MaCuMBA

Marine Microorganisms: Cultivation Methods for Improving their Biotechnological Applications

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Deliverable D5.5

Assessment of improved culture efficiency by using a living marine microbiota

Organisation name of lead contractor: DTU – partner 14

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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)	**X**

List of reviewers

Issue	Date	Implemented by
v.1	27/7	

Summary

Objective(s): Study the impact of living marine microbiota as background to improve cultivability of marine micro-organisms.

Rationale: The dynamics that drive population growth in the marine ecosystem are driven by living organisms. While cell-cell signals play a major role in shaping these communities, other factors that are produced by the living cells must also be considered. For this reason, the development of systems to utilise living microbiota to improve the culturability of marine isolates is the focus of this deliverable. In tandem with the identification of signal producing pioneer organisms, and the optimisation of culture conditions with abiotic factors, together these advances will provide a platform for the isolation of diverse marine microbial communities.

Results: Partner [14] DTU has embedded AHL-producing marine bacteria in agar and utilised MicroDish custom culture chips to assess culture efficiency. Also, the MicroDish co-culture chamber has been tested. This latter technology has already been developed by MicroDish using algal substrates in the inner chamber. However, due to leakage from the culture chamber, further validation using microbial organisms in a contained system is required before it can be used submerged *in situ* in e.g. harbour-areas. **Partner [4] AQP** was initially involved in this deliverable but has not provided any work/input and has withdrawn from the project. To some extend their role in this task has been taken over by **partner [6] MD,** however, this has caused delays. **Partner [19] PHM** has explored the effect of abiotic factors such as culture volume, age of fermentation, and the use of inorganic elements such as trace elements and salts in culture media. These data will lead to improved and optimised conditions with which to utilise the living microbiota to improve culturability.

Partner(s) involved in Deliverable production:
[4][6][14] [19]

Deliverable D5.5

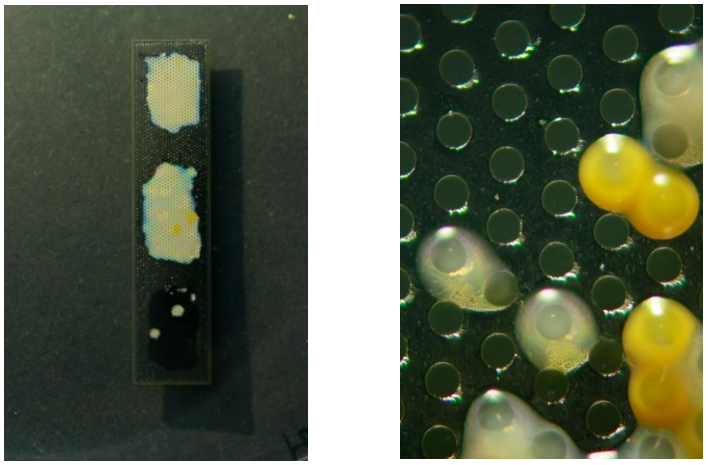
***Assessment of improved culture efficiency by using living marine microbiota*.**

Partner [14] has embedded an AHL-producing marine bacterium (*Phaeobacter inhibens*) in agar. An AHL-knock-out mutant of the same strain has also been similarly embedded in agar, while agar with no bacteria embedded is used as a negative control. This experimental design will ensure that observations can be classified as QS dependent or independent. MicroDish culture chips were placed on top of the embedded agar and marine samples were spread. Preliminary conclusions from these experiments have established the suitability of this approach, although conslusions on culture efficiency will need to be further investigated (Figure 1). **Partner [14]** is currently advancing this work using several dilutions of marine samples to establish quantitative improvements in culture efficiency. The involvement of MicroDish (**partner [6]**) in these experiments has been a valuable interaction, replacing AquaPharm who were initially tasked with providing the experimental set-up.

Figure 1.

Dilution series of a marine algal sample on MD culture chip (left). Closeup demonstrating growth of individual colonies in chip compartments

(data by Sonia Giubergia and MicroDish)



Partner [6] has also worked using its own biochamber (Figure 2). In this instance this partner used algal substrates in the inner chamber. This chamber is being further developed in collaboration with **Partner [14]** to allow having a living culture inside the inner chamber and the submerging in marine waters. However, further system improvements (elimination of leakage) are required before the technology can be used submerged in e.g. harbour-areas.

Figure 2.
The MicroDish biochamber allowing placement of algal extracts or live bacteria in an inner chamber and diffusion – on one side – through filters to the outside environment. On the other side, the contents of the chamber is not allowed to diffuse to the outside

Partner [19] has explored the effect of abiotic factors such as culture volume, age of fermentation, and supplementation with inorganic elements as trace elements and salts in culture media as well as biotic factors, mainly a range of carbon sources (dextrose, glycerol, mannitol, dextrin and other partially hydrolysed starch), nitrogen (malt extract, peptone, soyflour, corn steep power and dried yeast). These conditions are being tested for their impact on growth and secondary metabolite prodution in marine bacteria. All media contained artificial seawater in different concentrations (**Table 1**).

Stressing conditions of excess carbon source, e.g. **mannitol** (M1), has the effect of increasing the rate of bioactivity (specifically antitumor activity) in strains belonging to *Actinomadura* and *Streptosporangium*. Partially hydrolysed starch, such as **dextrines** or glucidex® (M2, M3 and M4) had a similar effect in increasing activity in *Micromonospora*, *Myceligerans* and *Nonomuraea*. This information feed into the development of culture conditions for living microbiota that are being considered for the MicroDish and DTU experiments described above.

Table 1. Percentage of active (antitumour screening) species belonging to deep-sea actinomycetes isolated from IG2 or MUR expeditions. The rate represents the % of active species in the specific media conditions.

Genera	Production media					
Genera	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**
Actinomadura	80%	0%	67%	0%	0%	-
Micromonospora	0%	18%	3%	0%	0%	15%
Myceligenerans	0%	100%	100%	0%	0%	-
Nocardia	10%	0%	25%	0%	0%	0%
Nocardiopsis	25%	25%	0%	0%	0%	0%
Nonomuraea	-	-	20%	50%	0%	-
Streptomyces	45%	31%	51%	49%	9%	17%
Streptosporangium	33%	0%	0%	0%	0%	33%

References: work from this deliverable has not yet been published