity 10.6 GBq mmol^{-1} (Amersham Corp.), as radiolabeled substrate. For both decompressed and undecompressed samples the final concentration was 5.8 nmol glucose l^{-1}.

After labeling, 150 ml subsamples were formalin fixed (final concentration 1%), at zero-time (control), 30, 60, 90, 120 and 180 min. Fixed samples were filtered through 0.2 µm polycarbonate filters. Radioactivity was counted by liquid scintillation (Beckman LS 1800). Counting efficiency and quenching correction were determined by use of internal standards. Data were corrected against those from control samples fixed immediately after label addition.

Preliminary studies showed that in these deep Mediterranean waters a 5 nM glucose concentration was above the saturation concentration of the glucose uptake systems. Therefore we estimated the maximal carbon incorporation rate (Vm, g C l^{-1} h^{-1}) according to:

$$Vm = \frac{\text{dpm incorporated} \times \text{g C dpm}^{-1} \times 1000}{\text{Incubated volume (ml)} \times \text{Incubation time (h)}}$$

Glucose assimilation rates were not normalized to bacterial numbers, as epifluorescence microscopy does not allow the determination of the actual percentage of bacteria able to use ^{14}C-glucose.

Bacterial numbers. Samples for bacterial counts were fixed in 2% formalin, stored at 4 °C and stained with diamidinophenylindole (Porter & Feig 1980). Filters were enumerated by epifluorescence microscopy (Olympus, BH2).

RESULTS

Bacterial activity in surface waters

Bacterial densities range between 3.6 and 7.6 × 10^5 cells ml^{-1} in the surface-water and between 0.9 and 9.3 × 10^4 cells ml^{-1} in deep-water samples collected in the Cassidagne Trench and in the Ligurian Sea (Table 1).

Samples collected at 20 m depth in the Cassidagne Trench area during fall appeared moderately active in ^{14}C-glucose incorporation, with a mean of 251 ± 35.1 pg C l^{-1} h^{-1} (±SE, n = 10) (Table 1). Samples collected at the same depth in the Ligurian Sea during winter appeared less active than those collected under fall conditions (at equal temperature conditions) in the Cassidagne Trench, with a mean value of 174.3 ± 24.2 pg C l^{-1} h^{-1} (n = 6). Samples collected in the same area in summer (T = 20 °C) appeared more active in ^{14}C-glucose incorporation (706.6 ± 73.4 pg C l^{-1} h^{-1}, n = 4).

Simulation of surface water sinking

Table 1 shows that when winter surface-water samples were pressurized at 11.14 MPa, simulating a sinking of water to 1100 m depth, carbon incorporation into

Table 1 ^{14}C-glucose incorporation rates (pg C l^{-1} h^{-1}) for Mediterranean seawater samples incubated under different hydrostatic pressure conditions. Mean values ± SE. Number of samples given in parentheses. Temp.. *in situ* temperature is also incubation temperature; Bacteria: total number (epifluorescence microscopy); Atm. pressure: samples decompressed during retrieval and incubated under atmospheric pressure conditions; nd: not determined

Sampling area	Hydrological conditions	Sampling depth (m)	Temp. (°C)	Bacteria (10^4 ml^{-1})	Glucose uptake rates Atm. pressure	11.4 MPa
Cassidagne Trench	Stratified	20	13	54.0 ± 4.5	251.0 ± 35 (10)	22.1 ± 7 (3)[a]
Ligurian Sea	Mixed	20	13	35.8 ± 3.8	174.3 ± 24 (6)	30.5 ± 4 (3)[a]
Ligurian Sea	Stratified	20	20	57.6 ± 4.3	706.6 ± 73 (4)	nd
Cassidagne Trench	Stratified	1100	13	2.2 ± 0.4	30.7 ± 10 (6)	78.5 ± 24 (6)[b]
Ligurian Sea	Stratified	1100	13	3.4 ± 0.6	23.6 ± 14 (8)	59.5 ± 11 (8)[b]
Ligurian Sea	Mixed	1100	13	7.3 ± 2.1	421.2 ± 43 (3)	14.5 ± 6 (3)[b]

[a] Samples compressed under laboratory conditions
[b] Samples undecompressed during retrieval and incubation

the microbial biomass at 13 °C decreased drastically (22.1 ± 7.3 and 30.5 ± 4.4 pg C l^{-1} h^{-1} for Cassidagne Trench and Ligurian Sea samples, respectively).

Bacterial activity in deep samples

Bacteria collected at 1100 m depth in the Cassidagne Trench, under stratified-water conditions at 13 °C, were less active than surface-water bacteria. Carbon incorporation rates (Table 1) appeared significantly higher in the undecompressed samples (78.5 ± 24.4 pg C l^{-1} h^{-1}, n = 6), than in the decompressed ones (30.7 ± 10.2 SE, n = 6). Difference appeared significant at $p < 0.05$.

Four samples in both winter and summer were collected in the Ligurian Sea at 1100 m depth in stratified-water conditions. For all these samples, incorporation rates were lower than those exerted by the Cassidagne Trench samples (Table 1). The 'dpm incorporated' of some samples were not higher than formalin killed blanks, as already observed in this area by Cahet & Jacques (1976). When active, these microflora exhibited a barophilic behavior, as did the Cassidagne Trench deep-sea microflora, being significantly (at $p < 0.05$) more active when incubated without decompression (59.5 ± 10.9 pg C l^{-1} h^{-1}, n = 8) than under atmospheric pressure (23.6 ± 14.4 pg C l^{-1} h^{-1}, n = 8).

Bacteria collected at the same sampling station at the same depth in the Ligurian Sea during the mixed-water period responded to pressure in the opposite way that microorganisms sampled in stratified-water conditions did. In these samples, we observed an adverse pressure effect on carbon assimilation (Table 1). Bacteria decompressed during retrieval and incubated at atmospheric pressure appeared significantly more active (421.2 ± 43.3 pg C l^{-1} h^{-1} at $p < 0.005$) than the undecompressed ones (14.5 ± 6.3 pg C l^{-1} h^{-1}, n = 3).

Generally, bacterial densities did not vary significantly in the samples during the 3 h incubation period, regardless of the pressure conditions.

DISCUSSION

Microbial densities in seawater samples collected at 1100 m depth varied with the geographical sampling area and with time. Even under deep-sea conditions, bacteria are not homogeneously distributed in both space and time.

Microbial activity measurements showed a large discrepancy between surface-water and deep-water samples. Surface-water bacteria are not adapted to growth at high pressure, as observed by Turley (1993). In our study, activity in deep-water samples depended on hydrological conditions. Rheinheimer et al. (1989) reported differences of nearly 2 orders of magnitude between mixed layer and 'winter water' in the Baltic Sea. Poremba (1994) observed recently that microbial degradation of phytodetritus in deep-sea sediments was positively influenced by elevated pressure mostly in summer.

Measurements of glucose assimilation without decompression or under atmospheric pressure conditions showed that hydrostatic pressure influences microbial activity. Pressure effects varied with the origin and the history of the microbial communities. Activity of surface-borne bacteria appeared to be affected by increased hydrostatic pressure. This adverse effect was observed on samples collected in the surface layers of the ocean (20 m depth), as well as on samples collected deeper in the water column (1100 m), when the seawater bodies rapidly sank to this depth. These bacteria exhibited lower metabolic activity when pressurized at the sampling depth pressure (11.14 MPa) than when under atmospheric pressure conditions. Our data are

limited to the carbon assimilation rates, therefore we do not know the influence of increasing depth on microbial respiration rates. It is likely, however, that as any other stress (Tison & Pope 1980, Griffiths et al. 1984), hydrostatic pressure also influences the respiratory rates.

These results contradict the previous observations by Wada et al. (1975) that nitrate metabolism in surface water is not substantially inactivated by exposure to high pressure (up to 600 atm). But these authors used peptone and yeast extract enrichment (5.0 and 1.0 g l^{-1}, respectively) and incubation periods of 128 or 212 h. These experimental conditions could have caused drastic modifications of the natural microbial community.

During the mixed-water period, bacterial concentration and activity at 1100 m depth were higher than usually found in this area at this depth. The samples decompressed during retrieval appeared 30-fold more active than the undecompressed ones. This pattern of bacterial activity lets us suggest that bacteria sampled during mixed-water column conditions are surface-water bacteria mixed into the deeper waters by hydrological processes. When incubated under atmospheric pressure, carbon assimilation by these samples appeared significantly higher than under stratified conditions in the Cassidagne Trench. More surprisingly, these bacteria collected at 1100 m appeared more active than the corresponding surface bacteria. We could hypothetize that some of the surface-borne microorganisms could be irreversibly damaged by the successive pressure stresses, releasing organic compounds into the incubation vessel. This enrichment could stimulate the metabolic activity of the surviving surface bacteria when relieved from the inhibitory effect of hydrostatic pressure.

Bacteria collected in deep-water masses in stratified-water conditions exhibited higher assimilation rates when incubated without decompression than at atmospheric pressure. The ratio undecompressed/decompressed is similar (≈ 2.5) for the 2 sets of samples collected at the same depth in the Cassidagne Trench and in the Ligurian Sea. This ratio indicates that in stratified conditions the deep-sea bacteria are well adapted to the hydrostatic pressure conditions exerted in their natural environment.

In these experiments we were able to compare the microbial activity of surface-water and deep-sea samples using the same incubation temperature. In the Mediterranean Sea, during the late fall and winter period, temperature conditions (13 °C) vary only slightly or not at all over the whole water column. In this way, the Mediterranean offers an exceptional opportunity to compare the microbial activity throughout the water column under increasing pressure but constant temperature conditions. These conditions permit one to distinguish between the low-temperature and the high-pressure effects that are usually linked in cold deep-sea environments (ZoBell & Johnson 1949, Jannasch & Wirsen 1973, Wada et al. 1975, Yayanos & Dietz 1982).

Decreasing the natural hydrostatic pressure conditions during sample retrieval and incubation can provoke either inhibitory or stimulatory effects on microbial cells. Our results demonstrate that these 2 opposite patterns could depend on the origin of the bacterial communities. In the marine environment, large scale transportations of free-living bacteria are mainly linked to hydrological conditions. Therefore the pressure effect, like temperature effects on natural communities (Bianchi 1987, Bird & Karl 1991), depends on the history of seawater masses. The knowledge of hydrological conditions could explain some controversial results in deep-sea microbial ecology.

CONCLUSIONS

Until now, most of the barophilic activities already described in the literature correspond to bacteria linked to the large particles sinking through the water column (Deming & Colwell 1985), to bacteria associated with the digestive tracts of deep-sea invertebrates (Schwarz et al. 1976, Yayanos et al. 1979, Deming et al. 1981), or to microorganisms acting at the water-sediment boundary layer (Cahet & Sibuet 1986). Free-living bacteria and bacteria attached to sinking particles react differently to increased hydrostatic pressure (Turley 1993). The 2 sampling devices used here, the Niskin sampler and the pressure-retaining sampler, do not collect the large sinking particles and, therefore, bacteria attached to large sinking particles would have been excluded. Our experiments showed clearly that the free-living bacteria and bacteria linked to small particles, comprising over 90% of the heterotrophic activity in marine waters (Azam & Hodson 1977), are sensitive to pressure conditions.

These experiments demonstrated that, regardless of the hydrological conditions, microbial activity measurements on decompressed samples do not reflect the actual activity of the microbial consortia in the deep ocean. Depending on the mixing conditions, 'conventional' decompressed samples will yield either an under- or an overestimation of the actual microbial activity. It is difficult to assess a precise depth limit where pressure affects bacterial activity. Nevertheless our experiments showed that, in the Mediterranean environment, microbial activity was clearly affected by pressure conditions at 1100 m depth.

Acknowledgements. The authors thank the 'Société Nationale Elf Aquitaine' for providing the pressure-retaining device (Convention SNEA #8280). We are grateful to the captains and crews of RV 'Antedon' and RV 'Korotneff' for cruise assistance. Special thanks to Gerhard Herndl and to 3 reviewers for their helpful comments and suggestions to improve this manuscript. This work was supported by the Institut National des Sciences de l'Univers, Programmes Flux Océaniques EUMELI and DYFAMED.

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This article was submitted to the editor

Manuscript first received: February 7, 1994
Revised version accepted: June 1, 1994