Journal of Experimental Marine Biology and Ecology 445 (2013) 13–20

Contents lists available at SciVerse ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Mortality and survival of cultured surface-ocean flagellates under simulated deep-sea conditions

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ARTICLE INFO

Article history:
Received 7 November 2012
Received in revised form 29 March 2013
Accepted 29 March 2013
Available online 24 April 2013

Keywords:
Cafeteria roenbergensis
Microbial eukaryotes
Neobodo designis
Piezotolerance
Pressure effects

ABSTRACT

The vast majority of the marine environment consists of dark, cold, high-pressure environments into which microbes from the surface ocean are continuously transported by advection or by attachment to sinking particles. Little is known about how the low temperature and high pressure of the deep ocean affect the abundance and activity of surface organisms, in particular microbial eukaryotes. Cultures of two flagellate species, *Cafeteria roenbergensis* and *Neobodo designis*, both isolated from surface waters where they are considered cosmopolitan, were incubated in titanium chambers for one to two weeks under typical deep-sea temperature (2 °C) and pressure (50 MPa, representing 5000 m water depth). Samples were taken daily with minimal loss of pressure in the culture vessel, and flagellates were subsequently enumerated, along with prokaryotic prey when possible. The abundance of protists declined in all treatments, with a significantly greater rate of mortality under combined cold temperature and high pressure conditions than in the cold temperature-only conditions. However, 1.6 (SD = 3.8)% of *C. roenbergensis* and 10.0 (6.1)% of *N. designis* cells survived on average in the high pressure treatments, indicating that some fraction of sinking protists can survive transport to the deep ocean. In addition, after a period of acclimation, positive growth rates were measured in some cases, suggesting that surface-adapted flagellates cannot only survive under deep-sea conditions but are able to reproduce and potentially provide seed populations to cold, high-pressure environments.

1. Introduction

The aphotic zone of the deep ocean accounts for 95% of the marine habitat by volume (Aristegui et al., 2009), but has not been studied to the same extent as surface waters due to the difficulties of sampling such a distant and sparsely inhabited environment. Pressure effects, and the adaptation to high pressures known as piezophily, have been studied in all three domains of life. Most work has focused on Bacteria, with many piezophilic strains isolated and maintained in laboratory high pressure vessels (Bartlett, 2002). Inspired by the lack of decomposition of food left in the submersible Alvin when it remained at a depth of 1500 m for 10 months, Jannasch and colleagues performed a series of experiments in the 1970s on bacterial respiration under deep-sea temperature and pressure conditions (reviewed in Jannasch and Taylor, 1984). They found that community microbial respiration decreased by one to two orders of magnitude under deep-sea pressure (Jannasch, 1979). In addition, pressure effects on specific cell components and processes have been examined in Bacteria (reviewed by Bartlett, 2002; Simonato et al., 2006). Cell morphology, membrane structure, flagellar motility, protein function, and gene expression are all influenced by high pressure (reviewed by Bartlett, 2002; Simonato et al., 2006). At the same time, high-pressure environments have been suggested as the habitat where life began (Daniel et al., 2006), making studies of organisms in this habitat particularly interesting.

Pressure effects are known to a much lesser extent for the other two domains (Archaea and Eukarya). Among the Archaea, a few piezophilic organisms have been isolated, but these few organisms represent a broad diversity within the domain, as compared to the fairly narrow taxonomic representation of piezophilic bacterial isolates (Bartlett, 2002; Kato and Qureshi, 1999). Most of the eukaryotes that have been studied under pressure conditions were metazoans, especially the crustaceans and fish that inhabit the deep ocean, as well as hydrothermal vent fauna (reviewed by MacDonald, 1997; Sebert, 2002). An important exception, and the only single-cell eukaryote specifically investigated for pressure effects was the yeast *Saccharomyces cerevisiae*, which has been used as a model organism in several studies of pressure effects on metabolism (Sharma et al., 2002) and gene expression (Miura et al., 2006). Mortality of *S. cerevisiae* under high pressure has been attributed to damage to intracellular membranes (Brul et al., 2000), cell membranes and the cell wall (Marx et al., 2011), though metabolic processes continued at pressures

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http://dx.doi.org/10.1016/j.jembe.2013.03.017

above 1 GPa (Sharma et al., 2002), a pressure an order of magnitude greater than that in the deepest ocean trench.

Membrane structure is affected by pressure, as mono- and poly-unsaturated fatty acid contents are increased in order to maintain the fluidity of membranes in deep-sea organisms (Allen et al., 1999; DeLong and Yayanos, 1985). Changes in the distribution of structural membrane proteins, respiratory chain trans-membrane proteins, and transporters have also been observed in response to high pressure (Vezzi et al., 2005) and protein folding has been shown to be inhibited at 400 MPa (Gross and Jaenicke, 1994). While these pressures are much higher than those experienced in the deep sea, function has also been shown to be affected at pressures less than 110 MPa, which exist in the ocean's deepest regions (Simonato et al., 2006). The effect of pressure on proteins is caused by changes not only in protein conformation, but also in interactions with water molecules, which are necessary for protein function (Balny et al., 1997). Bacterial motility can be reduced, as both formation and function of bacterial flagella are degraded under high pressure (Meganathan and Marquis, 1973; Welch et al., 1993). Enzymes can lose their function at pressures characteristic of deep sea environments, including mitochondrial and chloroplast F_1F_0 ATP-synthases, which are inactivated at 50 MPa (Dreyfus et al., 1988; Souza et al., 2004). Pressures characteristic of ocean depths of 1000–5000 m can lead to physiological responses, including expression of heat and cold stress proteins when temperature stress is not present (Welch et al., 1993) and accumulation of osmolytes which may be used to stabilize proteins against pressure effects or reduce the energetic cost of osmoregulation under pressure (Kelly and Yancey, 1999).

Most previous studies of pressure effects on marine microbes are from short-term incubations, sometimes under pressures much higher than those observed in the deep ocean, using axenic cell cultures or isolated cell components (e.g. Miura et al., 2006; Welch et al., 1993). While these studies are necessary for understanding physiological responses to pressure, they do not replicate the conditions faced by organisms which are transferred from the surface to the deep ocean via advection or riding on sinking particles. Studies in which natural particle-affiliated prokaryote communities were subjected to increasing pressure over several days showed that function but not taxonomic structure of prokaryote communities was affected by pressure (Tamburini et al., 2006). In particular, degradation of fecal pellets by prokaryotic communities was inhibited by high hydrostatic pressure (Tamburini et al., 2009). However, this work was limited to Bacteria, and one cannot assume that the effects on eukaryotes will be the same, because the details of cell membrane structure, biochemical makeup, cell physiology, and reproduction differ among the three domains (Woese et al., 1990), and all of these factors can be affected by the temperature and pressure of the deep sea (Bartlett, 2002; Rivalain et al., 2010). Another important consideration is the length of time that organisms were incubated at high pressure. Most previous studies investigated acute effects of pressure on cells or cell components in short-term incubations (one to several hours) (e.g. Miura et al., 2006; Welch et al., 1993). However, longer timescales are important for studying population growth due to the slow rates of respiration and reproduction exhibited by deep-sea organisms (reviewed in Aristegui et al., 2009; Morita, 1984).

In this study, we incubated flagellate cultures under simulated deep-sea conditions for one to two weeks in order to examine not only immediate effects of pressure but also the medium-term recovery from the initial stress response. Two flagellate species, *Cafeteria roenbergensis* Fenchel and Patterson (1988) and *Neobodo designis* (Skuja) Vickerman (Moreira et al., 2004) – both isolates from surface waters – were incubated to record their population-level responses to temperature and pressure conditions representative of the deep ocean. We used a semi-continuous flow-through system, which allowed daily subsampling with minimal loss of pressure to the main culture chamber. Cultures were not acclimated to the experimental temperature and pressure conditions prior to the start of each experiment, so this work represents a perturbation experiment and not a study of steady-state conditions.

2. Methods

2.1. Pressure system

The high-pressure system (HPS) used in these experiments was built as described in Jannasch et al. (1996) and consisted of a 200-ml titanium culture vessel attached to a 14-ml stainless steel subsampler, with pressure maintained by a high-pressure liquid chromatography (HPLC) pump (Fig. 1). All components that came into contact with the culture medium or cultured organisms upstream of the subsampler were made of titanium or non-reactive plastics to prevent iron contamination, which can lead to overgrowth of iron-oxidizing bacteria in the pressure vessel. Medium used in these experiments was Instant Ocean brand artificial seawater at 34 ppt, autoclaved, cooled to 15 °C and aerated overnight to adjust alkalinity. Medium was held in a non-pressurized flask connected to the HPLC pump by 1/16″ PTFE tubing through a metal-free filter (Alltech) to prevent any particles from entering the HPS (Fig. 1). The HPLC pump (Rainin HPXL) pressurized the media, and routed it via 1/16″ titanium tubing to a 1/8″ titanium injection needle, which delivered the inflowing seawater to the bottom of the chamber (Fig. 1). Continuous washing of the HPLC pump head was maintained using a peristaltic pump (Fisher) supplying a continuous loop of deionized water from a flask which was exchanged with fresh deionized water approximately every other day. Outflow from the top of the pressure vessel (i.e. the opposite end of the inflow) was directed through another section of 1/8″ titanium tubing (inner diameter 0.094″) to the subsampler, with flow controlled by needle valves at both the top and the bottom of the subsampler. The subsampler contained a piston with distilled water above it to maintain pressure during sampling. The subsampler could be bypassed through the use of the needle valves to send culture directly to the outflow nozzle when pressure release was desired. A small autoclaved PTFE-coated stir bar was placed in the bottom of each pressure vessel prior to

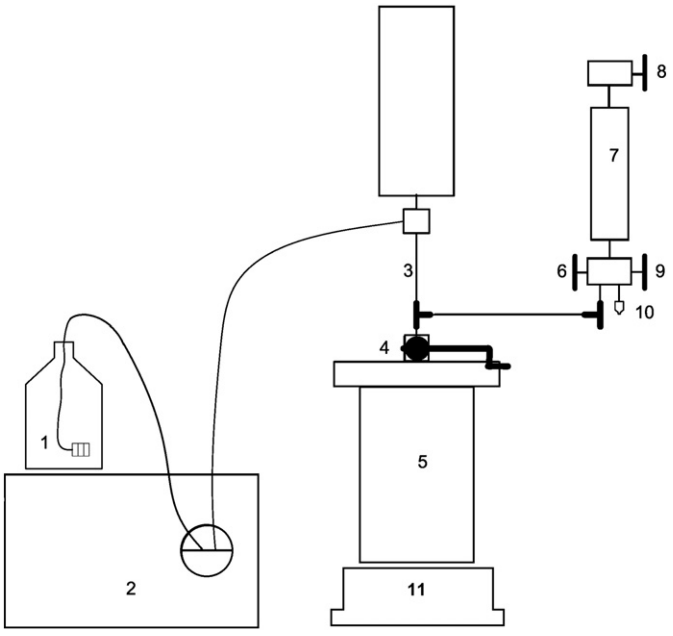

Fig. 1. Schematic of HPS system. 1: media flask, 2: high-pressure liquid chromatography (HPLC) pump, 3: titanium tubing to deliver artificial seawater to the bottom of the chamber (inflow), 4: outflow at top of chamber, 5: culture vessel, 6: needle valve to subsampler, 7: subsampler, 8: needle valve to hydraulic pump, 9: needle valve to outflow, 10: outflow nozzle, 11: magnetic stirring plate.