MaCuMBA

Marine Microorganisms: Cultivation Methods for Improving their Biotechnological Applications

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Deliverable D5.2

Profile of alkyl quinolone molecules in the marine environment.

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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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v.1		

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

Write a short informative summary of your Deliverable (2 pages maximum), which should include the following elements:

Objective(s): The objective of D5.2 was to profile the marine environment for the presence of alkylquinolone(AQ)-type molecules that can interface with the AQ signalling system in the model organism *Pseudomonas aeruginosa*. Previously only characterised in *P. aeruginosa*, *in silico* analysis strongly suggests the presence of AQ signalling systems in other organisms.

Rationale: A combination of functional screening and *in silico* analysis was used to profile collections of marine isolates and metagenomic databases for the presence of AQ-type signalling systems.

Functional screening: Both –lacZ (β-Galactosidase) and –lux (Bioluminescence) *pqsA*-promoter reporter fusions were used to identify AQ-activating compounds in a collection of >650 marine isolates isolated from marine sponges. Initially screened on solid agar overlays, cell-free supernatants were obtained from positive candidates after 3 and 6 days growth in marine broth. Culture based assays were then performed to validate the activation of the AQ-dependent *pqsA*-promoter fusions. These isolates are currently being identified through 16S rRNA gene sequencing. TLC analysis was used to perform preliminary profiling of the marine supernatants from AQ-type molecules while an Electrovoltammetric Detection system, recently developed in the BIOMERIT Research Centre (BRC) is also being employed for this purpose.

In silico analysis: The IMG database contains a rich resource of genomic and metagenomic datasets which were mined for AQ biosynthetic genes. As no COG or Pfam domains have been attributed to these biosynthetic genes as of yet, BLAST analysis was utilised to screen for homologous sequences. All five genes of the biosynthetic cluster were used in this analysis and the identification of putative encoding strains was based on (1) presence of at least 3 encoding genes, (2) genomic arrangement (e.g. operon/cluster or distantly encoded and (3) neighbourhood analysis (in genome sequences). Phylogenetic trees from genomic sequences were built using Neighbour Joining and Maximum Likelihood algorithms.

Results: Profiling of the marine isolate collection has resulted in the identification of AQ activating isolates, three of which have been further evaluated by TLC. Production of the AQ-type compound is temporal and strain specific and TLC analysis indicates that chemically these compounds may be distinct from the HHQ and PQS molecules of *P. aeruginosa*. Validation of the remaining positive candidates is ongoing as are efforts to isolate the active component of the marine supernatants.

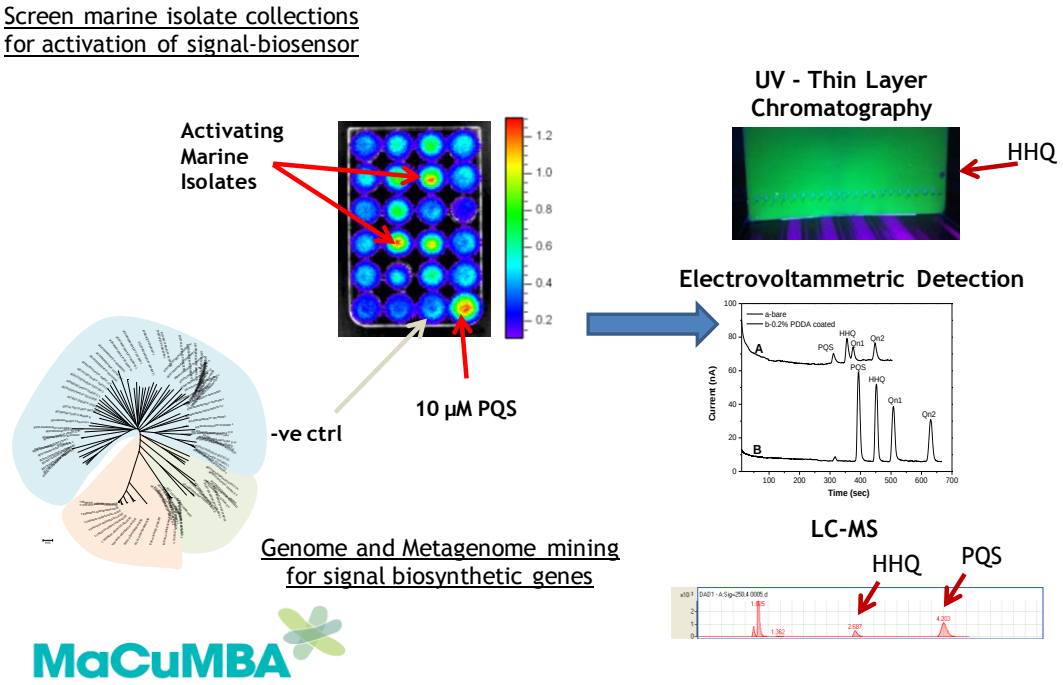

Figure 1. Methodology and results of AQ profiling of marine isolates. Activator screening using *pqsA*-promoter fusions in a PAO1 *pqsA* mutant strain was followed by validation using TLC and Miller Assays. *In silico* analysis has provided data for phylogenetic tree construction and mining of metagenomic databases.

In silico analysis has revealed the distribution of AQ biosynthetic genes among a broad spectrum of bacterial isolates, including those of marine origin. The biosynthetic genes have also been identified in metagenomic databases, although neighbourhood analysis has not been possible with these searches.

Partner(s) involved in Deliverable production: No other contributing partners.