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Survival of marine heterotrophic flagellates isolated from the surface and the deep sea at high hydrostatic pressure: Literature review and own experiments

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ABSTRACT

Although the abyssal seafloor represents the most common benthic environment on Earth, eukaryotic microbial life at abyssal depths is still an uncharted territory. This is in striking contrast to their potential importance regarding the material flux and bacteria consumption in the deep sea. Flagellate genotypes determined from sedimentary DNA deep-sea samples might originate from vital deep-sea populations or from cysts of organisms sedimented down from surface waters. The latter one may have never been active under deep-sea conditions. We wanted to analyze the principal ability of cultivable heterotrophic flagellates of different phylogenetic groups (choanoflagellates, ancyromonads, euglenids, kinetoplastids, bicosoecids, chrysomonads, and cercozoans) to survive exposure to high hydrostatic pressure (up to 670 bar). We summarized our own studies and the few available data from literature on pressure tolerances of flagellates isolated from different marine habitats. Our results demonstrated that many different flagellate species isolated from the surface waters and deep-sea sediments survived drastic changes in hydrostatic pressure. Barophilic behavior was also recorded for several species isolated from the deep sea indicating their possible genetic adaptation to high pressures. This is in accordance with records of heterotrophic flagellates present in environmental DNA surveys based on clone libraries established for deep-sea environments.

1. Introduction

Although the deep sea covers over half of the Earth's surface (Gage and Tyler, 1991), it remains one of the most unknown and unexplored habitats on Earth due to limited access caused by expensive and time-consuming ship time as well as extreme environmental conditions. The abyssal sea floor extends between 3000 and 6000 m (Bruun, 1956). Deep-sea organisms have to cope with extreme environmental conditions including low food resources, lower temperatures, darkness and high pressure, making life more challenging in the deep sea in comparison to surface waters. Despite the vastness of this biotope, the most studies of marine ecosystems are based on protists inhabiting the euphotic zone, while deep-sea protistan assemblages remain largely uncharacterized (Countway et al., 2007; Schoenle et al., 2016). However, there are some exceptions, such as deep-sea foraminiferans, who have received considerable attention because of their geological significance and morphological diversity (Countway et al., 2007; Pawlowski et al., 2011; Gooday and Jorissen, 2012).

Flagellated protists play an important role in microbial food webs (Jürgens and Massana, 2008). Heterotrophic flagellates (HF) are known as important grazers of bacteria in many aquatic ecosystems (Arndt et al., 2000; Boenigk and Arndt, 2002) with capabilities of regenerating nutrients and other inorganic molecules, enhancing the bioavailability of these compounds to other organisms (Fenchel, 1982; Sherr et al., 1983; Caron and Goldman, 1990). In shallow benthic and pelagic marine ecosystems, the importance of protozoan associations in energy transfer through aquatic food webs has been well established (Azam et al., 1983; Alldredge et al., 1986; Patterson et al., 1993). Although primary production is limited to the euphotic zone, delivery of fixed carbon to the deep sea via sinking detritus and carcasses provides a link between surface-associated and deep-sea detritus based microbial food webs (Caron et al., 1982; Gooday, 2002; Arndt et al., 2003). Whether all protists from euphotic surface waters can grow under deep-sea conditions is still unknown. According to Morgan-Smith et al. (2013), some

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surface isolates of *Cafeteria roenbergensis* and *Neobodo designis* were able to survive after exposure to 2°C and 500 bar (50 MPa) and even positive growth rates were recorded under these conditions. Turley et al. (1988) found a barophilic (better growth at high pressure) bodonid flagellate indicating an adaptation to deep-sea conditions (450 atm; 2 °C). Another flagellate (*Neobodo curvifilus*) was shown to have a wide barotolerance and its reproduction was recorded at pressures of 300 atm; a *Cercomonas*-like species isolated from the deep sea only grew at pressures of ≥300 atm (Turley and Carstens, 1991). Furthermore, Atkins et al. (1998) observed that deep-sea isolates of *Caecitellus parvulus* and *Rhynchomonas nasuta* had a higher growth rate at higher pressures (up to 300 atm) than their shallow-water counterparts. Protists can also form cysts in adverse conditions. A choanoflagellate isolate (*Monosiga* sp.) was observed to encyst at pressures greater than 50 atm (Atkins et al., 1998).

Environmental DNA surveys based on clonal libraries and next generation sequencing have revealed an enormous genotypical diversity of heterotrophic flagellates collected from the deep sea (Lopez-Garcia et al., 2001; Edgcomb et al., 2009; Scheckenbach et al., 2010; Pawlowski et al., 2011; Salani et al., 2012). However, for most genotypes it is not clear whether they originate from vital deep-sea populations or from cysts of organisms sedimented down from surface waters, never being active in the deep sea. Thus, a combination of ecological studies together with molecular surveys is necessary to understand the function of deep-sea heterotrophic flagellate communities. Here, we present ecological studies on pressure tolerance of different flagellate strains isolated from different marine habitats at different depths to check for their ability to survive exposure to high hydrostatic pressures. These data sets expand the few available studies (e.g., Turley and Carstens, 1991; Atkins et al., 1998; Morgan-Smith et al., 2013). We aimed to summarize available knowledge on pressure tolerance of HF and to add a significant number of additional experiments on strains belonging to a very wide range of taxonomic groups surviving hydrostatic pressure of up to 670 bar.

2. Material and methods

We summarized available studies from literature (Turley et al., 1988; Turley and Carstens, 1991; Atkins et al., 1998; Morgan-Smith et al., 2013) and compared these with our own studies. The conditions in the experiments were principally similar, though some basic parameters differed (Table 1).

In general, literature and own studies were carried out in a similar way: deep-sea and surface water samples were subsampled and cultivated in sea-water medium (≈34–35 PSU). Monocultures (except for Turley and Carstens (1991), who used mixed cultures) were established and stored for long-term cultivation at atmospheric pressure. Before performing pressure experiments, cultures were pre-cultivated at the respective experimental temperature until flagellates reached the exponential growth phase. Except in experiments done by Morgan-Smith et al. (2013), cultures were not acclimated to the experimental temperature and they used a continuous-flow chemostat to grow the flagellates in high density. Experimental cultures were enriched by autochthonous bacteria using additions of organics (cereal grains or glucose) or direct additions of bacteria obtained from cultures (*Pseudomonas putida*, *Holomonas halodurans*). In all experiments, it was assumed that food concentrations for flagellates were high enough to ensure ad libitum conditions. To determine whether heterotrophic flagellates (HF) were able to survive or even grow at experimental conditions, HF abundance was determined in subsamples taken from the experimental vessels and control vessels at the beginning, daily and at the end of experiments. The number of replicates (experimental vessels) varied between 3 and 14. Exposure to the experimental hydrostatic pressure was conducted in two ways: experimental vessels were exposed either directly to the final experimental pressure (end point; EP, see Table 1) or via different steps (time point; TP), and HF abundance and survival were analyzed after each step. In the latter, experimental vessels were released from pressure and exposed to the next higher pressure immediately after subsampling. Atkins et al. (1998) showed that time point experiments revealed a pressure tolerance of HF lying in the same range of end point experiments. In most experiments (see Table 1), end point sampling was used. Daily sampling, with minimum loss of pressure, was done only in experiments from Morgan-Smith et al. (2013). Except for Turley et al. (1988) and Morgan-Smith et al. (2013) live-counting was performed. Control vessels were investigated which were left at atmospheric pressure (all studies except for those by Morgan-Smith et al., 2013). Morgan-Smith et al. (2013) exposed control vessels at 0.8 MPa (8 bar).

2.1. Origin and cultivation of heterotrophic flagellates (HF)

We collected surface water samples from the Atlantic, Pacific Ocean, and the Baltic Sea. Deep-sea samples were obtained from different depths using a Multicorer system (Table 1). Samples were collected during different cruises with the research vessels R/V *Sonne* (SO223T, 2012; SO237, 2014/2015), R/V *Meteor* (ME 71/2, 2007; ME79/1, 2009), and R/V *Polarstern* (PS 62, 2002). Only corers with undisturbed sediment and overlaying water were used for further analyses. Once on deck, cores were immediately processed. Defined volumes of sediment (2 ml) or surface water (1 ml) were transferred into 50 ml-tissueculture flasks (Sarstedt, Nümbrecht, Germany) filled with 30 ml autoclaved seawater (35 PSU) and two quinoa grains to ensure growth of autochthonous bacteria. In the home laboratory, monocultures of different species were established by serial dilution or with the help of a micromanipulator (PatchMan NP 2 from Eppendorf, Germany) under an inverted microscope (ZEISS Axiovert 25). Isolated species were cultivated in 50 ml-tissue-culture flasks (Sarstedt, Nümbrecht, Germany) filled with 30 ml of autoclaved 35 PSU Schmaltz-Pratt medium (a liter contained 28.15 g NaCl, 0.67 g KCl, 5.51 g $MgCl_2$ x 6 H_2O, 6.92 g $MgSO_4$ x 7 H_2O, 1.45 g $CaCl_2$ x 2 H_2O, 0.10 g KNO_3, and 0.01 g K_2HPO_4 x 3 H_2O) and supplied with quinoa or wheat grains as a carbon source for autochthonous bacteria. Long-term cultivation conditions were 10 °C at 23-35 PSU.

2.2. Pressure experiments

Experiments on pressure tolerance of HF were carried out with a pressure generating system at pressures of up to 670 bar. The system consisted of three stainless steel pressure chambers (ø 30 mm, depth 50 mm) and a pressure pump with a transfer ratio of 1:400 bar (SITEC M 189-2 L, AMATIC-DIETRICH GmbH). Experimental cultures were adapted for two weeks either to 2 °C (deep sea of Atlantic), 13 °C (deep sea of the Mediterranean) or 20 °C (room temperature). To ensure unlimited food conditions, cultures were provided with quinoa, wheat or canola grains (long-term experiments) or with a glucose solution (3 ml of 2 g l^{-1} glucose added to 30-ml-batch culture; short-term experiments) to support growth of coexisting bacteria. One set of experiments (undetermined choanoflagellate HFCC 824) was carried out with a direct addition of bacteria (*Pseudomonas putida*).

We exposed isolated flagellate strains either directly (end point; EP) or stepwise (time point; TP) to different hydrostatic pressures. In experiments exposed to a direct increase of hydrostatic pressure (EP), all samples were observed at the beginning and after a period of 1 to 7 days of exposure to different pressures ranging from 50 to 670 bar. The pressure was established within a few minutes. In the other sets of experiments, a stepwise increase of pressure (TP) was applied (Table 1). Flagellates were exposed to pressure in total for 6 or 7 days and were decompressed every two days for taking subsamples and were then exposed to higher pressure again. Survival of the flagellates was recorded when the active movement of flagella was observed after exposure to a given hydrostatic pressure.

All experiments were run with 3-10 replicates. The same number of

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