MaCuMBA

Marine Microorganisms: Cultivation Methods for Improving their Biotechnological Applications

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Models for pure culture derived from sequence information for relevant selected microbes

Organisation name of lead contractor: UMH – partner 11

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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**

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Issue	Date	Implemented by
v.1		

The following document is related to this deliverable:

Please attach deliverable documents and any additional material if needed.

Summary

Objective(s): Models for pure culture derived from sequence information for relevant selected microbes.

Rationale: Due to the well documented issues with culturing bacteria, there are many groups that lack proper characterization. WP6 is tasked with utilizing innovative genomic strategies, in order to target these microbes that remain elusive. Specifically task three in WP6 focuses on analysing sequence data for cultivability. Thus it is the aim of this deliverable, to outline how sequencing data can be used to help determine specific growth strategies to more efficiently target specific microorganisms.

Results: Based on what is known about the genomes of two important members of pelagic marine communities: *Ca*. Actinomarinales and *Thaumarchaeota*, three enrichment cultures have been developed to isolate these microbes.

Partner(s) involved in Deliverable production:

Partner Name	Partner Number	Participation
UvA	2	NP
MATIS	10	NP
UMH	11	Y
UCC	12	NP
UW	13	Y
UF	18	NP
RIBO	22	NP

NP, no participation in the deliverable

NA, not applicable to current work but will use protocols in future work

Y, reported models for pure culture derived from sequence information

Partner 11 (UMH) has used the genomic approach to enrich for two groups of marine microbes, namely, *Ca*. Actinomarina and marine *Thaumarchaeota*. Using a combination of metagenomics, flow cytometry and FISH, **Partner 11 (UMH)** described a widely distributed novel clade of marine Actinobacteria that have the lowest GC content (32%) reported so far as well as the smallest cells found among free-living prokaryotes (even smaller than the cosmopolitan marine photoheterotroph, '*Candidatus* Pelagibacter ubique' (Rohit et al, 2013). This microbe was named *Candidatus* Actinomarina minuta. Metagenomic fosmids allowed a virtual genome reconstruction that also indicated very small genomes below 1 Mb (Figure 1).

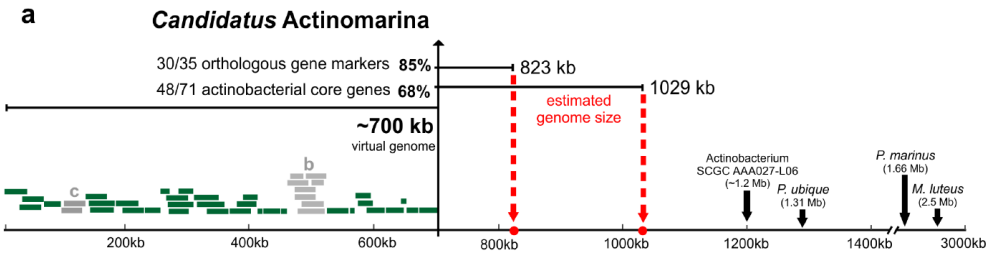

Figure. 1- Linear representation of '*Candidatus* Actinomarina' contigs showing their overlaps. Estimates of the genome size based on different indicators are shown to the right with some reference small genome sizes. Two groups of contigs are highlighted in grey and are shown in greater detail in the panels below.

From the information obtained from the genome the identification of a specific mannitol transporter was possible. Marine bacteria can use mannitol as a compatible solute and osmoprotectant. In order to obtain a pure culture of this microbe **Partner 11 (UMH)** designed enrichment experiments in which Mediterranean seawater was enriched with a modified mannitol media modified (autoclaved seawater, 0.1% mannitol; 0.01% peptone; 50 µg/ml gentamicin). Cultures were then incubated for two weeks at 15°C with 12 h light/dark cycles with constant stirring and finally microbial DNA was extracted for metagenomic sequencing. Attempts at isolating *Ca*. Actinomarina by this approach are ongoing. The genome of *Ca*. Actinomarina minuta also encoded a gene for a cyanophycinase. Cyanophycin is an amino acid polymer used as carbon and nitrogen storage material by several cyanobacteria, e.g. *Synechococcus,* that have large populations in the photic zone of the marine habitat. We also designed culture media (autoclaved seawater, 0.01% aspartic acid, 0.01% arginine and 50 µg/ml gentamicin) using the degradation products of cyanophycin in another attempt to enrich this microbe. Also cyanophycin is in the process to be obtained from cyanobacterial biomass for use as a substrate.

The oxidation of ammonia is the first step towards nitrification that ultimately results in the formation of nitrate, a critical component of the global nitrogen cycle. This step of the cycle is understood to be performed by archaea. Much less is understood regarding archaea in the marine habitat than for bacteria. Among the few marine archaea for which complete genome information is available are Thaumarchaeota, e.g. *Nitrosopumilus maritimus* (Konneke 2005). These are well-known ammonia-oxidising microbes. We designed culture media towards isolation of additional ammonia oxidising microbes. The media composition was as follow (autoclaved seawater, 10mM ammonium chloride, 0.1mM potassium phosphate, 0.2mM magnesium sulphate, 1mm sodium hydrogen carbonate and vitamin solution).

References:

Ghai R., Megumi C., Picazo A., Camacho A., Rodriguez-Valera F. Metagenomics uncovers a new group of low GC and ultra-small marine Actinobacteria. 2013. Scientific Reports; 3. DOI: 10.1038/srep02471

Könneke M., Bernhard A.E., de la Torre J. R., Walker C. B., Waterbury J. B., Stahl D. A. Isolation of an autotrophic ammonia-oxidizing marine archaeon. 2005. Nature; 437, 543-546. DOI:10.1038/nature03911

Partner 13. Marine *Synechococcus* are important primary producers with a ubiquitous distribution throughout the world's oceans. Due to their fluorescent patterns a great deal is now known about the spatial and seasonal distribution of *Synechococcus* as a group. However, over the past decade it has become evident that the widespread distribution of this group can be attributed to a high degree of genetic and genomic diversity. This latter work has been facilitated by the isolation and culture of representatives of several of the major phylogenetic lineages of marine *Synechococcus*, as defined using the 16S rRNA gene. Recent work by **partner 13 (UW)** using a molecular marker with a higher genetic resolution, *petB*, encoding the cytochrome b_6 subunit of the cytochrome b_6f complex, has allowed a fine-scale community structure analysis of natural marine *Synechococcus* populations (see Fig. 1). This revealed the occurrence of several novel environmentally abundant clades without cultured representatives, despite more than 30 years of culturing effort on this genus. Using a range of approaches for culturing marine picocyanobacteria (e.g. natural seawater from the site of strain isolation spiked with low concentrations of

growth media) we have isolated a range of picocyanobacteria on recent Atlantic Meridional Transects undertaken between the UK and South America. Over 50 isolates are now in clonal culture including several sub-lineages with no previously isolated representatives.

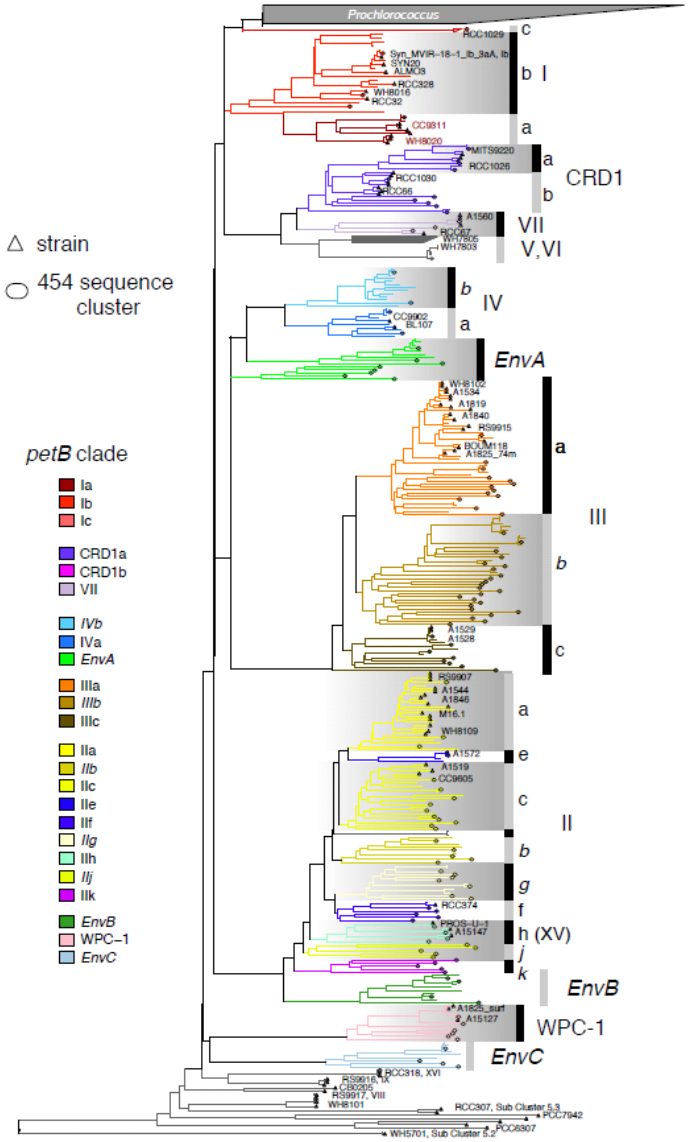

Figure 1. Phylogenetic tree of marine picocyanobacterial *petB* sequences from both cultures (indicated with △) or *petB* amplicons (454 sequence clusters O) from environmental DNA.