

Effects of hydrostatic pressure on yeasts isolated from deep-sea hydrothermal vents

Gaëtan Burgaud^{a,*}, Nguyen Thi Minh Hué^b, Danielle Arzur^a, Monika Coton^a,
Jean-Marie Perrier-Cornet^b, Mohamed Jebbar^{c,d,e}, Georges Barbier^a

^a Université de Brest, EA 3882, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, ESIAB, Technopôle Brest-Iroise, 29280 Plouzané, France

^b Université de Bourgogne, AgroSup Dijon, Dimacell Imaging Ressource Center, UMR A 02.102 PAM, F-21000 Dijon, France

^c Université de Bretagne Occidentale, UMR 6197-Laboratoire de Microbiologie des Environnements Extrêmes (LM2E), Institut Universitaire Européen de la Mer (IUEM), rue Dumont d'Urville, 29 280 Plouzané, France

^d CNRS, UMR 6197-Laboratoire de Microbiologie des Environnements Extrêmes (LM2E), Institut Universitaire Européen de la Mer (IUEM), rue Dumont d'Urville, 29 280 Plouzané, France

^e Ifremer, Centre de Brest, REM/EEP/LM2E, Technopôle Brest-Iroise, BP70, 29 280 Plouzané, France

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Abstract

Hydrostatic pressure plays a significant role in the distribution of life in the biosphere. Knowledge of deep-sea piezotolerant and (hyper) piezophilic bacteria and archaea diversity has been well documented, along with their specific adaptations to cope with high hydrostatic pressure (HHP). Recent investigations of deep-sea microbial community compositions have shown unexpected micro-eukaryotic communities, mainly dominated by fungi. Molecular methods such as next-generation sequencing have been used for SSU rRNA gene sequencing to reveal fungal taxa. Currently, a difficult but fascinating challenge for marine mycologists is to create deep-sea marine fungus culture collections and assess their ability to cope with pressure. Indeed, although there is no universal genetic marker for piezoresistance, physiological analyses provide concrete relevant data for estimating their adaptations and understanding the role of fungal communities in the abyss. The present study investigated morphological and physiological responses of fungi to HHP using a collection of deep-sea yeasts as a model. The aim was to determine whether deep-sea yeasts were able to tolerate different HHP and if they were metabolically active. Here we report an unexpected taxonomic-based dichotomic response to pressure with piezosensitive ascomycetes and piezotolerant basidiomycetes, and distinct morphological switches triggered by pressure for certain strains.

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1. Introduction

The marine biome is considered to be the largest in the world, covering three-fourths of the Earth's surface. The average depth of the marine biome is 3800 m [1], indicating, as a Gaussian distribution, that most of the biosphere is

subjected to a pressure of 38 megapascals (MPa), i.e. 380-fold higher than atmospheric pressure (0.1 MPa). Many factors regulate the biodiversity encountered in oceans, but hydrostatic pressure appears to be a key physical parameter in the dark cold abyss [2]. Deep-sea microorganisms have been classified by their cardinal growth pressures into the following categories: piezosensitive, piezotolerant, piezophiles or hyperpiezophiles [3–5]. The absolute record for the ability to grow at very high hydrostatic pressure is currently held by *Pyrococcus yayanosii*, an obligate piezophilic hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent,

* Corresponding author. Laboratoire Universitaire de Biodiversité et d'Ecologie Microbienne, Parvis Blaise-Pascal, Technopôle Brest-Iroise, 29280 Plouzané, France. Tel.: +33 02 90 91 51 00; fax: +33 02 90 91 51 01.

E-mail address: gaetan.burgaud@univ-brest.fr (G. Burgaud).

with growth recorded from 20 to 120 MPa [6,7]. Such results have highlighted that hydrostatic pressure plays a significant role in prokaryotic life distribution within the deep-sea.

Recent studies have shown that deep-sea microbial communities are composed of a “not only prokaryotic” world. Indeed, numerous DNA-based studies have shown fungal communities to be present in several deep-sea ecosystems, i.e. deep sediments [8–12], hydrothermal vents [13–16], sunken woods [17], cold seeps [18,19] and even deep hypersaline anoxic basins [20–22]. More recently, next-generation sequencing technologies have led to rRNA and mRNA-based approaches to reveal: (i) metabolically active parts of these communities [10,12], and (ii) their important ecological roles, e.g. in organic carbon recycling in deep seafloor sediments or their interactions with prokaryotes and antibiotic defense mechanisms [23]. Sequences recovered in these datasets were mainly affiliated with *Dikarya*, a subkingdom embracing the two Ascomycota and Basidiomycota phyla. To a lesser extent, some signatures of the basal *Chytridiomycota* and *Cryptomycota* phyla were also obtained. However, as such studies are based on molecular signatures, it is almost impossible to know whether fungi represented by these nucleotidic sequences are specifically adapted to deep-sea habitats. In this context, culture-based methods can be used to isolate deep-sea fungal strains and then to evaluate their ability to tolerate HHP. This will expand our knowledge on deep-sea fungi activities in situ and their ecological roles in such extreme environments.

High pressure effects on biological processes are diverse, with (i) pressure-sensitive lipids modifying fluidity, permeability and cell membrane functions, (ii) pressure-sensitive proteins affecting multimer associations and stability, thus impacting motility and cell division, and (iii) pressure-stabilized DNA hydrogen bonds affecting replication and transcription steps [24]. The impact of HHP on cell physiology has been extensively studied in the model yeast *Saccharomyces cerevisiae*. *S. cerevisiae* is piezosensitive, meaning that HHP is perceived as a stress and induces cell stress responses. The ability of *S. cerevisiae* cells to grow at moderate pressure, between 15 and 25 MPa, was shown to be directly connected to tryptophan auxotrophy and the availability of this amino acid. Cell growth arrest occurred at pressures above 50 MPa regardless of the amino acid auxotrophy of the strain. Pressures ranging from 100 to 200 MPa killed *S. cerevisiae* cells by disrupting microtubule ultrastructures, actin filaments or nuclear membranes [25]. Pressures greater than 200 MPa caused leakage of internal substrates and ions from cells. HHP also induced cytoplasmic petite mutations and tetraploid or homozygous diploid forms [1].

Few studies have specifically focused on growth of marine fungi under hydrostatic pressure. Amongst these studies, some focused on different species affiliated with the *Aspergillus* genera [26,27], while others dealt with marine yeasts such as *Rhodotorula rubra*, *Debaryomyces hansenii* and *Rhodospiridium sphaerocarum* [28]. All studied fungal isolates were characterized as piezosensitive, as growth was systematically better at atmospheric pressure (0.1 MPa) compared to high

hydrostatic pressures. A major result to be highlighted was that the 3 marine yeasts cultivated under HHP were able to grow up to at least 20 MPa, while only the basidiomycetous species *R. rubra* and *R. sphaerocarum* were able to grow at 40 MPa, suggesting better piezotolerance of basidiomycetes compared to ascomycetes. Since the analyzed strains were all ubiquitous in these studies, our goal here was to test different yeast species recently isolated from deep-sea hydrothermal vents [29], including *Candida oceani*, a novel obligate marine yeast [30]. Yeasts isolated in these studies appear well adapted to deep-sea marine conditions based on ecophysiological parameters (temperature, salinity) and the fact that some were directly observed on hydrothermal samples using fluorescent in situ hybridization.

The aim of our study was to resolve several questions of ecological interest: (i) are yeast strains able to tolerate hydrostatic pressure encountered at their isolation site; (ii) are strains considered piezosensitive, piezotolerant or piezophile; and (iii) are strains metabolically active under elevated HHP?

2. Materials and methods

2.1. Selected strains

A collection of deep-sea yeasts was previously created from hydrothermal vent samples [29]. Amongst the 32 isolated yeasts, 21 were identified as basidiomycetes and represented by 5 different species, while 11 were defined as ascomycetes and belonged to 7 different species. For each taxonomic group, one representative isolate was selected to evaluate growth under HHP (Table 1). All isolates are available in the UBO Culture Collection (<http://www.univ-brest.fr/ubocc>).

2.2. Cultivation under elevated hydrostatic pressure

Yeasts were cultured under HHP in batch using a simple system based on steel cylinders filled with hydraulic fluids as previously described [31]. Yeast cells were grown in GYPS broth (0.1% glucose, 0.1% yeast extract, 0.1% peptone, 0.1% starch and 3% sea salts) at 25 °C for 24–48 h. Exponentially growing cells were then resuspended in GYPS broth to a concentration of about 1.10^6 cells/ml and placed in 6 ml plastic tubes (Nunc[®] cryotubes) in triplicate under oxic conditions. After sealing with sterile parafilm, tubes were placed in pressure vessels filled with sterile water (used as the hydraulic fluid) and subjected to hydrostatic pressure [32]. The required hydrostatic pressures, i.e. 7 MPa (only for *Pichia guilliermondii* Ex15), 24.5 MPa, 60 MPa and 0.1 MPa (control condition, also incubated in HP tubes), were reached in less than 2 min using a hand-pump. Pressure vessels were then incubated at 25 °C for 96 h.

After incubation, pressure was released in approximately 15 s. Plastic tubes were immediately placed on ice. Then, the content of each tube was directly used for: (i) biomass quantification, (ii) yeast cell ribosomal activity estimate coupled with DAPI and calcofluor staining; and (iii) morphological analyses.

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