WAMSI PROJECT REPORT

Project Details

Project Number and Title:	5.4 Microbial Quorum Sensing
Node Leader:	Howard Shawcross
Project Leader:	Assoc. Prof. David Sutton
Project Team:	Assoc. Prof. David Sutton, Prof. Barbara Chang, Prof Emilio Ghisalbeti, Ms Jamie Summerfield
Project Start Date:	1 Jan 2008
Project End Date:	30 June 2011
Due Date for Final Report:	

1. Project Objectives and Achievement Criteria

Confirmation of the project objectives and the delivery of milestones against the Key Performance Indicators:

Project objectives

(i) to purify and identify quorum quenching compounds (*QQCs*) from marine invertebrates and bacteria;
(ii) to assess the diversity of quorum quenching effects and mechanisms of action amongst *QQCs*.
(iii) to investigate ecological aspects of the formation and activity of *QQCs*
(iv) to assess potential applications of *QQCs*.

#	Milestone	Date	Status
5.4.1	Commence PhD	Mar 07	Completed
5.4.2	Collect, screen and purify marine bacteria with QQ activity; begin purification of QQCs	Mar 08	Completed
5.4.3	Submit Progress Report to Supervisor/WAMSI	Apr 08	Completed
5.4.4	Continue purification of QQCs and begin identification of selected QQCs; develop additional QQ assays	July 08	Completed
5.4.5	Identify bacteria producing QQCs	Sept 08	Completed
5.4.6	Submit Progress Report to Supervisor/WAMSI	Apr 09	Completed
5.4.7	Continue identification of QQCs	May 09	Completed
5.4.8	Assess diversity of quorum quenching effects and mechanisms of action amongst QQCs	Aug 09	Completed
5.4.9	Investigate ecological aspects of the formation and activity of QQC's	Dec 09	Completed
5.4.10	Assess possible applications of QQCs	Feb 10	Completed
5.4.11	Submit draft of PhD; Submit Progress Report to Supervisor/WAMSI	Mar 10	Progress reporting completed. Thesis drafting still underway.
5.4.12	Academic Exit Seminar	Aug 10	Scheduled for second half of 2011.

2. Research Chapter(s)

Introduction

The project investigated aspects of the diversity, ecology and application of novel marine natural products termed *quorum quenching compounds (QQCs)*, obtained from marine bacteria. These products have potential pharmacological and commercial uses in the control of bacterial activities (including human and aquaculture disease, biofilm formation, and biofouling), and also have important ecological roles, including in invertebrate and algal settlement.

3. Methodology

Bioassay for detection of QQ bacteria:
Natural marine samples were screened for the presence of bacteria with QQ activity using a bioluminescence inhibition assay developed specifically for the purpose.

Bacterial isolation, culture and purification:
Bacteria testing positive in the screening assay were isolated, purified and preserved using standard microbiological culturing techniques.

Bacterial identification:
Bacterial isolates were identified using exhaustive biochemical testing to establish phenotypic characteristics, volatile fatty acid analysis, and molecular techniques based on 16S RNA analysis of genomic DNA. Test results were compared with published data on bacterial phenotypes (phenotypic characters) and online BLAST sequences (molecular analysis). Known and unknown isolates were grouped using phylogenetic analysis.

Confirmation of QQ activity and qualitative QQ assay:
The purified bacterial isolates were assessed for QQ activity in a quantitative assay (based on spectrophotometric methods to detect both growth and bioluminescence, enabling assessment of relative bioluminescence inhibition.

Extraction, purification and identification of QQCs:
Using a bioassay-directed procedure the QQCs from selected bacterial isolates were purified and identified using a combination of chemical procedures including solvent extraction, HPLC, GC, NMR and MS.

Assay of QQCs against additional quorum sensing processes:
Purified QQCs from the selected bacterial isolates were screened for their effects in a range of QQ assays including inhibition of bioluminescence by *Vibrio harvey*i, pigment production by *Serratia marcescens* and *Chromobacterium violaceum*, and biofilm formation by *Pseudomonas aeruginosa.*

4. Results

1. Bacteria inhibiting bioluminescence and/or growth of *Vibrio harveyi* strain Vh1 were found to be abundant in marine and estuarine waters of the Perth metropolitan area.

2. A total of 89 bioluminescence inhibiting and 18 growth inhibiting marine bacteria were isolated from marine and estuarine waters collected from 4 sites in the Perth metropolitan area. These bacteria were identified as *Vibrio* (58), *Pseudoalteromonas* (31), *Enterovibrio* (5), *Bacillus* (3), *Marinomonas* (2) and *Photobacterium* (2), as well as *Reinekea, Proteus, Flammeovirga* and *Halomonas* (1 of each).

3. Methanol extracts of media used to grow marine isolates K1 and B2 (chosen for their strong bioactivity) inhibited the quorum sensing-regulated processes of bioluminescence and pigment production in quantitative assays, indicating that the extracts were likely to contain quorum quenching compounds.

4. The bioactive compounds produced by isolates K1 and B2 were purified from the growth media extracts and identified as desferrioxamine compounds. Commercially available analogues of the desferrioxamine compounds exhibited the same bioactivity as the extracts in qualitative tests.

5. A desferrioxamine compound analogue was assessed for its affect on diverse quorum sensing-regulated processes in various quorum sensing bacteria using quantitative assays. In most assays the desferrioxamine compound either affected growth of the quorum sensing bacteria, or had no effect on the quorum sensing-regulated processes, indicating that the desferrioxamine

compound was not directly a quorum quenching compound. Evidence from other studies that indicate that Fe may be associated with regulation of quorum sensing processes suggest that the desferrioxamine compound may affect quorum sensing through involvement in Fe availability.

6. The marine isolates were tested for the production of desferrioxamine-like compounds in a qualitative assay. Five isolates, belonging to the genera *Bacillus, Reinekea* and *Flammeovirga*, did not produce desferrioxamine-like compounds and so remain likely candidates for the production of quorum quenching compounds.

7. Attempts were made to determine how desferrioxamine inhibits bioluminescence of *Vibrio harveyi* strain Vh1, however the results indicate the process is very complicated and it was not able to be fully elucidated as part of this project.

5. Discussion

Implications for Management and Advancement of the Field:

The project has enhanced understanding of the production of novel marine bacterial metabolites with potential human health and industrial applications.

Problems encountered:

1. Introducing a study of the numbers and diversity of bacteria producing quorum quenching compounds to objective (i) increased the amount of work required This study required additional sampling at 2 time points and resulted in the isolation of a further 98 isolates, all of which required processing and identification.
2. Some of the isolates repeatedly failed to produce PCR products from colony lysates and so these isolates required genetic extraction. Fatty acid analysis provided further information about each isolate but could not provide identification to the species level. Therefore biochemical and enzymatic testing was necessary. It took several weeks to determine which biochemical tests were required for each genera, as no comprehensive information has been published for the little researched genera. Also, some of the earlier journal articles identifying species were not available online.
3. Attempting to purify compounds from the bacterial isolates was difficult due to the low quantities of the bioactive compounds being produced. This entailed multiple cycles of crude extract preparation and separation, which was very time-consuming. The complexity of the extracts also required a number of sophisticated separation techniques to be employed and optimised to allow isolation of pure compounds for identification purposes. Once purified extracts were obtained, it took 10 weeks for analysis by high resolution mass spectrometry.
4. A lot of difficulty was encountered in making a specific assay (CAS) solution, which took some time to resolve.
5. When it became clear that the compound DFO B was unlikely to be inhibiting quorum sensing, and was probably moderating iron levels, iron-free culture media had to be developed. This was very difficult because the constituents used to make bacterial media all have trace amounts of iron in them, as does water. These trace amounts were able to support bacterial growth, and so had to be removed; this was a difficult and time consuming process.
6. The PhD student involved in the project suffered a debilitating and chronic wrist condition in 2010, which required an operation and reduced her capacity to undertake lab work for 4-6 months.

New Research Directions:

The major differences from what was originally proposed were (a) the decision to focus on marine bacteria as a source of QQCs, made because the production of these compounds by bacteria greatly simplified (because of the capacity to grow the bacteria in culture) the generation of material for chemical analysis; and (b) less of a focus on objectives (iii) and (iv) in preference to investigating possible mechanisms of action (objective ii). This was in part necessitated by the complexity of the analysis of mechanisms of action.

6. Overall Project Accomplishments

Students supported:

Jamie Summerfield (PhD candidate). Jamie carried out the study under the supervision of the other participants.

PhD theses, Dissertations and Student Placement:

Jamie's PhD thesis is expected to be submitted early 2012.

Publications:

Nil to date, but several publications are expected to follow submission of the thesis.

Presentations:

Scientific Posters Presented:

- Quorum Quenching Compounds. Summerfield J, Sutton D, Chang B. BBCS Research Forum 2007 Perth.
- Quorum Quenching Compounds: A novel approach to bacterial control. Summerfield J, Sutton D, Chang B, Ghisalberti E, Flematti G. ASM Annual Scientific Meeting 2008 Melbourne.
- Marine bacterial quorum quenching compounds. Summerfield J, Sutton D, Chang B, Ghisalberti E, Flematti G. Combined Biological Sciences Meeting 2009 Perth.

Seminars Presented:

- Quorum Quenching Compounds: A novel approach to bacterial control. Summerfield J. Combined Biological Sciences Meeting 2008 Perth.
- Marine Bacterial Quorum Quenching Compounds. Summerfield J. ASM Annual Scientific Meeting 2009 Perth.

Other Communications Achievements - Interviews, press releases, etc.

Nil

7. Overall Project Benefits Please note: Benefits go beyond Results and Accomplishments to provide information on direct physical, environmental, economic or social gains realised as a result of a research project or outreach activity.

Discovery and Application of New Products and Processes (if applicable)

This project comprised an initial survey of the production of a unique class of chemical compounds (QQCs) by marine bacteria, and a preliminary assessment of the mechanisms and scope of activity of any compounds identified. Consequently, it was not expected that commercially useful products would be generated, although this was a possibility. However, the project has clearly demonstrated that there is the potential to discover and develop novel QQCs from marine bacteria, and suggests that this is an area worth pursuing.

Tools, Technologies and Information for Improved Ecosystem Management

NA

Forecasting for Natural Resource Management Decisions

NA

Impacts:

Although still in a developmental stage, the research has focussed on alternative ways of controlling harmful and detrimental microbial activities, and indicates the potential for new natural products to be found from marine bacteria to address important industrial and medical issues, including antibiotic resistance.

8. Project Metadata and Data Generated

These must be available at an open access repository/data centre/iVEC.

The project has generated bacterial isolates that are preserved in the Discipline of Microbiology and immunology, UWA. **http://waodn.ivec.org/geonetwork/srv/en/metadata.show?uuid=c424f38e-457a-4213-9a55-26dbecee50d0**

9. Linkages to Associated Projects – can be WAMSI and non-WAMSI

Potential linkages to the Marine Bioresources Library, although this facility has no capacity to manage living cultures such as those generated in this study.

10. Other Comments and General Discussion

11. Annexures

- **Sub-project reports presented**
- **Additional attachments**