Microbial food webs under severe nutrient limitations: Life in the deep sea

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ABSTRACT

Particulate organic matter (POM) settling to the floor of the deep-sea is the nutritional basis for benthic life. This material loses most of its labile organic fraction during descent in the water column. Therefore, survival of bacteria in the deep-sea environment depends to a large extent on the production and regulation of extracellular enzymes. The amount of active extracellular enzymes, as well as the relative proportions of enzymes, changes as POM changes in relation to water depth. Studies carried out from shelf to deep sea in the Laptev Sea (Arctic Ocean) and in the Arabian Sea (Indian Ocean) have shown that different levels of food supply are reflected by different concentrations of hydrolytic enzymes. In deep-sea sediment samples, experimental additions of specific substrates induce the production of glucosidases and chitobiases, or repress the activity and production of peptidases. These differences in regulation imply different functional roles of these enzymes in the environment. Enzyme activities in the sediment show significant correlations with parameters related to organic carbon input and may potentially be used as indicators of organic matter turnover in the sediment.

Introduction

The deep sea is not only the largest ecosystem on earth, but exhibits a number of fundamental differences compared to most other ecosystems. There is no primary production of new organic matter, except for the globally small productivity at hydrothermal vents and cold seeps. All organic material has to be transported to the deep sea by sedimentation from the productive upper water column, or lateral transport from continental shelves. During this transport a large proportion of the labile organic material is lost [22]. It can be assumed that the scarce supply of organic material from the productive zones, rather than high pressures or low temperatures, are the main controlling factors in the deep-sea environment. The abundant life proliferating at hydrothermal vents and cold seeps is proof that, provided sufficient substrate is available, high production is possible. For deep-sea microbial communities competition for available food resources is presumably a much more important selective pressure than avoidance of predation, and relevant adaptive strategies had to be developed. Thus, efficient and economic utilisation of the sedimenting POM is the key to survival of deep-sea bacteria, and extracellular hydrolysation is the first process required to make this material available for bacterial consumption. The aim of this paper is to show that deep-sea bacteria regulate the production of extracellular enzymes according to availability of substrates in order to not only minimise energy expenditure, but to also allow maximal use of deposited organic particles. As this

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regulation is directly related to the influx of biologically utilisable organic matter to the deep-sea floor, it may be used as a proxy for assessment of the input of labile organic matter in this particularly inaccessible environment.

Degradation of Particulate Organic Matter in the Deep Water Column

On descent through the water column, microbial degradation [11, 17, 18] and zooplankton consumption reduce the amount of sinking POM exponentially [see e.g., 20]. Wakeham and Lee [1] found a 25 and 60 fold reduction in amino acids and fatty acids respectively in sediment traps located at 389 m and 5068 m, while total POC decreased by only 5 fold. Thus, with depth the more labile components, such as proteins, storage carbohydrates and lipids, disappear and POM reaching the deep sea is enriched in organic matter having longer half-lives, such as cellulose, chitin, structural proteins and various other cell wall components [5].

On particles collected from different depths in the Arabian Sea the activity of extracellular enzymes (see [9] for methods) changed not only in absolute values but also in relative contribution (Fig. 1). The sum of activities of the four measured enzymes decreased by a factor of 10 in samples from 60 m depth compared to those from below 1000 m depth. Aminopeptidase was in all cases by far the most active enzyme. The proportions of α- and β-glucosidases decreased and chitobiase increased with depth. This change in enzymatic pattern probably reflects changes in the chemical composition of POM. The cell specific activities only declined by a factor of 3-5 between 60m and 250 m. Chitobiase and peptidase per bacterial cell even increased in the deeper samples compared to the 250 m samples. Similarly, Hoppe et al. [10] found a 2-3-fold increase in peptidase activity per cell in water depths of 0-500 m.

Degradation of POM in Deep-sea Sediments

POM reaching the deep-sea floor is composed of macromolecules with long half-lives. Virtually all soluble organic material is lost [22]. Therefore, hydrolysation of the particulate material is a prerequisite for survival of bacteria, and their dependence on extracellular enzymes is more extreme in this environment than in the upper water column. Seasonal events of massive sedimentation of phytoplankton detritus to the deep sea [16, 19] necessitate fast reaction to these sudden food inputs. There is a fierce competition for this phytodetritus as most deep-sea organisms, from bacteria to holothuria, depend on this food. Therefore, bacteria have to be able to increase their set of enzymatic 'tools' in order to utilise this resource. However, they can only afford high enzymes stocks if the supply of labile organic matter is sufficient to cover the 'costs' of enzyme production [21]. Regulation of enzyme production is most likely one of the crucial capabilities required to survive in the deep-sea environment.

It can be shown that deep-sea microbial communities are in fact capable of processing relatively large quantities of particulate matter. In experiments carried out according to the methods described in Lochte [13], surface sediment samples amended with increasing amounts of particulate ^{14}C-labelled algal material showed a linear increase in production of $^{14}CO_2$. Even when up to 15 times (75 mg C m^{-2} d^{-1}) the average organic carbon flux rate (5 mg C m^{-2} d^{-1}) at this station was added, no saturation of the mineralization rate was observed. This indicates that the microbial population had the capacity to degrade a much

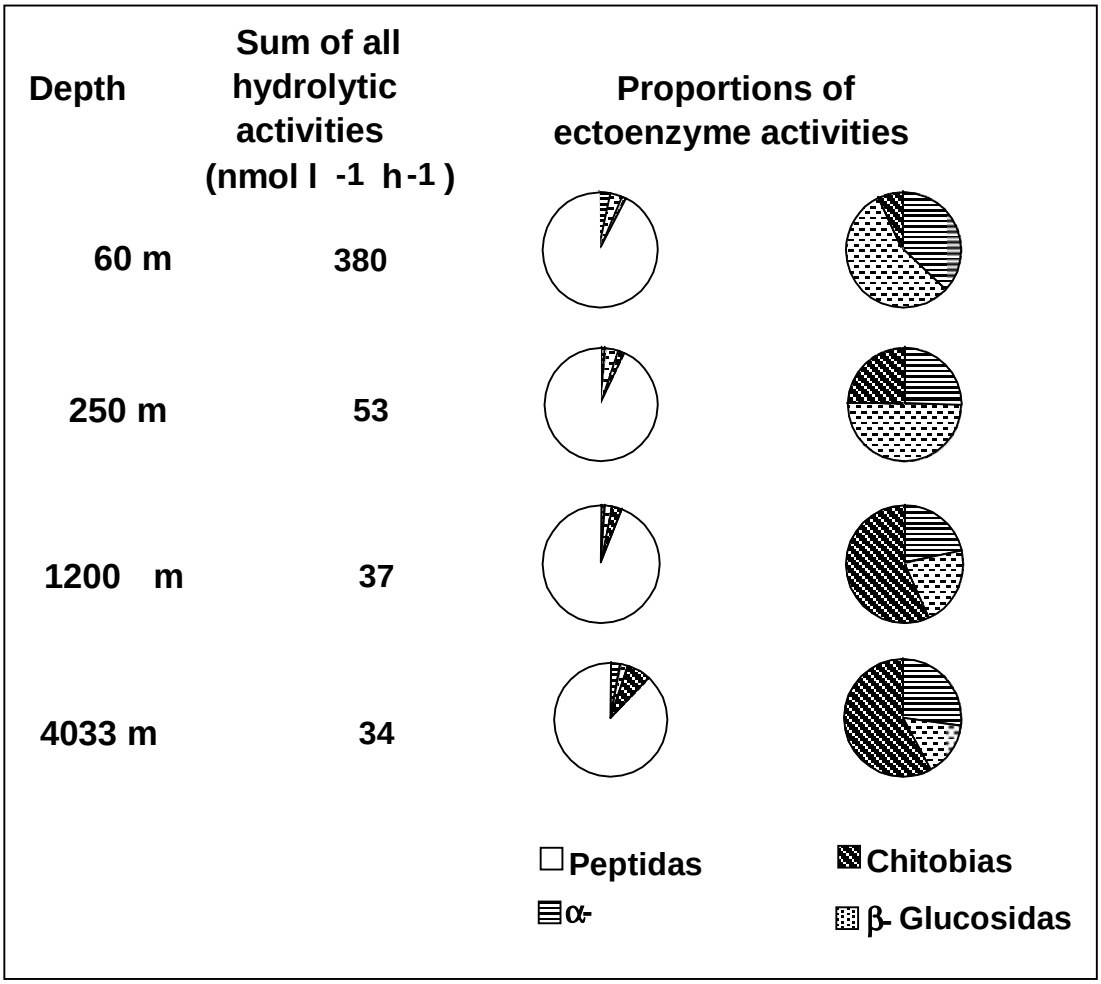

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Fig. 1

Fig. 1. Activities of four extracellular enzymes on particles collected in different depths (60 m, 250 m, 1200 m, 4033 m, 10m above bottom) at station WAST in the Arabian Sea in March 1997. Sea water was enriched with particles approximately 1:2 by tangential flow and incubated in the dark at 15 °C and atmospheric pressure for up to 27 days. The sum of hydrolysis rates of the four enzymes is given and the relative proportion of each enzyme is shown in pie diagrams. In the left column of the pie diagrams all four enzymes are shown, in the right column the dominant peptidase is omitted to show the changes in the other three extracellular enzymes. Enzyme activity was measured at saturating concentrations (see [9] for methods).

greater amount of organic matter than what would be supplied by the average sedimentation rate. This may be an adaptation to make optimal use of sudden pulses of sedimentation when large amounts of material are deposited within a short time.

Two case studies to investigate whether the amount of enzymes in sediments is related to different levels of organic matter supply are presented below.

Laptev Sea

In surface sediments collected from the continental slope of the Laptev Sea, Arctica, in the area from the mouth of the river Lena (25 m depth) to the deep Arctic Basins (3500 m depth), concentrations of chloroplastic pigments, proteins and dissolved free amino acids

decreased from shallow to deep stations [3, 5]. Activities of α- and β-glucosidases and chitobiases in surface sediments (see [1] for methods) generally declined with depth similar to the decline in organic material, while bacterial numbers and biomass, determined by microscopic counts, remained fairly constant. This indicates that bacterial communities maintain different levels of enzyme activity depending on the food supply.

Peptidase activity showed a completely different pattern increasing up to 6 fold with depth [3]. At concentrations of dissolved free amino acids above 10µg glycine equivalents cm^{-3}, peptidase activity remained low but increased greatly when amino acid concentrations fell below this threshold. Since bacterial numbers and biomass showed little change, the specific peptidase activity follows the same trend and was much higher in samples from the deep sea than from the shelf. We interpret this as a response to limitation of biologically available nitrogen compounds in the deep-sea sediment [2, 3].

Arabian Sea

Five stations in the Arabian Sea were investigated during 1995-1998 as part of the deep-sea research programme BIGSET. These stations are influenced to different degrees by monsoon-induced sedimentation. Station WAST in the western part is the most affected and has the highest flux rates [7], while the southern station SAST is least affected and the other stations (NAST, CAST, EAST) are intermediate (Table 1). Accordingly, concentrations of chloroplastic pigments in the surface sediments, indicating input of phytodetrital matter, were significantly higher at WAST compared to SAST [Pfannkuche, unpublished data]. All microbiological parameters in the surface sediments declined similarly from WAST to SAST. These parameters included total microbial biomass, estimated from phospholipids as a measure of all small organisms from bacteria to meiofauna, activities of β-glucosidase and chitobiase, and respiration of ^{14}C-labelled algae,

Table 1: Regional variability at four stations in the Arabian Sea and, for comparison, a station in the NE Atlantic (BIOTRANS).*

Stations	**WAST**	**CAST**	**EAST**	**SAST**	**BIOTRANS**
Latitude	16° 20' N	14° 24' N	15° 36' N	09° 59' N	47° 10' N
Longitude	60° 30' E	64° 33' E	68° 34' E	64° 59' E	19° 35°W
Depth	4040 m	3950 m	3820 m	4420 m	4450 m
POC flux (mg C m^{-2} a^{-1})	3.2	1.9	2.1	1.2	1.4
CPE (mg m^{-2})	53.8	7.5	12.4	3.1	3.0
Microbial Biomass (g C m^{-2})	4.5	0.8	2.8	0.7	0.6
β-glucosidase activity (µmol m^{-2} hr^{-1})	8.0	5.5	4.8	2.5	2.0
chitobiase activity (µmol m^{-2} hr^{-1})	44.7	22.8	22.1	11.4	10.0
Respired ^{14}C algae (% d^{-1})	40	25	16	28	19

*All benthic data represent the 0-1 cm sediment layer. Particulate organic carbon flux in sediment traps 1000 m above bottom [7, 8, 12], chloroplastic pigments in the surface sediment [15, and Pfannkuche unpublished data], total microbial biomass estimated from phospholipids (see [1] for methods), activity of β-glucosidase and chitobiase (see [1] for methods), mineralisation of ^{14}C-labelled algae (see [13] for methods).

as a measure for turnover rates of organic material. Thus total microbial biomass as well as indicators of microbial activity roughly follow the regional pattern of sedimentation of organic matter.

Regulation of Extracellular Enzyme Activity by Substrate Supply

Experimental enrichment of sediments with cellulose or chitin increased the specific activity (i.e., activity per biomass unit) of β-glucosidase or chitobiase, respectively (Fig. 2a,b). This implies that enzyme production is induced by the relevant substrate. The enzyme production was found to be approximately proportional to the amount of material added [1, Boetius, unpublished data).

A notable exception to this pattern of regulation is aminopeptidase. Addition of different substrates did not increase its activity above that of the control (Fig. 2c). Less peptidase activity was found in samples enriched with either easily utilisable substrates, such as glycine or glucose, or enriched with substrates containing nitrogen, such as albumine or chitin. Glycine suppressed peptidase activity immediately due to end product inhibition [2]. Production of peptidases is obviously not inducible. This extracellular enzyme seems to be constitutive and is regulated differently than the other enzymes.

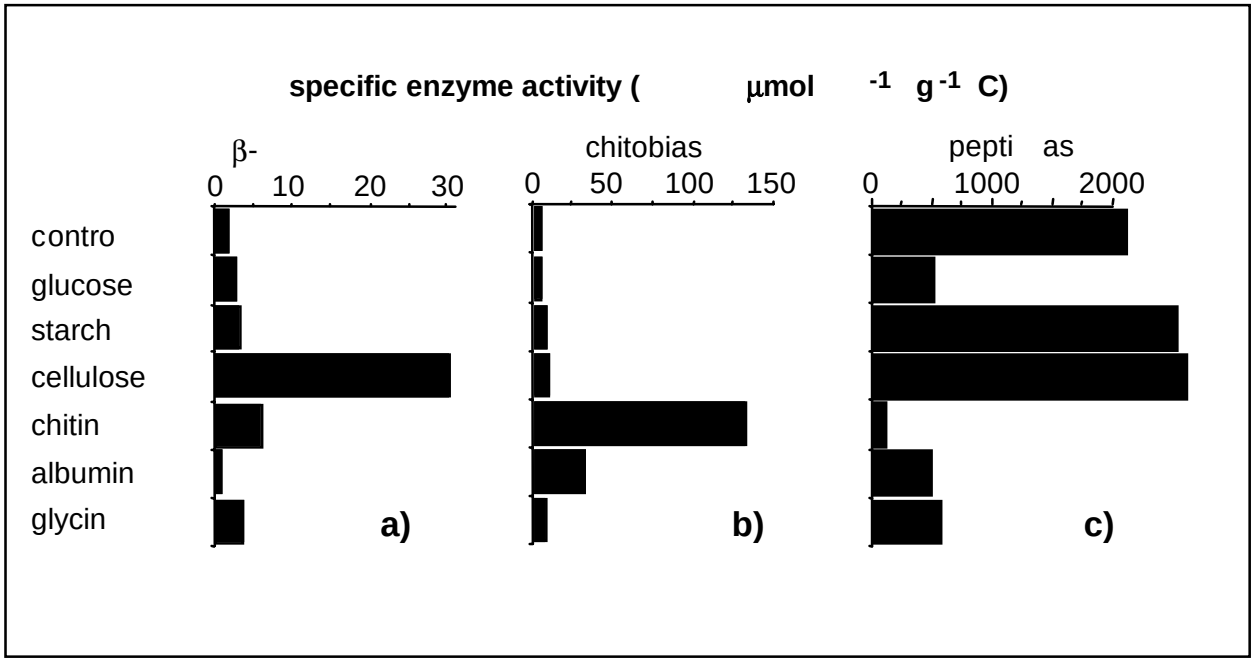

Fig. 2. Specific activities of extracellular enzymes in surface sediments from the arctic continental slope (82° 09'N/42° 02'E, 1013 m) amended with different substrates after 10 days incubation at -1°C and atmospheric pressure: a) ß-glucosidase, b) chitobiase, c) aminopeptidase. (see [1, 2] for methods). The specific activity per g biomass carbon is shown for different added substrates; additions were equivalent to 0.5 to 2 mg C cm^{-3} sediment. (data modified after [2]).

Correlation of Enzymatic Activities With Organic Matter at the Sea Floor

Activity of β-glucosidase in sediments shows a significant correlation with chloroplastic pigments for such widely differing oceanic regions as the Laptev Sea, Mediterranean Sea,

NE-Atlantic and Arabian Sea (Fig. 3). A significant correlation between respiration rates and rates of hydrolysis (sum of α-, β-glucosidase, chitobiase, lipase) was also found ($y = 0.14 + 0.13x$, $r^2=0.828$) [5]. However these data only cover the Arctic continental slope and additional data from other oceanic regions are necessary before conclusions about the generality of this relationship can be drawn.

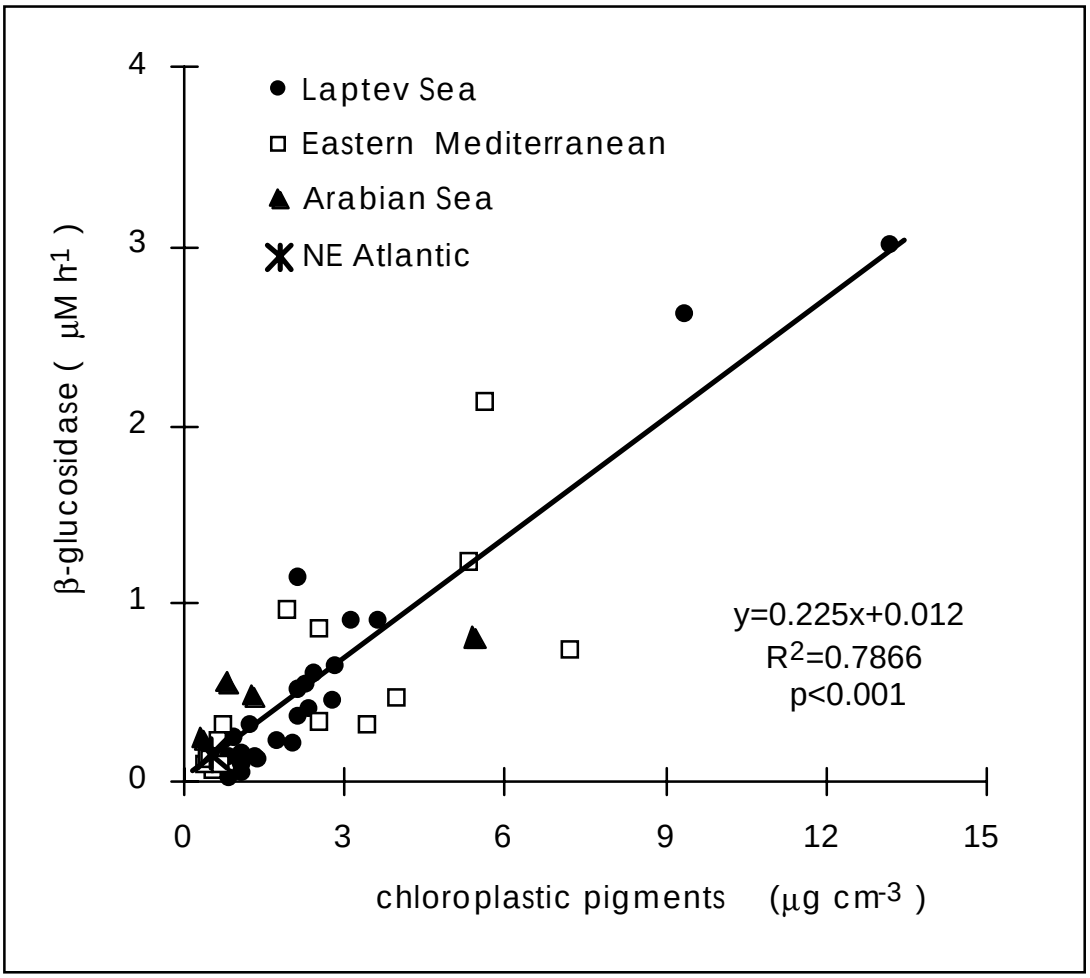

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Fig. 3

Fig. 3. Correlation of β-glucosidase activity with chloroplastic pigments in surface sediments (data from [1, 3, 4, 5]), pigment data from the Arabian Sea [Pfannkuche, unpublished data]).

These correlations are not based on a direct causal relationship, but all of these parameters are controlled by the same factor, i.e., deposition of biologically utilisable organic matter at the sea floor. Sedimenting POM is composed of macromolecules and we have to assume that the chemical composition (types of chemical bonds) of the sedimenting POM is roughly similar. We could show that the production of some of these enzymes is strictly regulated by the relevant substrates. Under these conditions sedimentation of organic matter will induce enzyme production proportional to the amount of labile material

and result in a linear relationship between variables of phytodetritus deposition (e.g., chloroplastic pigments), turnover rates (respiration) and enzymatic activity. Such a relationship could be demonstrated here for β-glucosidase and may also exist for chitobiase and other extracellular enzymes. However, peptidases have a different mechanism of regulation and probably assume a different function in the environment.

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