



MaCuMBA

Marine Microorganisms: Cultivation Methods for Improving their
Biotechnological Applications

Project number: 311957

Start of the project (duration): August 1st, 2012 (48 months)

Collaborative Project
Seventh Framework Programme
Cooperation, KBBE

Deliverable D5.1

**Profile of AHL based quorum sensing molecules in the
marine environment.**

Organisation name of lead contractor: UCC (12)

Due date of deliverable: M08

Actual submission date: M12

Revision: V.1

Project co-funded by the European Commission within the Seventh Framework Programme (2007-2013)	
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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List of reviewers

Issue	Date	Implemented by
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): Detection and identification of AHL-based quorum sensing molecules from the marine environment.

Rationale: The paradigm for quorum (QS) sensing systems is the bioluminescent LuxIR system of *Vibrio fischeri*, a bacterium of marine origin that engages symbiotically with the squid *Euprymna scolopes*. While the spectrum of characterised QS systems has grown considerably since then, the AHL-based LuxR regulatory protein appears to be the most widely encoded. Therefore, there is a strong likelihood that AHL production is a major factor in controlling population dynamics within the marine ecosystem. High throughput screening of marine isolate collections (>650 marine isolates) was therefore undertaken to profile the existence of AHL-like molecules in the marine niche. In tandem with this analysis, *in silico* mining of genomic and metagenomic databases has also been undertaken to profile the distribution of AHL-associated loci in publically available sequences.

Results:

Functional Screening: Screening of >650 marine organisms isolated from marine sponges using a set of three biosensor strains (*Chromobacterium violaceum* CV026, *Serratia marcescens* SP15, and *Agrobacterium tumefaciens* traG::lacZ reporter fusion) has been completed. AHL producing marine isolates have been identified and confirmed using the *S. marcescens* SP15 (10 isolates) and *A. tumefaciens* (3 isolates) biosensor strains indicating that the micro-organisms possess short and long chain AHLs. No positive isolates were identified using the AHL *C. violaceum* biosensor. These isolates are currently being identified through 16S rRNA gene sequencing. AHLs are being isolated from these marine isolates and have been sent for characterisation to DTU. Screening of metagenomic libraries generated from marine sponges is ongoing.

Screening strategy

- Biosensors that are unable to produce indigenous AHLs.

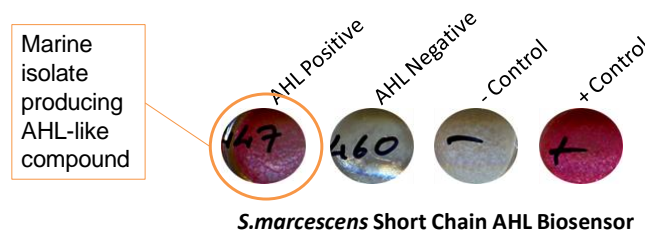


Figure 1. Use of *S. marcescens* biosensor strain to identify AHL positive strains from the marine niche. Of >650 marine isolates that have been screened, 10 long chain AHL producers and 3 short chain AHL producers have been identified.

DTU has continued work on elucidating the nature/structure of the QS inducing compound in *P. luteoviolaceae*. Extracts have been compared (LC-MS) to all available AHLs and a lactone ring is not detected, even though a *luxI* analogue was identified in 3 of 12 strains of *P. luteoviolaceae*. The presence of the *luxI* homologue correlated with production of the antibiotic indolmycin.

Mutagenesis *P. luteoviolaceae* to knock out the presumed luxI-related gene has not yet been possible and DTU are currently developing a sorbitol washing procedure before electroporation. Potentially, the organism may be producing a new QS type molecule.

In silico analysis: UCC have commenced screening of available genome and metagenome datasets for AHL pathways, identifying a considerable prevalence of AHL synthase gene homologues among marine metagenomes.

Partner(s) involved in Deliverable production:

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