Univerza v Mariboru

Fakulteta za kemijo in kemijsko tehnologijo

Effect of high hydrostatic pressure on selected Black Yeast species

"Emerging Potential of Black Yeasts“, 14-16 May 2010, Ljubljana

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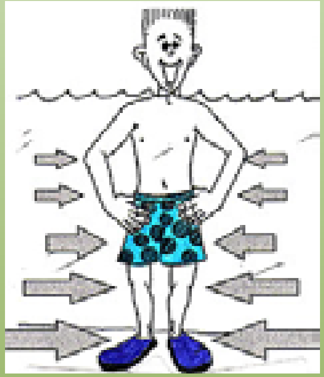
Overview

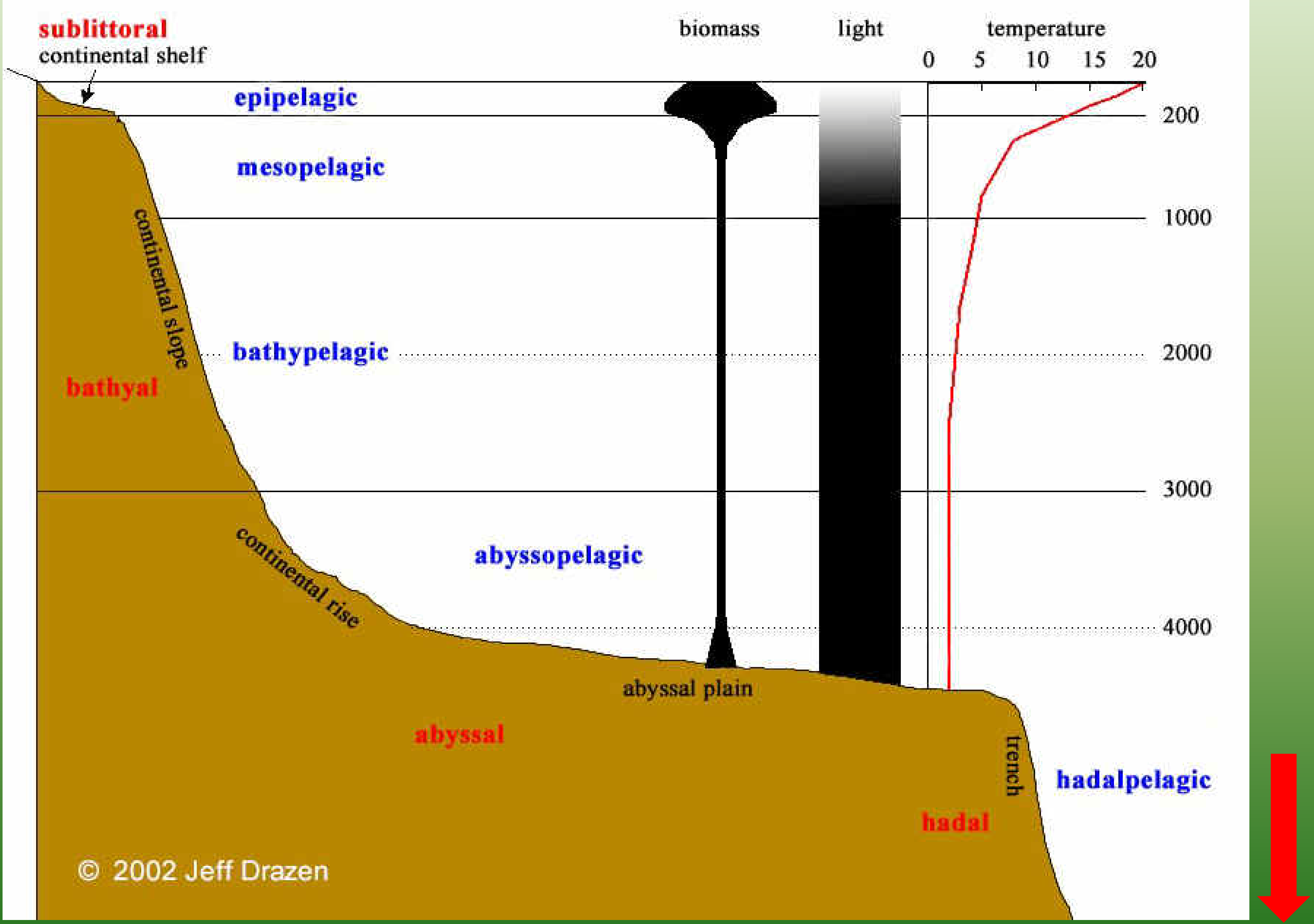
- Introduction
  - hydrostatic pressure
  - microbial diversity

- Experimental design
- Results
- Conclusions and future directions

Hydrostatic pressure



- Pressure increases with depth
  (oceans or underground)
- 1000m----100atm=101,325bar=10MPa
- Average depth of oceans 3800m (38MPa), maximum depth 11000m (110MPa)
- Deep sea - water depth 1000m and below
- High pressure biotopes: above 100MPa pressure (10.000m)


sublittoral
continental shelf
biomass
light
temperature
0 5 10 15 20
epipelagic
200
mesopelagic
1000
continental slope
bathypelagic
2000
bathyal
3000
continental rise
abyssopelagic
4000
abyssal plain
abyssal
trench
hadalpelagic
hadal
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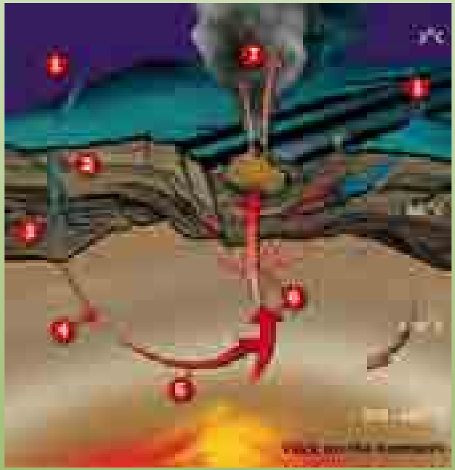
High pressure biotopes and microbial diversity



http://www.divediscover.whoi.edu/vents/vent-chemistry.html

- Until 19th century: no life below 600m
- 1940 high pressure biology (ZoBell and Morita)
- 1977 hidrotermal vents (2600m, invertebrae)
- 1979 first isolate of barophilic bacteria (Yayanos et al)
- 1993 first obligate barophilic anaerobic archaea (Erusto at al)
- 2009 fungal diversity in deep sea Hydrotermal Ecosystem (Le Calvez et al.)

Prefix “baro” = “piezo”

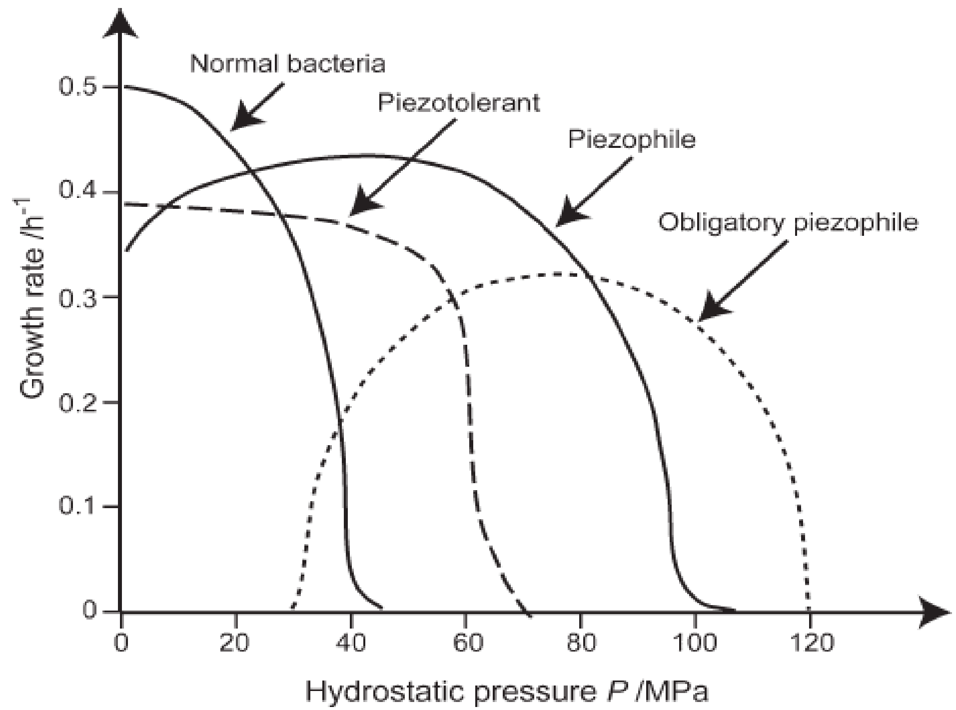


Fig. 3 Definitions of the relations between growth rate of microorganisms and pressure. Piezophile microorganisms display a maximum growth at high pressure. They can either grow at atmospheric pressure or not, and are called strictly piezophile in the latter case. Piezotolerant microorganism grow best at atmospheric pressure, but can sustain high pressure, whereas mesophile or piezosensitive microorganisms totally stop growing at 40–50 MPa. (R. Margesin and Y. Nogi, *Cell. Mol. Biol.*, 2004, **50**, 42

Origins of life and biochemistry under high-pressure conditions

Isabelle Daniel,* Philippe Oger and Roland Winter

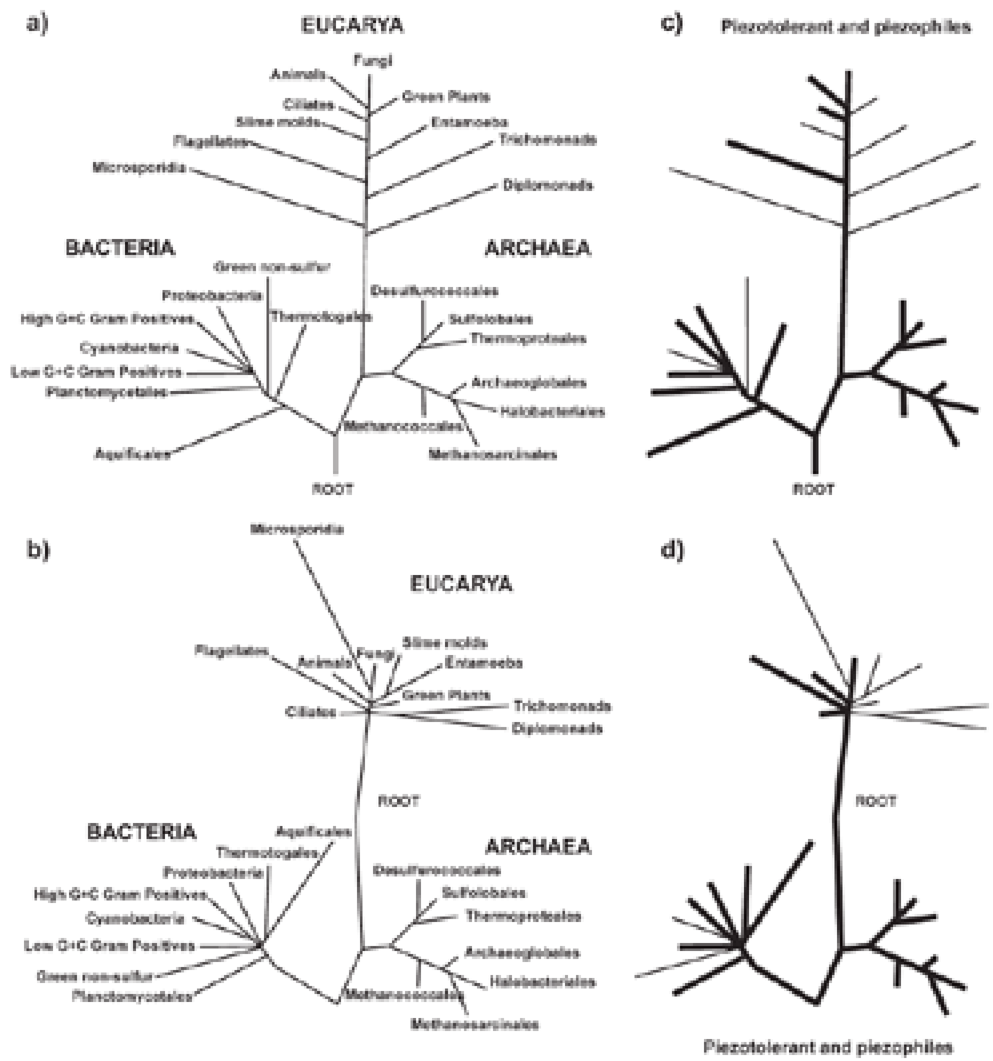


Fig. 2 Piezophily in the tree of life. (a) The classical view of the tree of life. The topology of the tree is mainly based on rDNA comparison. (b) A revised topology of the universal tree of life, after correction of phylogenetic pitfalls, such as long branch attraction or the removal of uninformative site from the analysis. (c) and (d) Distribution of piezophilic and piezotolerant organisms in the two trees of life. Thick blacks lines highlight bacterial groups in which piezotolerant and piezophile organisms have been characterized. Thin black lines highlight groups for which only piezotolerant species are known.

Adapation mechanisms I

BACTERIA & ARCHAEA	Source	Depth (m)	Adaptation	Reference
Shewanella benthica	Suruga Bay Ryukyu Trench Japan Trench Izu-Bonin Trench Ryukyu Trench	From 2485 to 6499	All strains tested became more barophilic at higher temperatures.	Kato C, Sato T, Horikoshi K: **Isolation and properties of barophilic and barotolerant bacteria from deep-sea mud samples**. *Biodiv Conserv* 1995 Kato C, Masui N, Horikoshi K: **Properties of obligatory barophilic bacteria isolated from a sample of deep-sea sediment from the Izu-Bonin trench.**.l *Mar Biotechnol* 1996 Li L, Kato C, Horikoshi K: **Distribution of the pressure regulated -operons in deep-sea bacteria.** *FEMS Microbiol Lett* 1998
Moritella japonica	Japan Trench	6356		
DSK25 Gram-positive spore-forming bacterium	Japan Trench	6500		
Methanococcus jannaschii (arhaea)			Resistant proteases	Michels PC, Clark DS: **Pressure-enhanced activity and stability of a hyperthermophilic protease from a deep-sea methanogen.** *Appl Environ Microbio 1997*
Strain 2D2 Strain 16C1	intestinal contents of the deep-sea fish	6100 3100	General shift from saturated to unsaturated fatty acids. Furthermore, these adaptive changes in response were also observed in response to low temperature.	Yutaka Yano, Akihiko Nakayama, Kenji Ishihara, and Hiroaki Saito Adaptive Changes in Membrane **Lipids of Barophilic Bacteria in Response to Changes in Growth Pressure.** Appl Environ Microbiol, February 1998
Photobacterium profundum	Ryukyu Trench		Differences in respiratory chain (expression of certian cytochromes, amount of quinol oxidases) Acumulation of protein-stabilizing solutes	Qureshi, M.H., Kato, C., Horikoshi, K.,. **Purification of a cbb type quinol oxidase specifically induced in a deep-sea barophilic bacterium, *Shewanella sp. strain DB-1***72F. *Extremophiles 2* 1998 Martin, D.D, Barlett, D.H, Roberts, M.F. Solute accumulation in dep-sea bacterium *Photobacterium profundum*. Extremophiles, 2002.

Subject of detail gene expression profiling experiments

Adaptation mechansims II

FUNGI	Optimal growth properties (MPa)	Depth (m)	References
Aspergillus ustus	10	860	Raghukumar C, Raghukumar S. **Barotolerance of fungi isolated from deep-seasediments of the Indian Ocean.** AQUATIC MICROBIAL ECOLOGY 1998.
Graphium sp	10	965	
Debaryomyces hansenii, Rhodotorula rubra and Rhodosporidium sphaerocarpum	20-40		LORENZ R. ; MOLITORIS H. P. ; **Cultivation of fungi under simulated deep sea conditions.** Mycological research , 1997
181 cultures of fungi (25 representative)	20 (30)	5000	Damare S, Raghukumar C, Raghukumar S. **Fungi in deep-sea sediments of the Central Indian Basin.** Deep-sea research part i-oceanographic research papers, 2006
deep-sea *Aspergillus* isolates	20	5000	Samir R. Damare, Manju Nagarajan and Chandralata Raghukumar **Spore germination of fungi belonging to *Aspergillus* species under deep-sea conditions** . In Deep Sea Research Part I: Oceanographic Research Papers, 2008
Saccharomyces cerevisiae (MODEL)	18		Damare S, Raghukumar C, Raghukumar S **Effect of temperature on the role of Hsp104 and trehalose in barotolerance of Saccharomyces cerevisiae.** FEBS Letters, 1997

- morphology
- abillity to germinate

Effect of high pressure on *S. cerevisiae*

- **On gene expression:**
  - Up-regulation of genes involved in stress tolerance and carbohydrate metabolism
  - Down-regulation of genes involved in cellular transcription, protein synthesis and cell cycle

- **On protein synthesis**:
  - RNA synthesis is maintained
  - Improved piezoresistance AFTER being exposed to a mild stress

- **On fluidity of the membrane:**
  - Increase of unsaturated fatty acids

- **On cell wall and cytoskeleton:**
  - Up-regulation of Hsp12p in cell wall, where it was proposed that acts as a plasticizer

- **Intracellular changes:**
  - Acidification of cytoplasm and vacuole

F. Abe, *Cell. Mol. Biol.*, 2004, **50**, 437.

Table 1 Pressure effects on yeast physiology[30]

Pressure/MPa	Effects
0.1–50 MPa	Arrest of cell growth
	Metabolic changes
	Inhibition of amino acid uptake
	Stress-inducible expression
50 MPa–	Inhibition of ethanol fermentation
	Internal acidification
	Stress-inducible expression
100 MPa–	Reduction in viability
	Membrane, and cell wall perturbations
	Acquired piezotolerance
	Stress-inducible expression
200 MPa–	Alteration of genome preservation
	Shrinkage and leakage of cells
	Stress-inducible expression

Time of exposure to HP is important factor!
(50MPa for 24hrs results in 100% mortality)

HP treatment of food products (300-500MPa)

Multiple response of black yeasts to stress

- production of melanin

  -osmoadaptation

- Meristematic/microcolonial growth (keeping the optimal volume-to-surface ratio)

Studied fungi

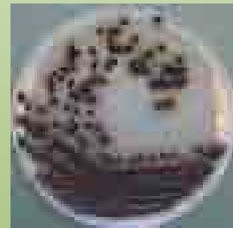
1.	***Hortaea werneckii***	EXF - 225	**Salterns**
2.	***Trimmatostroma salinum***	EXF - 295	**Salterns**
3.	***Phaeotheca triangularis***	EXF-206	**Salterns**
4.	***Cladosporium halotolerans***	EXF - 572	**Salterns**
5.	***Cladosporium spinulosum***	EXF - 334	**Salterns**
6.	***Candida parapsilosis***	EXF - 517	**Salterns**
7.	***Pichia guillermondii***	EXF - 1496	**Arctic**
8.	***Pichia guillermondii***	EXF - 518	**Salterns**
9.	***Aureobasidium pullulans var. pullulans***	EXF-150	**Salterns**
10.	***Aureobasidium pullulans var. pullulans***	EXF-1668	**Arctic**
11.	***Aureobasidium pullulans var. melanogenum***	EXF - 924	**Arctic**
12.	***Aureobasidium pullulans var. melanogenum***	EXF - 3382	**Deep sea**
13.	***Aureobasidium pullulans var. subglaciale***	EXF - 2481	**Arctic**
14.	***Aureobasidium pullulans var. subglaciale***	EXF-2479	**Arctic**
15.	***Aureobasidium pullulans var. namibiae***	EXF - 3398	**Namibia**
16.	***Hormonema 1***	EXF – 914	**Arctic**
17.	***Hormonema 2***	EXF - 2500	**Arctic**
18.	***Cryptococcus liquefaciens***	EXF-3584	**Arctic**
19.	***Rhodotorula mucilaginosa***	EXF 1630	**Arctic**
20.	***Rhodotorula mucilaginosa***	EXF-3790	**Salterns**
21.	***Cryptococcus diffluens***	EXF-3772	**Salterns**
22.	***Cryptococcus albidus***	EXF-3477	**Arctic**
23.	***Penicillium crustosum***	EXF 763	**Salterns**
24.	***Penicillium crustosum***	EXF-1329	**Arctic, CREA +**
25.	***Penicillium crustosum***	EXF-1005	**Arctic, CREA -**
26.	***Debaryomyces hansenii***	EXF-589	**Salterns**
27.	***Debaryomyces hansenii***	EXF-1590	**Arctic**
28.	***Wallemia ichthyophaga***	EXF-994	**Salterns**
29.	***Wallemia muriae***	EXF-951	**Salterns**
30.	***Wallemia sebi***	EXF-958	**Pumpkin seed**
31.	***Saccharomyces cerevisiae***	EXF-531	**control**

Selected black yeasts under hydrostatic pressure

- *Trimmatostroma salinum*
  EXF – 295, Solar salterns
- *Aureobasidium pullulans var. melanogenum*
  EXF – 924, Arctic ice
- *Hormonema 1*
  EXF – 914, Arctic ice
- *Saccharomyces cerevisiae* (positive control)

Experimental design

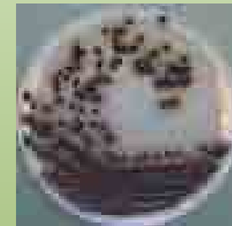
7 days of growth



MEA

Cells in 0,9%NaCl solution

10 days of growth



MEA + 10% NaCl

Cells in 10 %NaCl solution

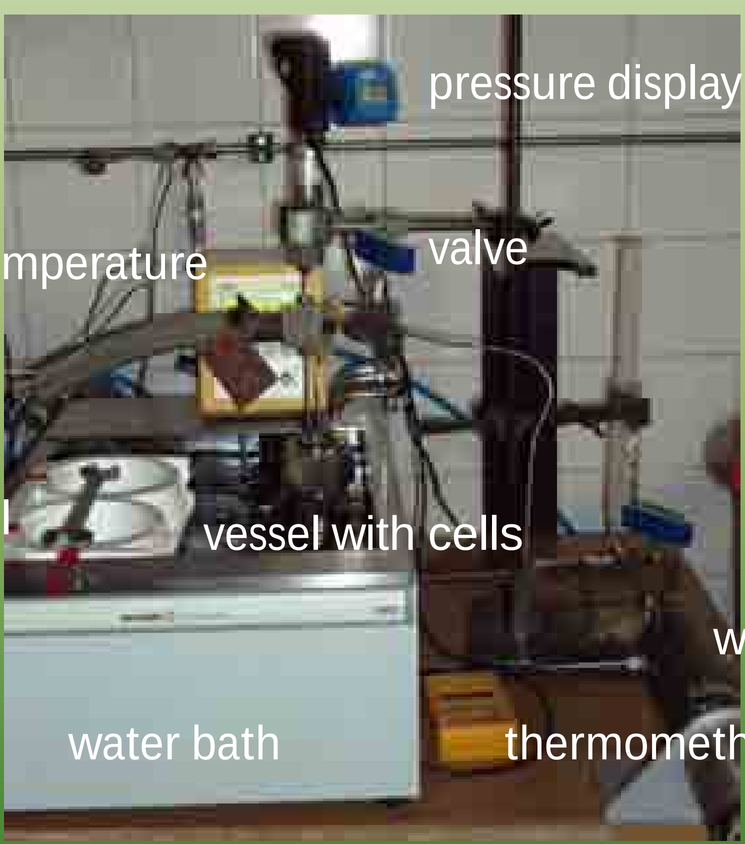
immediate pressurization

High pressure vessel
24hrs, 300 bar (30MPa) room temperature (23°C)

MEA

MEA + 10% NaCl

High pressure equipment



High pressure equipment (UniMb)

Results

Trimmatostroma salinum

MEA

control

after exposure to HP

↓ number

MEA + 10% NaCl

control

after exposure to HP

↓ number

↑ colony diameter

Results

Aureobasidium pullulans var. melanogenum

MEA

control | after exposure to HP

No differences observed

MEA + 10% NaCl

control | after exposure to HP

↓ number

↑ colony diameter

Results

Hormonema 1

MEA | **MEA + 10% NaCl**

control | after exposure to HP | after exposure to HP | control

No differences observed

↓ number

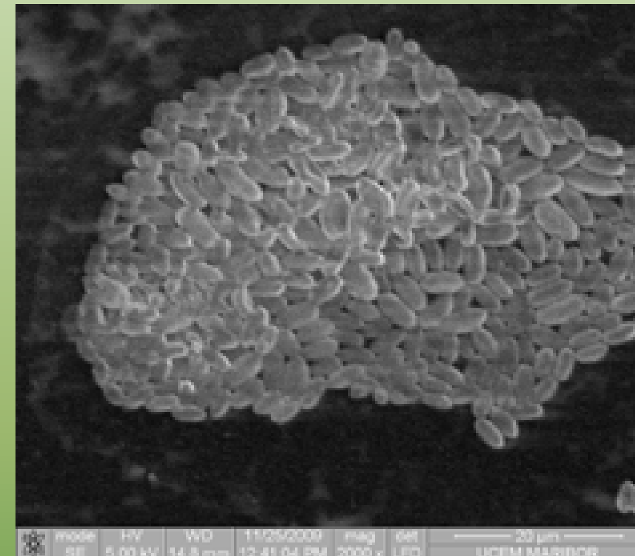
↑ colony diameter

SEM

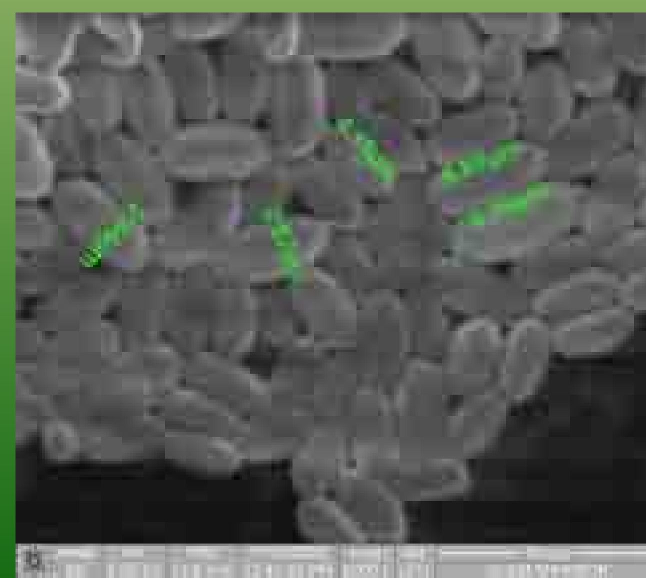
Hormonema 1

control

after exposure to HP



MEA

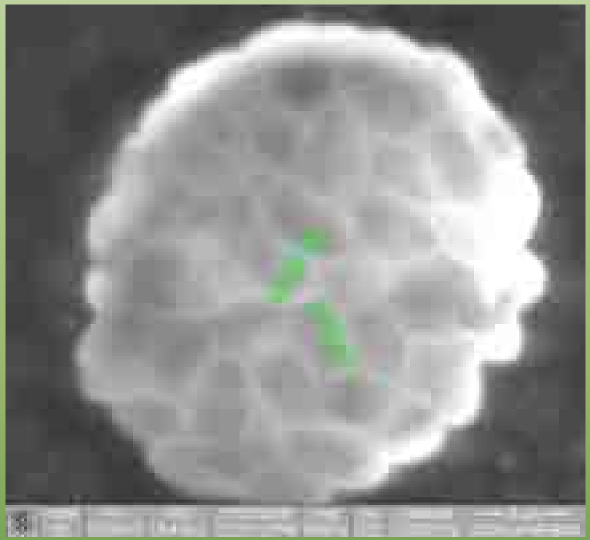


SEM

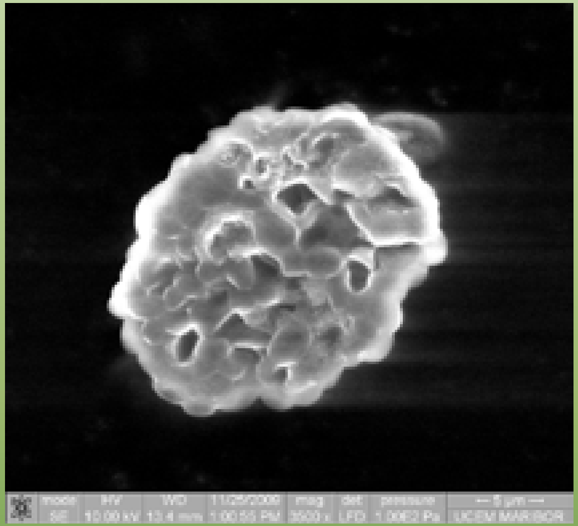
Hormonema 1

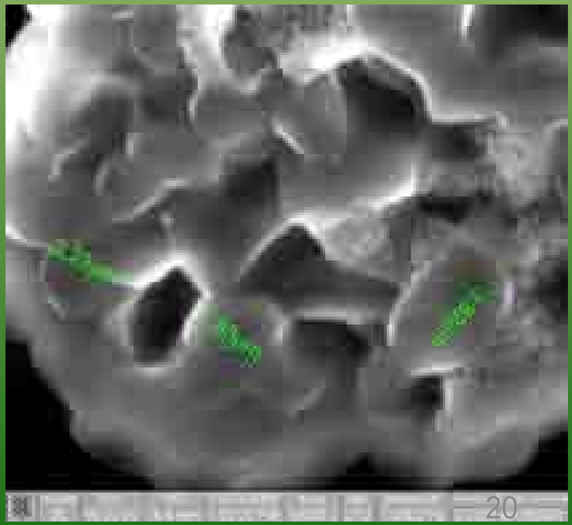
control

after exposure to HP



MEA + 10%NaCl





Clumps:
-High concentration of salt
-HP

Results

Saccharomyces cerevisiae

MEA

after exposure to HP control

MEA + 10% NaCl

after exposure to HP control

Conclusions and future directions

- Cells of selected black yeasts are able to survive exposure to 30 MPa
- Exposure to 30MPa pressure can cause changes in colony appearance
- Abillity of subglacial *Hormonema* to adjust volume to surface ratio (long and narrow shape of cells after exposure to HP; SEM)
- Future plan - increase of hydrostatic pressure!!!

Thank you for your attention!