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Assimilation of marine extracellular polymeric substances by deep-sea prokaryotes in the NW Mediterranean Sea

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Running title: EPS assimilation by deep-sea prokaryotes

Abstract

This study examined total uptake of extracellular polymeric substances (EPS) and glucose and the percentage of prokaryotic cells (Bacteria, Crenarchaea and Euryarchaea) consuming these compounds in the major water masses at the DYFAMED site (NW Mediterranean Sea). The potential assimilation rates of EPS at 10m-depth were higher but on the same order of magnitude as those at 2000m-depth (from 43.4 to 29.0 pmol L^{-1} h^{-1}). In contrast, glucose assimilation rates decreased with depth from 49.4 to 0.07 pmol L^{-1} h^{-1} at 10 and 2000m-depth, respectively. Microautoradiography analyses indicated similar percentages of active cells assimilating EPS at 10 and 2000 m-depth (13% and 10% of the total-cells). The combination of microautoradiography and catalyzed reporter deposition fluorescence *in situ* hybridization (MICRO-CARD-FISH) analyses revealed that the percentages of Bacteria assimilating ^{3}H-carbohydrates decreased with depth by 2-fold for EPS. In contrast, the contribution by Euryarchaea to EPS consumption increased with depth by 6-fold. At 2000m, 50% of active cells consuming ^{3}H-carbohydrates were Euryarchaea. These data highlight potential differences in the roles of Bacteria and Archaea in the deep sea biogeochemical cycles and shed light on the importance of deep-sea Euryarchaea in the degradation of dissolved organic matter.

1 *Introduction*

Over the last decade, research has unequivocally shown that prokaryotes (Bacteria and Archaea) are the main drivers of marine biogeochemical cycles in the ocean. The dark ocean, below the euphotic zone, is characterized by the increase of hydrostatic pressure with depth, the decrease of temperature and the sporadic input of organic matter. Deep-sea microorganisms display unique metabolic capabilities with respect to the degradation of complex organic matter (Vezzi et al., 2005; DeLong et al., 2006), molecular architectures (Lauro and Bartlett, 2007) and metabolic activities adapted to *in situ* conditions (Deming et al., 1980; Tabor et al., 1981; Jannasch and Wirsen, 1982; Tamburini et al., 2003) that allow them to thrive under cold and high-pressure conditions. Data from fluorescence *in situ* hybridization (FISH) studies indicates that Archaea make up a larger fraction of total prokaryotic abundance in the dark ocean than in the surface waters of the North Pacific and North Atlantic Oceans (Karner et al., 2001; Teira et al., 2004; Herndl et al., 2005) and of the Mediterranean Sea (Tamburini et al., 2009; Winter et al., 2009). However, little is still known about the metabolic capacity and biogeochemical roles of both Bacteria and Archaea in the dark ocean. Using microautoradiography with FISH (or catalyzed reporter deposition-FISH, CARD-FISH), it is possible to link assimilation of specific radiolabeled compounds to individual members of the prokaryotic community. Recent studies suggest that some Archaea are chemoautotrophic, able to fix CO_2 (Herndl et al., 2005) and some are heterotrophic (or mixotrophic) assimilating for example amino acids in the North Atlantic Ocean (Herndl et

al., 2005; Teira et al., 2006b). However, the role of marine Archaea in assimilating other organic compounds is still not clear.

Semi-labile dissolved organic matter (DOM) is defined as the fraction of the total DOM pool that escapes rapid degradation by marine heterotrophs in surface waters and is available for export to the dark ocean (Carlson et al., 1994). Semi-labile DOM is made up of a variety of compounds including extracellular polymeric substances (EPS) which includes complex organic macromolecules such as lipids, nucleic acids, proteins and carbohydrates. Carbohydrates are the most abundant component, generally representing 40 to 95% of the EPS in marine environments (Hoagland et al., 1993; Bhaskar et al., 2005). Chemically, EPS are rich in high molecular weight polysaccharides, thus, EPS are considered as a part of high molecular weight dissolved organic matter (HMW-DOM > 1kDa) (Aluwihare et al., 1999; Bhaskar et al., 2005). Within high molecular weight (HMW)-DOM, carbohydrates represents 40 to 50% in surface waters of DOM and 10 to 30% in the deep-sea waters (Benner et al., 1992; Aluwihare et al., 1997). Lastly, the deep Mediterranean water masses may represent a peculiar case since they are regularly enriched by organic carbon input during deep water formation events (open-sea convection as well as dense shelf water cascading) (Canals et al., 2006; Santinelli et al., 2010). The aim of this study was to determine the capacity of deep-sea prokaryotes to degrade and assimilate complex carbohydrates (^{3}H-EPS) by measuring both bulk and single-cell activity measurements. We used a simple carbohydrate (^{3}H-glucose) as an indicator of the assimilation of labile compounds. Experiments were conducted in triplicate at three depths in the water column at the

DYFAMED site (NW Mediterranean Sea). To accurately measure prokaryotic activities in the deep-sea waters, we performed our sampling, retrieval and incubation under *in situ* temperature and pressure conditions (see Supporting Information for more details) using our high-pressure sampler (Bianchi et al., 1999; Tamburini et al., 2003).

2 Results and discussion

Microbial assimilation rates

The potential assimilation rates of EPS ranged from 43.4 pmol L^{-1} h^{-1} at 10m to 29.0 pmol L^{-1} h^{-1} at 2000m-depth (Table 1). In contrast, glucose assimilation rates (glucose being added at "trace" concentration) diminished by three orders of magnitude from the surface to the deep waters (from 49.4 to 0.07 pmol L^{-1} h^{-1}). In order to determine the contribution of the total prokaryotic community to ^{3}H-EPS and ^{3}H-glucose assimilation, we divided the number of DAPI-positive cells that were silver grain positive as revealed by microautoradiography by the total-cells counts determined by DAPI staining (Table 1). The percentage of the prokaryotic community actively assimilating ^{3}H-EPS was similar at 10 and 2000m-depth (13 ± 6% and 10 ± 1%). In contrast, the percentage of total prokaryotes actively assimilating ^{3}H-glucose decreased with depth from 34% at 10 m to 5% at 2000m-depth (Table 1). In bathypelagic waters of North Atlantic Ocean, microautoradiography analyses (Herndl et al., 2005; Teira et al., 2006b) indicated that about 5% and 16% of the heterotrophic prokaryotic community take up D- and L-aspartic acid and leucine. The potential assimilation of ^{3}H-EPS and ^{3}H-glucose has been studied only in the surface waters of the Delaware Estuary (Elifantz et al., 2005) and of the

Arctic Ocean (Elifantz et al., 2007; Kirchman et al., 2007). In the Arctic, a higher fraction of cells assimilated EPS (35 to 50%) than glucose (10 to 30 %) while most of the surface bacterial groups in the Delaware were more active in assimilating glucose than ^{3}H-EPS (Kirchman et al., 2007). Different trophic conditions in surface waters of Delaware Estuary and Arctic Ocean could explain differences found in glucose and EPS assimilation by prokaryotic communities (Elifantz et al., 2007; Kirchman et al., 2007). In the present study, we estimated that the percentage of cells assimilating glucose is 3 times higher than the percentage of cells assimilating ^{3}H-EPS in surface waters while in deep waters the percentage of cells assimilating ^{3}H-EPS is 2 times higher than for ^{3}H-glucose (see Table 1).

^{3}H-compounds specific-cell activities

In order to determine the specific activity per cell according to the depth and the substrate assimilated, we divided the ^{3}H-EPS or ^{3}H-glucose assimilation rates by the number of silver grain-positive cells (consuming tritiated substrates), as revealed by microautoradiography. Deep-sea prokaryotes assimilated more EPS than surface prokaryotes ; the cell-specific assimilation rate increased (more than 6-fold) from 0.7 at 10m to 4.5 amol assimilating cell^{-1} h^{-1} at 2000m-depth (Figure 1A). For the glucose, the opposite trend was observed with a decrease of one order of magnitude from 0.2 at 10m to 0.01 amol cell^{-1} h^{-1} at 2000m-depth (Figure 1B). Growth of deep-sea prokaryotes is C-limited and deep-sea prokaryotes deployed high levels of ectoenzymatic activities to recover carbon and energy coming from complex organic matter (Nagata et al., 2000;

Hoppe et al., 2002; Tamburini et al., 2009; Baltar et al., 2010). Also, EPS being a complex mixture of compounds including mono- and polysaccharides, lipids, proteins and non-sugar moieties (Bhaskar et al., 2005), they provide a large spectrum of macromolecules to deep-sea prokaryotic assemblage. When faced with a lack of nutrient sources, heterotrophic prokaryotes could be better adapted to a "mixed substrate growth" as suggested by Egli (2010).

Contribution of phylogenetic groups assimilating ^{3}H-compounds

Using microautoradiography coupled with CARD-FISH, we compared throughout the water column the contribution of Bacteria, Crenarchaea and Euryarchaea to the degradation and assimilation of HMW and LMW compounds (^{3}H-EPS and ^{3}H-glucose). We calculated for each phylogenetic group and substrate the number of active cells that were probe and silver grain-positive. We normalized the contribution of each group to the number of total-cells that were silver grain-positive for ^{3}H-EPS and ^{3}H-glucose (Figure 2).

In the upper layer, Bacteria dominated the assimilation of ^{3}H-EPS and ^{3}H-glucose, representing 74% and 88% of cells consuming ^{3}H-compounds, respectively (Figure 2A and B), in concordance with data obtained in surface waters of the Arctic Ocean (Elifantz et al., 2005; Kirchman et al., 2007). Crenarchaea and Euryarchaea contributed less than 20% to the ^{3}H-EPS and 10% to the ^{3}H-glucose assimilation (Figure 2A and B).

At 2000m-depth, Bacteria represented 38% of the assimilation of ^{3}H-EPS and 32% of the assimilation of ^{3}H-glucose (Figure 2A and B). Crenarchaea contributed weakly to ^{3}H-

EPS and ^{3}H-glucose assimilation. Several authors have shown that Crenarchaea in the dark ocean are mixotrophic or chemoautotrophic (Herndl et al., 2005; Ingalls et al., 2006; Kirchman et al., 2007) and are ammonium-oxidizers as revealed by the presence of the *amoA* gene (Francis et al., 2005; Konneke et al., 2005; Hallam et al., 2006). Ingalls *et al.* (2006) suggested that the use of organic carbon through mixotrophy or heterotrophy might be associated with the need to derive energy. Herndl *et al.* (2005) showed that Euryarchaea also assimilate inorganic carbon, but their contribution decreased with depth. Their heterotrophy capacity has also been highlighted (Herndl et al., 2005; Teira et al., 2006a; Teira et al., 2006b), since this archaeal group is also able to assimilate amino acids and leucine. In this experiment, Euryarchaea were the main contributors to ^{3}H-EPS and ^{3}H-glucose assimilation at 2000m-depth, representing respectively 58% and 53% of the total cells actively assimilating ^{3}H-EPS and ^{3}H-glucose (Figure 2A and B). This result, provided from a snapshot view performed in deep-sea waters of the Mediterranean Sea at the DYFAMED Site, highlights a potential major role of Euryarchaea in the deep-sea waters. Consequently, more experiments are required to generalize the role of Euryarchaea in the carbon cycle.

3 Concluding remarks

Our study supplies new clues about the interactions between deep-sea prokaryotes and the degradation of different size classes of DOM. In this preliminary work, we have performed an original experiment taking into account *in situ* conditions (of pressure and

169 temperature). These conditions are potentially crucial to correctly estimating the deep-
170 sea prokaryotic capacity to degrade complex organic matter (Vezzi et al., 2005).
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184 **Figure captions**

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186 Figure 1: The specific-cell activity assimilating **(A)** ^{3}H-EPS and **(B)** ^{3}H-Glucose (amol cell^{-1} h^{-1}) at 10m,
187 500m and 2000m-depth. **Note the different scale between figure A and B.** Errors bars indicate standard
188 deviation (n=3)

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190 Figure 2: Contribution of Bacteria, Crenarchaea and Euryarchaea to ^{3}H-EPS degradation **(A)** and ^{3}H-
191 glucose assimilation **(B)** at 10, 500 and 2000m-depth, respectively, at DYFAMED site (NW Med. Sea).
192 Contribution expressed as percentage of total silver grain and DAPI-positive cells as determine by
193 microautoradiography. Errors bars indicate standard deviation (n=3)

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Table 1: Assimilation rates (pmol L^{-1} h^{-1}) and percentage of cells consuming ^{3}H-EPS and ^{3}H-Glucose by the total prokaryotic community at 10, 500 and 2000m-depth at the DYFAMED site in April 2008. Percentages assayed by micorautoradiography (DAPI silver grain-positive) and were calculated relative to the total prokaryotic community (DAPI total counts). Mean values ± SD are given (n=3).

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		Consumption rates (pmol L^{-1} h^{-1})	% of cell consuming ^{3}H-compounds (% from total DAPI-cells)
	10m	43.3 ± 4.2	13.1 ± 6.2
EPS	500m	33.9 ± 4.2	2.8 ± 1.7
	2000m	27.0 ± 3.4	10.6 ± 1.4
	10m	49.3 ± 0.84	34.1 ± 4.5
Glucose	500m	0.1 ± 0.01	2.6 ± 0.9
	2000m	0.1 ± 0.02	4.8 ± 1.1

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