MaCuMBA

Marine Microorganisms: Cultivation Methods for Improving their Biotechnological Applications

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Protocols for media and isolation of bacteria from extreme environments

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Dissemination Level	
PU Public	
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission Services)	
CO Confidential, only for members of the consortium (including the Commission Services)	**CO**

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List of reviewers

Issue	Date	Implemented by
v.1	24-Jan-14	Pauline Vannier & Viggo Thor Marteinsson

Indicate any document related to this deliverable (report, website, ppt etc) and give file name

** Please attach deliverable documents and any additional material if needed.*

Summary

Objective(s): The objectives are to develop cultivation systems that will mimic the natural environment of **extreme** microorganisms for enriching and isolating hitherto uncultured microorganisms with expected biotechnological potential. The deliverable is part of WP2, Task 2.7, subtask 8.

Rationale:

Samples were collected from a coastal hydrothermal vents located in the NW of Iceland, Reykjanes. The hydrothermal source located on the beach is about 80°C during high tides and is covered every 6 hours by cold seawater (4°C) following the high tide of the sea. This regular periodic hot and cold temperatures are constant every 6 hours.

This is a unique geothermal area because hot springs are submerged during high tide therefore creating a highly dynamic environment subject to constant periodic disturbance with steep gradients of temperature and salinity between the hot alkaline freshwater fluid and the cold seawater.

This environment also provides attractive conditions for the study of extremophilic heterotrophs due to profusion of algal vegetation and crustacean shells around the hot springs and due of extremely wide gap of temperature.

Results:

Samples from extreme environment were collected from a) Mixture of sand, gravels and shells, 78°C: Sample 1; b) sand and gravels, 50°C: Sample 2; pure sand, 57°C: Sample 3; c) Cyanobacteria and sand, 47°C: Sample 4 and d) pure hot water, 80°C: Sample 5 (Figure 1)

Sample 1

Sample 2

Sample 3

Sample 4

Sample 5

Figure 1: Photos of 5 different samples from the Reykjanes site.

An incubator was programmed to mimic these conditions in laboratory (Figure 2). The incubator was designed with light for phototrophs and carbon dioxide inlet to the cultures.

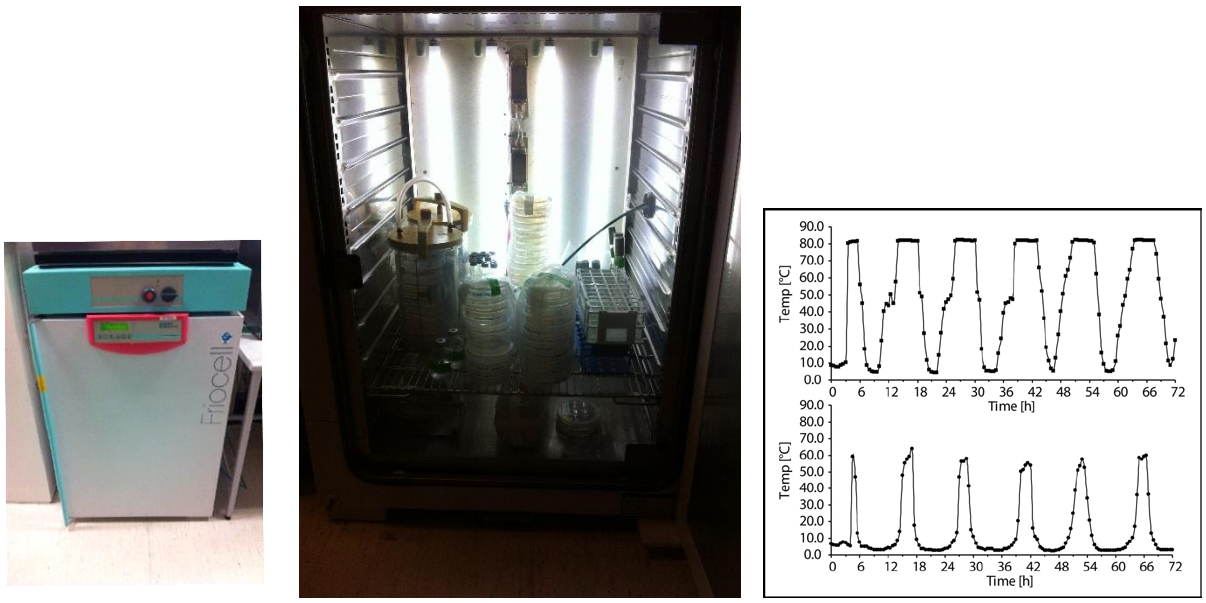

Figure 2. A photo of the incubator (a) outside and (b) inside (c) example of temperature measurements.

The samples were inoculated into five different media for aerobic cultures. Then, Petri dishes were incubated in a unique temperature cycle, from 4°C to 70°C, in order to imitate the *in situ* conditions on site. Besides, light was be turn on during the day, and turn off during the night in order to mimic the real sun conditions.
At least 86 isolates have been purified and a few identified which belong to at least 4 different genera.

For anaerobic cultures, one medium was used: a mix between Thermococcales Rich Medium (TRM) [1] and Ravot medium [2]($MgCl_2.6H_2O$: 0.2 g, $CaCl_2.2H_2O$: 0.1 g, KCl : 0.7 g, $CH_3COONa.3H_2O$: 0.83 g, Yeast Extract : 1.0 g, PIPES : 3.3 g, K_2HPO_4 : 0.5 g, KH_2PO_4 : 0.5 g, NaBr : 0.05 g, $(NH_4)_2SO_4$: 0.5 g, Tryptone : 4 g, Ferric citrate : 0.1 g). Cultures at anaerobic conditions (N_2, CO_2, H_2 : 80:10:10) were performed in liquid and solid medium at 4° up to 80°C, with or without sulfur, and at different salinity concentrations : 0, 1, 2, 3 and 4%. About 80 enrichment cultures were obtained and strains are being isolated with two methods: dilution to extinction in liquid medium and colonies transplanting on solid medium. Sequencing and storage in the ISCAR collection of each strain will be done.
New cultivation method is in progress by using adhesive 24-well permeable plates from Coylab laboratories in anaerobic jars (Figure 3).

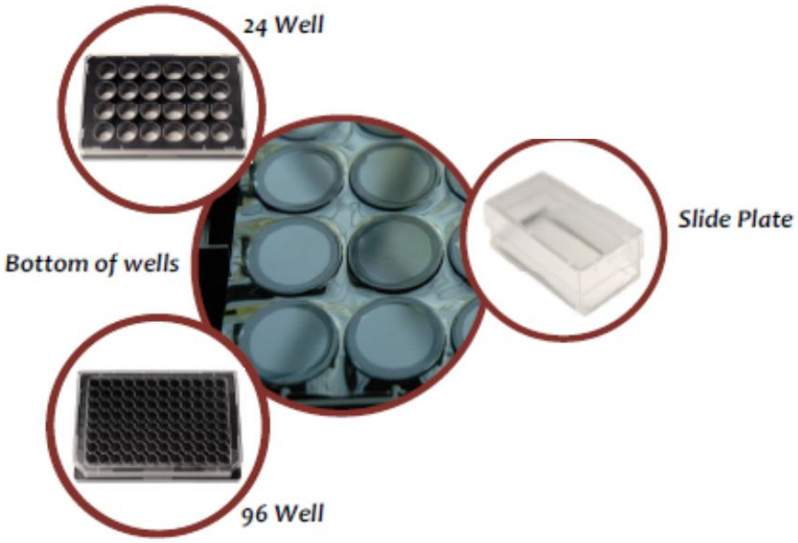

Figure 3 : Gas permeable plates from Coylab laboratories

These plates consist in a plastic plate covered by two polymer films, which permit a high gas transfer rate in retaining liquid. These plates are ideals for intermittent hypoxia studies where the cell microenvironments must change in response to rapid cycling of gaseous 0_2 levels. Furthermore, with its polymer film, adherent cells are able to grow.

1. Zeng, X., et al., *Pyrococcus CH1, an obligate piezophilic hyperthermophile: extending the upper pressure-temperature limits for life.* Isme Journal, 2009. **3**(7): p. 873-876.
2. Ravot, G., et al., *Thermotoga ELFII sp-nov, a novel thermophilic bacterium from an african oil-producing well.* International Journal of Systematic Bacteriology, 1995. **45**(2): p. 308-314.