MaCuMBA

Marine Microorganisms: Cultivation Methods for Improving their Biotechnological Applications

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Deliverable D3.6
Improved cultivation approach for selected microorganisms (from WP2, or already isolated) from coastal and deep extreme sites and the North Atlantic Ocean

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Dissemination Level	
PU Public	
PP Restricted to other programme participants (including the Commission Services)	**X**
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)	

List of reviewers

Issue	Date	Implemented by
v.1	01/02/2015	Viggó Thór Marteinsson
v.2	01/02/2015	Colin Ingham
v.3	23/02/2015	Gwenaelle Le Blay
v. 4	24/02/2015	Colin Ingham

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):
Develop a new cultivation method: MicroDish technique to improve cultivation from coastal and deep extreme sites and from the North Atlantic Ocean.

Rationale:
ACTIVITY ONE AND TWO: In order to increase growth of bacteria or Eukaryotic microorganisms, MicroDish Culture Chips (MDCC) were used (Figure 1 below). The MDCC is a disposable cultivation device based around a porous aluminium oxide base (PAO). MDCC180 chips were used (180 micron diameter wells in a hexagonal array with > 4000 wells/chip, see Figure 1 panels C and D for views of this type of well and Figure 2 for a wider view of a 8 x 36 mm chip). This allows cultivation *in situ* or at least under near natural conditions. After recovery of this device the microorganisms can be analysed by molecular methods (e.g. DNA sequencing) or further cultivation in the laboratory.
The MDCC can be placed in a stainless steel reusable cultivation device used to deploy it in the natural environment (see WP8). Alternatively, and more extensively used in this work, the MDCC can be placed on sea water gelled with agar or Gellan gum or a conventional growth medium. Microorganisms are inoculated onto the MDCC, after a period of time (days to months) the MDCC can be shifted to a new (marine related, low temperature) growth environment (ACTIVITY ONE, concentrating on low temperature growth of arctic samples) and growth reassessed by microscopy. ACTIVITY TWO used the MDCC180.10 for cultivation of thermophilic microorganisms.

During the course of screening a fungus was isolated which showed unusual morphology shifts during cultivation on MDCC (ACTIVITY THREE). This strain was investigated further using a variety of chip-based approaches to determining the surface topography that triggered changes in morphology as a novel method for cultivation optimisation where the texture/topology affects growth.

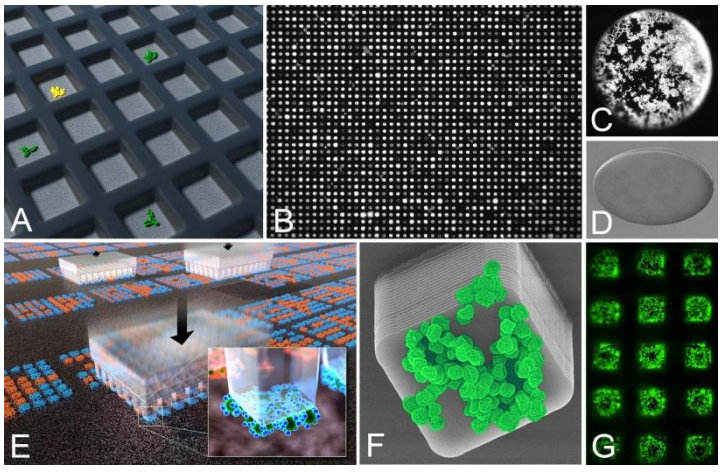

Figure 1. High throughput screening culture chip (MDCC) and printing system. A. Illustration of culture chip with a grid pattern of micron-scale square compartments with a porous aluminium oxide base. Green and yellow microcolonies of bacteria are shown. B. Array of microcolonies growing in 20 x 20 μm culture wells detected by fluorescence microscopy. Visible are over a thousand microcolonies (white, each containing > 50 bacteria) from a chip with 180,000 culture areas. C. Single well from alternative version of a culture chip

showing a single 180 µm diameter culture area with bacteria labelled with the fluorogenic dye Syto 9 and imaged by fluorescence microscopy. D. Scanning electron microscopy of same diameter culture well as panel C, viewed from above at a 45 degree angle. Illustration of printing method with PDMS (an elastomer) stamp dropped from a few microns height to ensure even contact with the PAO beneath. Shown are bacteria with quantum dots used to label the surface. F. PDMS pin loaded with fungal spores (coloured green). G. Image of 40 x 40 µm compartments (3 x 5 are shown) printed with bacteria using a PDMS stamp, the bacteria were labelled with Syto 9 before printing and visualised by fluorescence microscopy.

Results:

ACTIVITY ONE: Microorganisms (100 MD collection, from WP2) derived from North Atlantic (Roscoff) and deep sea Arctic samples were used to explore cultivation optimisation on MDCC chips. The strategy was to move the chips between nutrient environments and look for newly emerging microcolonies triggered by the new culture conditions. Pools of up to 10 isolates from the same environment (described in WP2) were created then inoculated on MDCC180.10, the latter placed on marine agar (or the equivalent with Gellan Gum) and grown at 4 °C for 2 months until no further growth was observed. MDCC were then moved to new medium (either the same medium as a control, or with an additional compound, particularly homoserine lactones) and observed for growth activation of otherwise poorly growing microcolonies. Two approaches were tried, either transfer to marine agar or a formulation in which the same water that the group of microbes was isolated from was used (gelled with Gellan Gum) as the culture medium. In both cases addition of a homoserine lactone was used as a possible growth trigger. MDCC are observed during incubation by low power microscopy (assessing colony number, colony size). Current work suggests that this approach leads to the activation of previously and otherwise poorly growing colonies when the original water sample is used as the growth medium supplemented with N-(3-Oxodecanoyl)-L-homoserine lactone (50 micrograms/ml, Sigma chemicals). Further analysis (identification, quantification of HSL-accelerated isolates) is in progress (**MD**).

ACTIVITY TWO: 5 µl of Icelandic Sea Waters sample were spread in MDCC180.10 placed on marine agar and incubated for one month at 4°C and 17°C (**MATIS**). Overgrowth was observed by microscopy at both temperatures, and samples were diluted. Unfortunately, due to the hydrophobic surface it was difficult to homogeneously spread the sample. **MD** has sent new MDCC (cleaned to make less hydrophobic) and Matís is isolating new thermophilic strains from Reykjanes site (60°C).

ACTIVITY THREE: During screening activities **MD** isolated a Eukaryotic microorganism from North Atlantic water. On an agar based culture medium [1% sea salts (w/v) from Sigma (59883), 5 g peptone/litre, 1g yeast extract/litre and 5 g/l kappa-carageenan] this microorganism appeared to be a rapidly growing fungus with mycelia ~1 microns across and rapidly producing abundant spores. PCR (18S and ribosomal spacer) based sequencing, scanning electron microscopy (SEM) and attempts at axenic culture have been performed. The fungus is an *Acremonium* spp. (based on 18S large subunit and internal transcribed spacer sequencing) with further identification in progress. By manipulating the geometry of the MDCC (well spacing, surface texture, coatings) the culture (biomass, morphology) can be optimised. The fungus formed mycelial bundles growing out the MDCC wells when the diameter was 100 to 300 µm (Figure 2). Imaging (SEM and light microscopy) indicated that in this situation both side branches and sporulation are less, changing the mycelial network from a flat branched network with abundant spores into a parallel bamboo-like mass of mycelia.

A new culture chip has been designed to investigate this further, with a wider range of culture chamber geometries than previously possible (Figure 3). Topology triggered changes in fungal growth are known, but little is known in this respect of sponge derived fungi and *Acremonium* spp. The current working hypothesis is that the MDCC chips are mimicking the texture of marine sponges. Figure 4 shows the growth of *Acremonium* spp. in MDCCVAR.

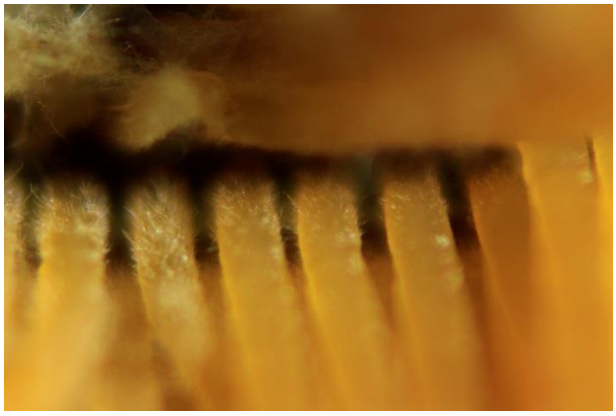

Figure 2. 180 micron diameter mycelial ropes growing out of MDCC180.10 wells. Orange colour comes from a food dye that is incorporated into the mycelial masses (to give contrast).

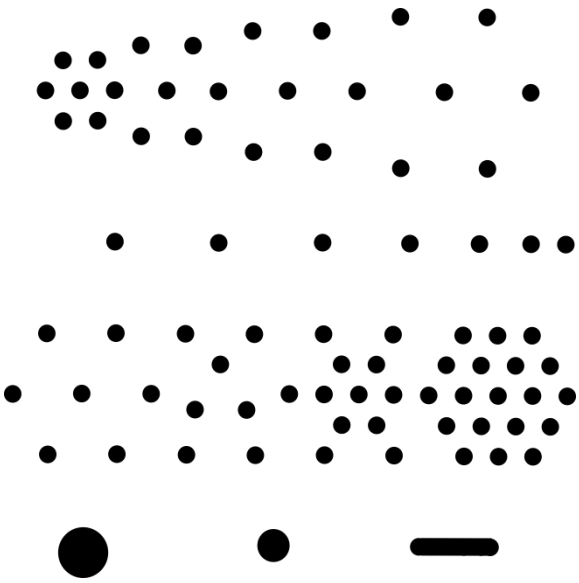

Figure 3. Shadow mask designed to create new MDCC (MDCC180.10VAR) with variable spaced wells (dark areas will create wells of 180 micron diameter for smaller circles, plus other geometries).

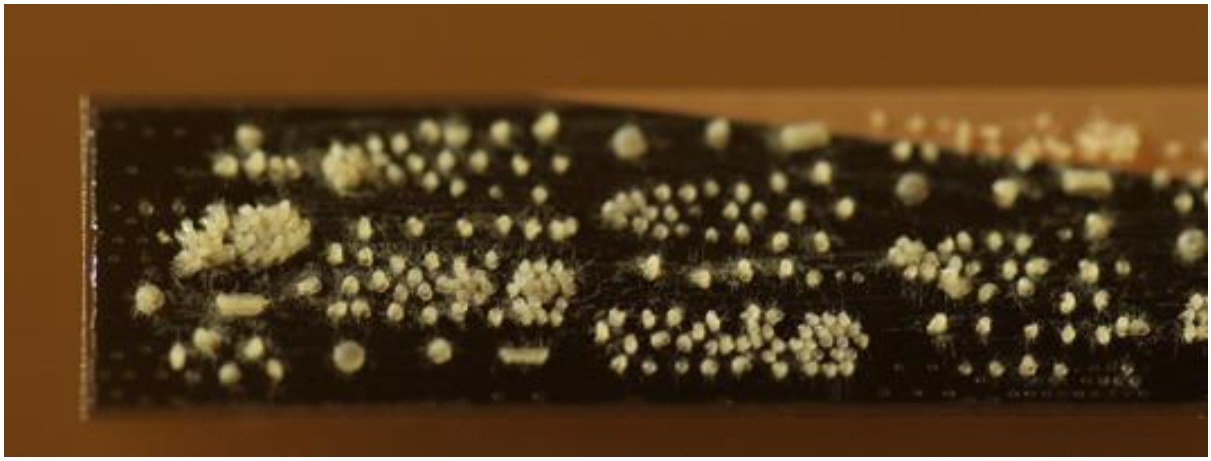

Figure 4. Cultivation of *Acremonium* spp. in MDCCVAR. Chip is 8 x 36 mm with 70% in view.

Partner(s) involved in Deliverable production:
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MicroDish (**MD**) – Colin Ingham