COURSE MANUAL

ENVIRONMENTAL MICROBIOLOGY (MICR3071)

MARINE MICROBIOLOGY (MICRO9071)

2017
School of Biotechnology and Biomolecular Sciences

MICR3071

Environmental Microbiology

MICRO9071

Marine Microbiology

Session 1 2017
I certify that:

- I have read and understood the University Rules in respect to Student Academic Misconduct and plagiarism.
- I have read and understood the assessment requirements for the course
- I have read and understood the laboratory safety procedures as set out in the laboratory protocol book

Signature: ..........................................................

Date: ...............................................................
Welcome to Environmental Microbiology (MICR3071)

Course staff:
- Convenor: Brendan Burns (brendan.burns@unsw.edu.au) Room 354A
- Technical Staff Member: Khaled Daud
- Demonstrators: James Charlesworth, Alan Wong

Course information:
This course is worth 6 units of credit

Environmental Microbiology MICRO3071 provides a comprehensive introduction to microbial ecology, fundamental aspects of microbial physiology and diversity, and selected themes that are essential features of applied and environmental microbiology. The course is designed to give you an up-to-date understanding of modern research in this field and the link between laboratory-based research and application in the field. As a higher-level subject, students gain an insight into the contemporary theory and practice of microbial ecology, which overcomes the significant limitations of classical microbiology.

The structure of the laboratory sessions is designed to give you training in the practical skills necessary for the workplace, and is applicable whether you plan to continue your career in academic research, industry or any work that requires contact with science and research. There is an emphasis on planning and carrying out practical tasks as members of a group, as this is a realistic parallel to “life after University”, however the writing of scientific reports on the results you obtain in the laboratory is an individual responsibility.

Learning outcomes

When you successfully complete this course you will be able to:

1) Discuss microbial ecology concepts including:
   a) Factors that limit microbial growth in natural habitats
   b) Methods for studying microbial populations and their function in the natural environment
   c) Ecological principles and mechanisms of microbial interactions within mixed microbial communities and between microorganisms and higher organisms
   d) The effect of microorganisms on the global environment eg: their role in cycling of elements
   e) The application of microbial ecological principles for industrial, environmental or public health benefits
2) Test scientific hypotheses via experimental design, analyse results and discuss outcomes in the light of the current body of knowledge (e.g. literature)
3) Critically evaluate scientific output. This includes self generated, peer generated and published literature
   4) Demonstrate effective written and verbal scientific communication skills

*This course is also badged as Marine Microbiology (MICRO9071), a postgraduate course listing. The content, outcomes, and running of the course is exactly the same as for MICRO3071.
# Lecture schedule

<table>
<thead>
<tr>
<th>Week</th>
<th>Date</th>
<th>Time</th>
<th>Topic</th>
<th>Lecturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mon 27/2</td>
<td>10-11 am</td>
<td>No lecture</td>
<td>BB</td>
</tr>
<tr>
<td></td>
<td>Wed 1/3</td>
<td>2-3 pm</td>
<td>Introduction to course and project</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Mon 6/3</td>
<td>10-11 am</td>
<td>Microbial communities and interactions 1</td>
<td>SE</td>
</tr>
<tr>
<td></td>
<td>Wed 8/3</td>
<td>2-3 pm</td>
<td>Microbial communities and interactions 2</td>
<td>SE</td>
</tr>
<tr>
<td>3</td>
<td>Mon 13/3</td>
<td>10-11 am</td>
<td>Microbial communities and interactions 3</td>
<td>SE</td>
</tr>
<tr>
<td></td>
<td>Wed 15/3</td>
<td>2-3 pm</td>
<td>The tree of life</td>
<td>BB</td>
</tr>
<tr>
<td>4</td>
<td>Mon 20/3</td>
<td>10-11 am</td>
<td>Stable Isotope Probing</td>
<td>BB</td>
</tr>
<tr>
<td></td>
<td>Wed 22/3</td>
<td>2-3 pm</td>
<td>Methods to detect microbes</td>
<td>BF</td>
</tr>
<tr>
<td>5</td>
<td>Mon 27/3</td>
<td>10-11 am</td>
<td>Biofilms 1</td>
<td>BB</td>
</tr>
<tr>
<td></td>
<td>Wed 29/3</td>
<td>2-3 pm</td>
<td>Biofilms 2</td>
<td>BB</td>
</tr>
<tr>
<td>6</td>
<td>Mon 3/4</td>
<td>10-11 am</td>
<td>Environmental genomics</td>
<td>TT</td>
</tr>
<tr>
<td></td>
<td>Wed 5/4</td>
<td>2-3 pm</td>
<td>Metaproteomics</td>
<td>TW</td>
</tr>
<tr>
<td>7</td>
<td>Mon 10/4</td>
<td>10-11 am</td>
<td>Bacterial sensing and adaptation</td>
<td>BB</td>
</tr>
<tr>
<td></td>
<td>Wed 12/4</td>
<td>2-3 pm</td>
<td>Bacterial sensing and adaptation</td>
<td>BB</td>
</tr>
</tbody>
</table>

**Mid-session break (Easter): 17/4 – 21/4**

| 8    | Mon 24/4   | 10-11 am | Microbes in extreme environments                | BB       |
|      | Wed 26/4   | 2-3 pm   | Microbial nutrient cycling - C                  | BB       |
| 9    | Mon 1/5    | 10-11 am | Ocean microbiology                              | MB       |
|      | Wed 3/5    | 2-3 pm   | Ocean microbiology                              | BB       |
| 10   | Mon 8/5    | 12-1 pm  | Microbial nutrient cycling - N                  | BB       |
|      | Wed 10/5   | 3-4 pm   | Microbial nutrient cycling - S                  | BB       |
| 11   | Mon 15/5   | 10-11 am | The future of studying the past: stroms         | BB       |
|      | Wed 17/5   | 2-3 pm   | Cyanobacteria and water quality                 | BB       |
| 12   | Mon 22/5   | 10-11 am | Microbial diversity and drug discovery          | BB       |
|      | Wed 24/5   | 2-3 pm   | Human microbiome                                | SL       |
| 13   | Mon 29/5   | 10-11 am | Waste and drinking water micro                  | MS       |
|      | Wed 31/5   | 2-3 pm   | Course summary                                  | BB       |

Lectures Mon 10-11 (CLB 5) and Wed 2-3 (CLB3)

Brendan Burns (BB); Suhelen Egan (SE); Torsten Thomas (TT); Belinda Ferrari (BF); Michael Storey (MS); Tim Williams (TM); Steven Leach (SL); Mark Brown (MB)
# MICR3071: Environmental Microbiology S1 2017

## Tutorial, lab, assessment schedule

<table>
<thead>
<tr>
<th>Week</th>
<th>Date</th>
<th>Task</th>
<th>Time</th>
<th>Details/Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thur 2/3</td>
<td>NA</td>
<td>NA</td>
<td>No tutorial</td>
</tr>
<tr>
<td></td>
<td>Fri 3/3</td>
<td>NA</td>
<td>NA</td>
<td>No lab</td>
</tr>
<tr>
<td>2</td>
<td>Thur 9/3</td>
<td>Tutorial</td>
<td>12-1</td>
<td>Lab intro talk (G08)</td>
</tr>
<tr>
<td></td>
<td>Fri 10/3</td>
<td>Lab</td>
<td>2-5</td>
<td>Lab intro, OHS, research plans (G08)</td>
</tr>
<tr>
<td>3</td>
<td>Thur 16/3</td>
<td>Tutorial</td>
<td>12-1</td>
<td>Biosensors</td>
</tr>
<tr>
<td></td>
<td>Fri 17/3</td>
<td>Lab</td>
<td>2-5</td>
<td>Work on Research Project</td>
</tr>
<tr>
<td></td>
<td>Fri 17/3</td>
<td>Assess</td>
<td></td>
<td>Major research plan due</td>
</tr>
<tr>
<td>4</td>
<td>Thur 23/3</td>
<td>Tutorial</td>
<td>12-1</td>
<td>Poster topic prep (GO8)</td>
</tr>
<tr>
<td></td>
<td>Fri 24/3</td>
<td>Lab</td>
<td>2-5</td>
<td>Work on Research Project</td>
</tr>
<tr>
<td>5</td>
<td>Thur 30/3</td>
<td>Tutorial</td>
<td>12-1</td>
<td>Mid session quiz (7%)</td>
</tr>
<tr>
<td></td>
<td>Fri 31/3</td>
<td>Lab</td>
<td>2-5</td>
<td>Work on research project</td>
</tr>
<tr>
<td>6</td>
<td>Thur 6/4</td>
<td>Tutorial</td>
<td>12-1</td>
<td>Report writing/new and views</td>
</tr>
<tr>
<td></td>
<td>Fri 7/4</td>
<td>Lab</td>
<td>2-5</td>
<td>Work on research project</td>
</tr>
<tr>
<td>7</td>
<td>Thur 13/4</td>
<td>Tutorial</td>
<td>12-1</td>
<td>TBA</td>
</tr>
<tr>
<td></td>
<td>Fri 14/4</td>
<td>Lab</td>
<td>2-5</td>
<td>No lab- GOOD FRIDAY</td>
</tr>
<tr>
<td></td>
<td>13/4</td>
<td>Assess</td>
<td></td>
<td>Draft research project report due (10%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mid-session break (Easter): 17/4 – 21/4</td>
</tr>
<tr>
<td>8</td>
<td>Thur 27/4</td>
<td>Tutorial</td>
<td>12-1</td>
<td>BLAST Sequences</td>
</tr>
<tr>
<td></td>
<td>Fri 28/4</td>
<td>Lab</td>
<td>2-5</td>
<td>Work on Research Project</td>
</tr>
<tr>
<td></td>
<td>Mon 24/4</td>
<td>Assess</td>
<td></td>
<td>News and views due (8%)</td>
</tr>
<tr>
<td>9</td>
<td>Thur 4/5</td>
<td>Tutorial</td>
<td>12-1</td>
<td>TBA</td>
</tr>
<tr>
<td></td>
<td>Fri 5/5</td>
<td>Lab</td>
<td>2-5</td>
<td>Work on Research Project</td>
</tr>
<tr>
<td>10</td>
<td>Thur 11/5</td>
<td>Tutorial</td>
<td>12-1</td>
<td>Poster preparation</td>
</tr>
<tr>
<td></td>
<td>Fri 12/5</td>
<td>Lab</td>
<td>2-5</td>
<td>Poster preparation</td>
</tr>
<tr>
<td></td>
<td>12/5</td>
<td>Assess</td>
<td></td>
<td>Final Research project report due (20%)</td>
</tr>
<tr>
<td>11</td>
<td>Thur 18/5</td>
<td>Tutorial</td>
<td>12-1</td>
<td>Poster preparation</td>
</tr>
<tr>
<td></td>
<td>Fri 19/5</td>
<td>Lab</td>
<td>2-5</td>
<td>Talk preparation</td>
</tr>
<tr>
<td>12</td>
<td>Thur 25/5</td>
<td>Tutorial</td>
<td>11-12</td>
<td>TBA</td>
</tr>
<tr>
<td></td>
<td>Fri 26/5</td>
<td>Lab</td>
<td>2-5</td>
<td>Posters and oral presentations (15%)</td>
</tr>
<tr>
<td>13</td>
<td>Thur 1/6</td>
<td>Tutorial</td>
<td>11-12</td>
<td>TBA</td>
</tr>
<tr>
<td></td>
<td>Fri 2/6</td>
<td>Lab</td>
<td>2-5</td>
<td>Posters and oral presentations</td>
</tr>
</tbody>
</table>

Tutorial location: To be advised depending on task (Thurs 12-1)
Lab location: Lab 110 (Bioscience building) (Fri 2-5)
Assessment information:

The mark for the course will be allocated as follows:

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous assessment</td>
<td>60%</td>
</tr>
<tr>
<td>Final examination</td>
<td>40%</td>
</tr>
</tbody>
</table>

In order to pass this subject, students must:

i. Satisfactorily complete all parts of the continuous assessment.

ii. Attend at least **eighty percent** of prescribed practical classes and tutorials.

**Note:** The practical classes are of **3h** duration. The roll may be marked during each class.

A 1-hour tutorial will be held most weeks, it is expected that you will participate in all tutorial discussions, and your efforts will be graded as **unsatisfactory or satisfactory** at the end of the session.

Components of the continuous assessment (60% total):

Assessment during session will consist of the following:

1. Quiz 7%
2. News and Views 8%
3. Major research project paper 30%
4. Poster & presentation 15%

**Quiz (7%):**

The purpose of this quiz is to provide students with the opportunity to assess their progress and understanding during the session. The quiz will be held in the tutorial time slot in week 4 and will consist of short answer questions, which will cover material from the lecture, tutorial and practical session and aim to assess learning outcomes 1 and 2 (see page 4).

**News and Views piece (8%):**

This assessment task is designed to test your literacy skills and capacity to write on a ‘hot’ topic in a manner that is easily digestable. This will be written in the style of a short ‘News and Views’ report as seen in the journals *Nature* or *Science*. Further details on this assessment task will be given in the tutorial in Week 6.
Major research project- scientific paper (total 30%):

This is a report on the research project that you and your group will be planning and carrying out during weeks 2-10. The assessment for this project is in three parts.

1) The submission of a proposal and risk assessment in week 3
2) The submission of a draft report in week 7 (worth 10%)
3) The final report submission in week 10 (worth 20%)

The final report will be in the format of a scientific paper and aims to assess learning outcomes 2, 3 and 4 (see page 1). Although the lab work will be done as a group, your report on that work must be done individually. You will be given detailed information on this project during the first lecture in week 1 and tutorial & laboratory sessions in week 2 and 3. You will be given guidance on scientific report writing during both the laboratory and tutorial sessions. This is a major assignment and so should not be left to the last minute to complete. Therefore, as part of the learning process you will be required to submit a draft report in week 7. More details on the requirements for your reports will be given throughout the course. You will be provided with feedback that can be incorporated into your final report.

Poster & presentation (total 15%):

This is intended as an exercise in information literacy and presentation skills and thus will be assessing learning outcomes 3 and 4 (see page 4). In a small group (of say 2-4) you will be asked to choose a topic of interest to you related to environmental microbiology. You will be give guidance in this during the laboratory and tutorial sessions. The assessment for this project is in two parts:

1) As a group you will produce a scientific poster to present your findings in this area. During the final 2 laboratory sessions (week 12 & 13).
2) Each small group will also present their topic to the class (10% coordinator mark plus 5% peer assessment).

Please note that we will be giving you the option to have your posters printed, if you would like this option the poster will need to be submitted electronically one week before (i.e. week 11)
Final examination (40% total):

Final examination:

The two-hour paper will consist of two parts worth equal marks:

Part A: A short answer section of 6 questions, each of which is worth 10 marks (ie: 60% of exam paper).

Part B: An essay section in which students will be asked to answer two, out of a choice of five questions, each 20 marks (ie: 40% of exam paper).

The essay section will include a question worded as follows:

Select an area in environmental microbiology. Clearly and in detail:

  a. Define why this area is of interest.
  b. Review current knowledge of the area and discuss continuing research approaches.

This question is designed to allow students the opportunity of in-depth study of areas they find particularly interesting. Those wishing to attempt it are advised to prepare by reading recent scientific literature, as successful candidates will need to demonstrate a knowledge of the area which is more extensive than can be provided in lectures. According to past experience, anyone attempting this question without prior preparation is likely to do very poorly. If you have not specifically prepared you would be best advised to choose from the set essay topics. YOU MAY NOT ANSWER THIS QUESTION IN THE AREA CHOOSEN FOR YOUR POSTER PROJECT. IF YOU DO SO, NO MARKS WILL BE AWARDED.
**Academic honesty and plagiarism:**

Plagiarism is the presentation of the thoughts or work of another as one’s own.*

Examples include:

- direct duplication of the thoughts or work of another, including by copying work, or knowingly permitting it to be copied. This includes copying material, ideas or concepts from a book, article, report or other written document (whether published or unpublished), composition, artwork, design, drawing, circuitry, computer program or software, website, Internet, other electronic resource, or another person’s assignment without appropriate acknowledgement;
- paraphrasing another person’s work with very minor changes keeping the meaning, form and/or progression of ideas of the original;
- piecing together sections of the work of others into a new whole;
- presenting an assessment item as independent work when it has been produced in whole or part in collusion with other people, for example, another student or a tutor; and,
- claiming credit for a proportion a work contributed to a group assessment item that is greater than that actually contributed.†

Submitting an assessment item that has already been submitted for academic credit elsewhere may also be considered plagiarism.

The inclusion of the thoughts or work of another with attribution appropriate to the academic discipline does not amount to plagiarism.

Students are reminded of their Rights and Responsibilities in respect of plagiarism, as set out in the University Undergraduate and Postgraduate Handbooks, and are encouraged to seek advice from academic staff whenever necessary to ensure they avoid plagiarism in all its forms.

The Learning Centre website is the central University online resource for staff and student information on plagiarism and academic honesty. It can be located at:

[www.lc.unsw.edu.au/plagiarism](http://www.lc.unsw.edu.au/plagiarism)

The Learning Centre also provides substantial educational written materials, workshops, and tutorials to aid students, for example, in:

- correct referencing practices;
- paraphrasing, summarising, essay writing, and time management;
- appropriate use of, and attribution for, a range of materials including text, images, formulae and concepts.

Individual assistance is available on request from The Learning Centre.

Students are also reminded that careful time management is an important part of study and one of the identified causes of plagiarism is poor time management. Students should allow sufficient time for research, drafting, and the proper referencing of sources in preparing all assessment items.

* Based on that proposed to the University of Newcastle by the St James Ethics Centre. Used with kind permission from the University of Newcastle.

† Based on that proposed to the University of Newcastle by the St James Ethics Centre. Used with kind permission from the University of Newcastle.
UNSW has strict rules regarding plagiarism. Plagiarism of any kind is not acceptable in this course.

If you are in any doubt about whether something constitutes plagiarism, always ask your tutor before handing in your assignment.

Resources for students:

There is no set text assigned to this course and you will receive recent articles on relevant topics throughout the course. However for those students wishing to have a text book Atlas and Bartha Microbial Ecology: fundamentals and applications 4th Edition 1998 Benjamin/ Cummings Publishing Company Inc, is recommended.

Recommended journals for extra reading

- The ISME journal
- Aquatic Microbial Ecology
- Advances in Microbial Ecology
- Current Opinions in Microbiology
- Applied and Environmental Microbiology
- Trends in Microbiology
- Environmental Microbiology
- FEMS Microbiology Ecology
- Marine Ecology Progress Series
- Microbial Biotechnology
- Microbial Ecology
- Microbiology
- Biofouling
- Trends in Biotechnology
- Extremophiles
- Journal of Bacteriology
- Molecular Microbiology
- PNAS

Equity and diversity

Those students who have a disability that requires some adjustment in their teaching or learning environment are encouraged to discuss their study needs with the course convenor prior to, or at the commencement of, their course, or with the Equity Officer (Disability) in the Equity and Diversity Unit (9385 4734 or www.equity.unsw.edu.au/disabil.html). Issues to be discussed may include access to materials, signers or note-takers, the provision of services and additional exam and assessment arrangements. Early notification is essential to enable any necessary adjustments to be made.

Continual course improvement

At the end of the course student evaluative feedback on the course may be gathered, using UNSW's Course and Teaching Evaluation and Improvement (CATEI) Process. Student feedback is taken seriously, and continual improvements are made to the course based in part on such feedback. Significant changes to the course will be communicated to subsequent cohorts of students taking the course. If you would like to make suggestions at any time during the course please feel free to discuss this with the course convenor.
SPECIAL CONSIDERATION AND FURTHER ASSESSMENT SEMESTER 1 2017

Students who believe that their performance, either during the session or in the end of session exams, may have been affected by illness or other circumstances may apply for special consideration. Applications can be made for compulsory class absences such as (laboratories and tutorials), in-session assessments tasks, and final examinations.

Students must make a formal application for Special Consideration for the course/s affected as soon as practicable after the problem occurs and within three working days of the assessment to which it refers. Students should consult the “Special Consideration” section of Moodle for specific instructions related to each BABS course they are studying. Further general information on special consideration can also be found at https://student.unsw.edu.au/special-consideration.

HOW TO APPLY FOR SPECIAL CONSIDERATION
Applications must be made via Online Services in myUNSW. You must obtain and attach Third Party documentation before submitting the application. Failure to do so will result in the application being rejected. Log into myUNSW and go to My Student Profile tab > My Student Services channel > Online Services > Special Consideration.

After applying online, students must also verify supporting their documentation by submitting to UNSW Student Central:
- Originals or certified copies of your supporting documentation (Student Central can certify your original documents), and
- A completed Professional Authority form (pdf - download here).

The supporting documentation must be submitted to Student Central for verification within three working days of the assessment or the period covered by the supporting documentation. Applications which are not verified will be rejected.

Students will be contacted via the online special consideration system as to the outcome of their application. Students will be notified via their official university email once an outcome has been recorded.

SUPPLEMENTARY EXAMINATIONS:
The University does not give deferred examinations. However, further assessment exams may be given to those students who were absent from the final exams through illness or misadventure. Special Consideration applications for final examinations and in-session tests will only be considered after the final examination period when lists of students sitting supplementary exams/tests for each course are determined at School Assessment Review Group Meetings. Students will be notified via the online special consideration system as to the outcome of their application. It is the responsibility of all students to regularly consult their official student email accounts and myUNSW in order to ascertain whether or not they have been granted further assessment.

For Semester 1 2017, BABS Supplementary Exams will be scheduled on:
- All BABS coded courses (except BABS1201): Wednesday 12 July
- All BIOC coded courses: Thursday 13 July
- BABS 1201 and MICR-coded courses: Friday 14 July

Further assessment exams will be offered on this day ONLY and failure to sit for the appropriate exam may result in an overall failure for the course. Further assessment will NOT be offered on any alternative dates.
Safety and laboratory procedures:

Biotechnology and Bio-molecular Sciences (BABS)
UNSW

RISK ASSESSMENT

Working in a laboratory is inevitably associated with certain risks. Good laboratory practice means working in such a way as to eliminate, or at least minimise, these hazards. In order to perform your work safely and to comply with government legislations, a risk assessment has been conducted on all of work that will be performed in this subject in the laboratory and the following potential risks have been identified:

Biological hazards: All microorganisms are potentially harmful if ingested or exposed to body surfaces. Some organisms used in this class may be opportunistic human pathogens, however none are considered to pose a significant risk if handled appropriately (see procedures below).

Chemical hazards: Most of the chemicals used in this subject (e.g. in solid and liquid media and most buffers) are not hazardous at the concentrations that are being used, however all chemicals should be considered potentially harmful. Some practicals employ hazardous chemicals. In these cases the hazard is described in the class directions for that specific exercise. The concentration of antibiotics in media are generally not harmful, however contact with skin should be avoided.

Note: Material Safety Data Sheets (MSDS) are available for all of the hazardous chemicals from your tutor. You should be familiar with the relevant MSDSs prior to commencing your practical work.

Physical hazards: Bunsen burners and heat from other sources such as water baths, breakable glassware, sharp objects such as plastic tips and needles.

Hazards involving work environments: The combination of large numbers of students performing laboratory work (e.g. with bunsen burners alight) and the necessity to wear protective clothing (see below), especially in summer weather, may cause discomfort to some students. In addition, the nature of laboratory design (benches and stools) may cause discomfort to some students.

PROCEDURES FOR DEALING WITH RISKS

In addition to the general risks that have been identified with laboratory work for this subject (see above), any additional risks associated with specific practicals are written in this manual at the beginning of each practical description. At the commencement of each new practical your tutor will review the risks with you. At the commencement of each class the procedures may be reviewed. You may be examined on your understanding. It is imperative that you are present at the beginning of each class to ensure that you are available to review safety procedures. If you are not present you may be excluded from the class.
Following are some simple rules that you must follow which will ensure good laboratory practice and minimise the consequences of risks:

i. Wear adequate protective clothing. This will protect you from contamination by cultures and chemicals as well as protecting the cultures and chemicals from contamination by you. A laboratory coat must always be worn while in the lab, and removed on leaving. Where necessary protective clothing also includes footwear. Fully enclosed shoes must be worn and thongs and other open, loose footwear are not permitted. Safety glasses may also be required. You should protect yourself from the possibility of falling equipment (glass) and spilling chemicals. Also there may be broken glass, spilled liquids, etc. in the floor. Students who do not comply with these stipulations will be asked to leave the laboratory.

ii. You must not eat, drink, smoke, apply make-up etc. in the lab. Neither should you bring food, drink etc. into the lab. Habits such as chewing the ends of pens and pencils, nail biting etc. are often difficult to avoid, but you should make a conscious effort not to do them. Do not sit on laboratory benches.

iii. Do not invite anyone into the lab. They may not be aware of the hazards and may themselves create additional hazards.

iv. Practice good aseptic techniques when handling micro-organisms. If you do not know what this involves you must ask.

v. Never pipette by mouth. Use plugged, sterile pipettes and mechanical pipetting devices to transfer cultures and solutions. This will prevent contamination in both directions. Take care to avoid breaking glass pipettes when inserting them into mechanical pipettors.

vi. Keep everything covered. Do not leave the plugs off flasks or caps off tubes and bottles. As well as minimising spillages this will prevent contamination of cultures and solutions.

vii. If there is an accident with a microbial culture, or hazardous chemical, ask a fellow student to call someone in authority immediately. Do not move and risk the spread of contamination. If there is a fire, remove yourself immediate danger and call someone in authority immediately.

viii. Place PIPETTES (ONLY) in the labelled containers of disinfectant. Pasteur pipettes should be disposed of in the pasteur pipette discard container. Place all other glassware in the containers provided as soon as possible after use. Broken glass, and only broken glass, should be placed in the “glass only” bins located at the front of the class. All other waste (infected or not), including used Petri dishes, should be placed in the plastic, autoclaved bags. These will be autoclaved before disposal. Never place any infectious material in the sinks, to avoid blockages, never drop anything solid into the sinks. Special discard bottles will be provided for particular chemicals.

ix. All materials for incubation or refrigeration should be adequately labelled and placed in the relevant containers provided.
x. Equipment such as centrifuges, baths, etc. should be operated carefully and correctly. If any doubt exists regarding the correct method of operation of any equipment, then consult a demonstrator before proceedings.

xi. Before leaving the laboratory tidy your bench, clean your bench area using disinfectant provided and wash your hands with Bioprep hand wash and water.

xii. If you feel discomfort from your work e.g. (heat exhaustion or back pain), consult your tutor or the subject authority.

xiii. If you feel you may have allergic reactions or be sensitive to any of the biological or chemical components (e.g. antibiotics) used in the practicals, consult your tutor or the subject authority.

xiv. If you get any biological or chemical substance (e.g. sodium hydroxide) in your eye, ensure that you immediately go to a tap and wash your eye.

While washing your eye, alert someone to your situation so that they can assist you and gain the attention of someone in authority. Continue to wash your eye until someone in authority indicates for you to do otherwise. It is imperative that you take this seriously as you may risk permanent eye damage if it is a harmful chemical. Note: you should always wear safety glasses when handling hazardous substances.

**Health and Safety Precautions for Electronic devices including laptop computers and mobile phones.**

**Mobile phones:** For your own safety when using your mobile phone in class please ensure it is placed in a plastic zip lock bag. Every student will be provided with a zip lock bag in their first practical class which they should continue to use during the session (keep it with your lab book). If you misplace or loose this bag you will be expected to provide your own zip lock bag.

**Computers and tablets:** this applies to either your own device or those supplied by the school. Please cut a section of benchcoat (this will be provided in the lab class) and place your device on this on the lab bench to separate it from your other laboratory work. DO NOT wear gloves when using these devices. If the lab is being used as a dedicated DRY LAB (please check signage on the door) you will not be required to do this as the benches will be cleaned and decontaminated before your class.

**WORKING OUT OF CLASS TIMES**

There is plenty of time to do all practical components during allocated laboratory times. However if required arrangements to do extra work must be made with one of the tutors. A book, recording the times of beginning and completing extra work, must be filled in and endorsed by a tutor who should be aware of the nature of the work to be done and who is willing to be available for advice if necessary.

Working out of class can be undertaken only **between 9:00am and 5:00pm.**
Major Research Project

Background
A research group at the University of New South Wales initiated a research project with the aim of developing new approaches for the prevention of biofouling on man-made surfaces submerged in seawater that would provide an alternative to toxic heavy metal-based paints. Their approach was to look for naturally occurring interactions between marine macroalgae, the microbial biofilms colonising their surfaces and the larvae of potential fouling invertebrates.

Divers observed that the surface of a marine red macroalga, *Delisea pulchra*, and that of the green alga *Ulva australis*, were relatively free of fouling organisms, to a degree that could not be explained simply by mechanical processes such as the shedding and sloughing off of surface tissue.

The hypotheses they considered were:

1) **Are there any mechanisms by which the algae or algal surface chemistry can influence the colonisation of the microbial community on its surface? For example, by:**
   a) Antimicrobial effects, or;
   b) Inhibition of colonisation by interference with bacterial signalling compounds (acylated homoserine lactones (AHLs)), which are known to regulate key colonisation phenotypes in many bacterial strains.

2) **Do the bacteria living on the surface of the algae produce a specialised biofilm that influences the settlement of macro-fouling organisms? Specifically do the bacteria directly influence the growth or colonisation of potential colonising bacteria through:**
   a) Antimicrobial effects, or;
   b) Bacterial signalling compounds (AHLs) or inhibitors of AHLs; or
   c) Other biologically active secondary metabolites.

If so, do they deter bacteria that induce settlement of macro-fouling organisms and/or promote the colonisation of bacteria with antifouling properties?

3) Does the surface of the marine algae contain antifouling properties that are inhibitory toward the settlement of marine macro-fouling organisms, produced by either:
   a) The algal tissue itself, or;
   b) The microbial community residing on the surface of the algae.

Your laboratory group has the task of addressing **Hypothesis 1 & 2 in experiments to be performed throughout the session.** The organisation, planning and running of experimental tasks should be done as a group of 3-4 people. However, each individual is required to hand in an experimental report with his or her own interpretation of the data collected as a group in Week 10. As part of this report you will need to consider how you could address Hypothesis 3 as part of future work based on information provided to you throughout the session and through your own literature searches.
You need to plan your work within the available resources. The resources available to you group are as follows:

A) Lab demonstrators, who will be available to help mediate and guide your group work, and to advise on experimental issues and concerns.

B) A CD containing some background theory and practical information on the issues and experimental techniques you will need for your investigation.

C) A laboratory protocol book that includes, among others, the experimental protocols mentioned on the CD.

D) The physical resources (given as lists of supplies on page 26).

E) Suggested literature and key words to get you started

Suggested literature:

Key Words
Biofouling, Antifouling, Antimicrobial, Bioactive molecules Bacterial signalling systems, Quorum sensing, Antifouling (bio)assays, interactions between marine surface associated bacteria and their host, host defence mechanisms, Delisea pulchra, Ulva lactuca, Ulva australis

Key authors who have published in the field: Steinberg, P., Kjelleberg, S., Egan, S., de Nys, R., Dworjanyn, S., Rasmussen, T., Dobretsov, S., Rao, D., Givskov, M.
WEEK 2 (10th March) – Introduction to course
Research project experimental planning

No wet laboratory component this week. You will be provided with course information and plan experiments for the research project. G08 computer laboratory will be available for looking at CD and answering questions provided. You may also begin with literature searches and designing experiments. For the research project you are expected to form into working groups of 4-5 students.

Note: You will be provided with environmental samples of *Delisea pulchra* and *Ulva australis* sometime in the first few weeks (availability is dependent on sampling/tides). Therefore, it is essential groups are flexible and ready to set up this series of experiments (4.2) when advised.

WEEK 3 (17th March) Research project
- Experimental planning
- Risk assessment

This week you will continue experimental planning for the major research project, and can also look at setting up the first of your experiments. You will also learn how to complete a risk assessment of the laboratory procedures you are planning and to modify them accordingly to reduce risk.

WEEK 4 (24th March) Research project

WEEK 5 (31st March) – Research project

WEEK 6 (7th April) – Research project

WEEK 7 (14th April) – No lab….GOOD FRIDAY

*Easter break*

WEEK 8 (25th April) – Research project

WEEK 9 (6th May) – Research project- Final week for experiments

This week students will have the opportunity to finalise any experiments please discuss your needs with a lab demonstrator. This week you should be collecting all of the data and working on your final report. There will be time to discuss the findings with lab demonstrators and fellow students.

WEEK 10 (15th May) – Research project- Final week

Final research project report due.
Environmental Microbiology Laboratory Protocols

For the Major Research Project you must address Hypotheses 1 & 2 outlined on page 15. Protocols that are commonly used in Environmental Microbiology have been outlined below for your reference. Each protocol contains a section where you must outline (and perform) appropriate experimental controls. Controls are a crucial part of scientific experimental design (without proper controls) results can be meaningless.

Isolation and 16S rDNA sequencing of bacteria from environmental samples

As part of your experiments you will be investigating the bacteria that colonise the surfaces of *Delisea pulchra* and *Ulva australis*. To make things easy for you we have in pure culture 10 bacterial isolates from each of the respective macroalgae species. We have sequenced the 16s rDNA for these isolates and you will use this sequence data to identify the bacterial species in a tutorial later in the session. Below is how the strains were isolated and sequenced; this is for your reference and has already been done for you.

*Delisea pulchra* was isolated from subtidal waters at Bare Island Botany Bay, Sydney and the intertidal green alga *Ulva australis* was collected from the rock platform at nearby Shark Point, Sydney. Samples were transported to UNSW in filtered seawater and processed immediately. The macroalgae samples were placed in respective tubes containing 10 mL sterile filtered seawater and vortexed for 45 sec at maximum speed. The supernatant was then aseptically transferred to marine agar plates in 100 μL aliquots and spread plated (as described in Appendix 1). Plates were incubated for 48 h at 20°C. Single colonies were subcultured onto marine agar until pure cultures were obtained.

Sequencing of bacterial isolates involved extracting Genomic DNA using a Pure Link Genomic DNA kit (Invitrogen) and amplifying the 16S ribosomal RNA gene sequences using PCR. EconoTaq® Polymerase was used with universal primers 27F and 1492R. Amplified PCR fragments were then purified and sequenced using 27F primer in a Big Dye Terminator reaction as per manufacturer’s instructions. DNA sequencing was carried out on an Applied Biosystems 3730 DNA Analyzer at the Ramaciotti Centre for Gene Function Analysis (University of New South Wales, Sydney, Australia).
1. Assay for assessing antibacterial activity of crude extracts

You have been provided with crude non-polar extracts of both *Delisea pulchra* and *Ulva australis*. These extracts were prepared by freeze-drying the sample, then extracting the compounds in ethyl acetate. The extract components (approximately 50% halogenated furanones) are then eluted in ethanol.

Controls used in this experiment: *(Hint: Ethanol is the chosen diluting agent in this experiment.)*

1) Create a well in a marine agar plate using the reverse end of a yellow tip ensuring all agar is removed from the well
2) Dilute crude extract in ethanol as appropriate (why dilute in ethanol not water?) and add 20 µl of extract at defined concentrations to the well plate
3) Allow the ethanol to evaporate at room temperature
4) Spread plate 100-200 µl of bacterial strain to be assessed for sensitivity on a marine agar plate (Hint: ensure you evenly distribute the bacterial sample)
5) Incubate at conditions appropriate for the bacterial strain being assayed (think about what strain(s) from those you have might give interesting and ecologically relevant information back…)

![Diagram of the assay process](image)
2. Assay for assessing antibacterial activity of bacterial isolates

There are many ways this can be performed. Please discuss with your tutor what method
would be best given available resources. Two methods are given below, and you could also
consider doing t-streaks (see demonstrator as needed).

Method 1
2) Onto the middle of a fresh MA plate inoculate 20 µl of the liquid culture and air dry. Incubate
the plates for one week at the appropriate conditions to allow colonies to form.
3) After incubation overlay the test plate with 4 ml of MA soft agar (0.6 % agar) containing 0.4 ml
of an overnight culture of a target bacterial strain.
4) Determine antibacterial activity after a further 24 h incubation by a zone of inhibition
surrounding the test strain.

Method 2
1) Ensure the optical density of the target strain is at 0.6 (Abs= 600 nm).
   ○ *The target strain is the one you will be testing for activity against.*
2) Inoculate 20 µl of target strain into 10 mL 0.75% soft marine agar
3) Pour the inoculated agar onto a fresh marine agar plate and allow soft agar to solidify
4) Harvest 50 µl of test strain at OD 0.6 (600nm) at 8500 rpm for 2 min and resuspend pellet in 20
   µl of fresh media (think why…to remove possible media effect!!)
5) Carefully cut a well into the agar plate using the reverse end of a yellow pipette tip (Hint: ask
   your demonstrator for advice on this - it is best not to remove all of the agar from the plate)
6) Add 20 µl of test strain to the well
   ○ *The test strain is the strain you want to test for antibacterial activity.*
7) Incubate plate upright for 48 h at room temperature
8) Determine if the test strain has antibacterial activity by looking for a zone of inhibition of the
   target strain (which surrounds the test strain)

Controls to be used in this experiment:
*(Hint: You will be provided with ‘known’ strains listed in the Materials section)*
*It is also possible to assess the antibacterial activity of compounds secreted by the test bacteria
using a modified version of the above protocol. Use the supernatant harvested in Step 4 that
contains the compounds secreted by the bacteria during growth. See the following article- Wilson,
3. Semi-quantification of bacterial biofilm biomass

You will be provided with overnight cultures of the marine bacteria strains. The optical density of the cultures have been adjusted to 0.6 (Abs600 nm). Think: why do we want all the strains to be normalised to the same OD????

1) Harvest 1ml of the cells by centrifugation at 8500 rpm for 2 min. Wash cells once in NSS (nine salts solution- wash solution to remove cell debris) and resuspend in 1 mL of ½ Marine Broth.

(Use a 15 ml tube to centrifuge and resuspend cells)

2) Transfer 150 µl aliquots of cells into the wells of a 96 well polystyrene microtitre plate (you should do at least three replicates for each strain). Incubate at room temperature for 1 week

3) Remove the supernatant by turning the plate upside down and ‘blotting’ on paper towel. Add 150 µl of 0.2% crystal violet solution (2% solution of Crystal Violet in ethanol diluted in distilled water) to each well. Stain 15 min at room temperature

4) Wash wells successively with NSS one to two times. Let plates air dry for 5 min

5) Add 150 µl of 95% ethanol to each stained well. Allow dye to solubilize by covering plates and incubating 5 min at room temperature

6) Measure the optical density (OD) at a wavelength of 600 nm

Think: How could you improve this experiment such that you could assess attachment of bacterial biofilms to a living marine surface? What surfaces are relevant to our investigation and what materials have we been provided with?
4. Assays for the detection of AHL’s and AHL inhibitors

The *Chromobacterium violaceum* CV026 assay system

Production of the purple pigment (violacein) by the bacterium *Chromobacterium violaceum* is under the regulation of acylated homoserine lactone (AHL)-mediated signalling (a type of quorum-sensing), thus it makes a useful tool for the detection of AHL and AHL-inhibitors from environmental samples. *Chromobacterium violaceum* CV026 reporter strain is a transposon mutant, unable to synthesize its own AHL(s) and thus does not express violacein unless an appropriate AHL is supplied in the medium. This strain can be used to detect AHLs in several different ways. Individual bacterial strains can be assayed for AHL production as well as assaying environmental samples (eg. rocks, fragments of higher organisms) for the presence of AHLs and thus presumably the presence of AHL-producing bacterial strains. Likewise inhibition of AHL-mediated signalling can be assessed by the addition of AHL to plates and then looking for inhibition of the AHL-mediated phenotype.

Please keep in mind the *C. violaceum* CV026 strain is a mutant, therefore its weaker than wild type strains. It has been noted to grow slowly under typical lab conditions and is sensitive to salt concentrations above 10 g/L (marine agar typically contains 20 g/L sodium chloride).

4.1 Screening bacterial strains for AHL signal production or AHL-signalling inhibitors

Because AHLs are small enough to diffuse through the agar medium, if an AHL-producing strain is streaked next to a streak of the reporter strain CV026, then the AHLs will stimulate the reporter to synthesise violacein. In a similar fashion if AHL producing bacteria are streaked onto an agar plate and then overlayed with a soft agar mixture containing the reporter strain the AHL will diffuse into the top agar and stimulate the expression of violacein, which is visualised as a purple colour in the top agar surrounding the test bacterium.

There are two methods that can be used to screen bacterial strains for AHL’s and AHL inhibitors:
**Option 1:**

1) Streak in single lines across an agar plate the bacteria to be tested for AHL production and incubate for 1 week at room temperature

2) After incubation mix 100 µl of an overnight culture of the reporter strain (CV026) with 10 ml of LB soft agar (1% agar) that has been cooled to 46°C in a water bath

3) Gently pour the soft agar mix on top of the agar plate and allow to cool completely before incubation at 30°C overnight

4) Observe if the reporter strain indicates that the bacteria produces AHL

To test for AHL-signalling inhibitors, perform steps 1-4 as outlined previously except add AHL’s to the soft agar overlay together with the reporter strain (CV026) in step 2. Add the AHL (in the case of *C. violaceum* the native AHL is 3-oxo-N-hexanoyl-L-homoserine lactone (abbreviated to OHHL)) to a final concentration of 10 µM and mix 100µl of the CV026 overnight culture then pour the soft agar over the test bacterial streaks as described above.

**Option 2:**

1) Inoculate 200µL overnight culture of the reporter strain (CV026) with 10 ml of LB soft agar (1% agar)

2) Gently pour the soft agar mix on top of a LB agar plate and allow to set for at least 1.5 hours

3) Carefully cut a well into the agar plate using the reverse end of a yellow pipette tip (Hint: ask your demonstrator for advice on this - it is best not to remove all of the agar from the plate)

4) Harvest 20 µl of the marine strain to be tested at OD 0.6 (Abs 600nm) at 8500 rpm for 5 min and resuspend in 20 µl of marine broth

5) Add the 20 µl aliquot of cells to the well of the agar plate. Incubate with the lid of the plate upwards at 30°C overnight

To test for AHL-signalling inhibitors, perform steps 1-5 as outlined above except add 10 µM AHL to the soft agar overlay together with the reporter strain (CV026) in step 1 and mix thoroughly
4.2 Detecting AHL signals or AHL-signalling inhibitors in environmental samples

It is possible to detect AHLs in natural samples, such as biofilms covering rocks and seaweeds, without having to isolate and grow individual bacterial strains.

1) Rinse the environmental sample (e.g., rock, plant, seaweed etc) three times in sterile MilliQ and air-dried (on Kimwipes). *Note: it is important to work quickly after air-drying the samples as compounds can degrade over time*

2) Place the sample on a fresh LB agar plate

3) Mix 100 µl of an overnight culture of the reporter strain (CV026) with 10 ml of LB soft agar (1% agar) that has been cooled to 46°C in a water bath

4) Gently pour the soft agar mix on top of the environmental sample and allow to cool completely before incubation at 30°C overnight.

To assay for AHL inhibitors present in environmental samples repeat steps 1-4 with the addition of AHL’s to the soft agar at a final concentration of 10 µM

4.3 Detecting AHL signals or AHL-signalling inhibitors in solvent extracts of environmental samples

Natural chemical compounds from living material can be extracted from the raw material using chemical solvents (such as methanol, DCM etc) and subsequently tested for AHL signals or AHL-signal inhibitors using a soft agar overlay method similar to that above.

1) Mix 100 µl of an overnight culture of the reporter strain (CV026) with 10 ml of LB soft agar (1% agar) that has been cooled to 46°C in a water bath

2) Gently pour the soft agar mix on top of a fresh LB agar plate and allow to cool completely. Once set use the end of a pipette tip to cut wells into the agar (limit to 6 wells per plate)

3) Dilute the concentrated stock of crude extract and appropriate controls with fresh media and add 20-50 µl of each sample into individual wells (depending on the size of the well you make the volumes may vary)

4) Incubate overnight at 30°C with the lid of the petri-dish facing upward

To assay for AHL inhibitors present in environmental samples repeat steps 1-4 with the addition of AHL’s to the soft agar at a final concentration of 10 µM (depending how daring you are, a range of AHL concentrations could also be tested….)
5. The *E. coli* GFP-based detection system

There are many different types of biosensors available for the detection of AHLs, with sensitivities to different types of AHLs or chain lengths. Biosensors can also differ based on their reporting mechanism such as the production of a pigment or beta-galactosidase. In this experiment we use an *Escherichia coli* strain harbouring a plasmid pJBA132 that responds to AHLs by producing Green Fluorescent Protein (GFP). This biosensor has a different range of sensitivity to CV026 the other biosensor used in this course.

Groups could look at comparing the *E. coli* biosensor with that of CV026, by performing similar assays such as directly overlaying bacterial isolates with the *E. coli* biosensor.

The *E. coli* biosensor grows on LB media and visualization of a response to AHL signals is via a phosphorimager (see demonstrator as needed).

**Relevant references:**


Materials available per lab group each week:

- **Streak plates per lab group:**
  - 1 x streak (marine agar) plate of each of the 5 *Delisea pulchra* isolates (D1 to D5)
  - 1 x streak (marine agar) plate of each of the 5 isolates from the *Ulva australis* isolates (U1 to U5)
  - 1 x streak (LB) plate of *Chromobacterium violaceum* CV026
  - 1x streak (LB) plate of *Chromobacterium violaceum* wild-type strain
  - 1 x streak (LB) plate of *Pseudomonas aeruginosa* PAO1
  - 1 x streak (LB) plate of *Serratia marcescens* MG1

- **Cultures per lab group:**
  - 2 x 10 ml overnight culture (Marine broth) of each of the 5 *Delisea pulchra* isolates.
  - 2 x 10 ml overnight culture (Marine broth) of each of the 5 *Ulva australis* isolates.
  - 2 x small (10 ml) O/N cultures (LB10) of *Chromobacterium violaceum* CV026.
  - 2 x small (10 ml) O/N cultures (LB10) of *Chromobacterium violaceum* wild type

- **Plates and medium per lab group:**
  - LB10 plates to use for overlay with soft agar (NB: these should not be filled with more than 20 ml media) plus some spares
  - Marine agar plates plus some spares
  - 3 x 500 ml Marine broth medium in Schott bottle
  - 3 x 500 ml ½ Marine broth media
  - 2 x 10 ml sterile LB medium (for dilution of extracts and AHL’s before placing in agar wells)
  - 40 x 10 ml aliquots of 1% LB soft agar stored in water-bath at 46°C (For AHL assays)
  - 40 x 10 ml Marine agar soft agar (0.6 % agar) stored in water-bath at 46°C
Other materials supplied per lab group:

- 30 x 96 well microtitre plates (non-tissue culture treated if possible)
- 0.2% Crystal Violet solution (2% solution of Crystal Violet in ethanol diluted in distilled water)
- 3x 200 µL aliquots Ampicillin (50 mg/mL) filter sterilised in MiliQ
- 3x 200 µL aliquots Streptomycin (100 mg/mL) filter sterilised in MiliQ
- 3 x 1mL aliquots of Chloramphenicol (34 mg/mL) filter sterilised in MiliQ
- 6 x 50 ml sterile NSS
- 2x 500 ml MiliQ (in Schott bottles)
- 1 x 10 ml Delisea pulchra extract (5 mg/ml concentration) dissolved in ethanol
- 1 x 10 ml Ulva australis extract (5 mg/ml concentration) dissolved in ethanol
- Fresh unprocessed samples of Delisea pulchra and Ulva australis
- 3 x aliquots ethanol (5 ml in Mac. bottle)
- 1 x 350 µl aliquot of 10 mM OHL (N-octanoyl-L-homoserine lactone) on ice
- 1 x 350 µl aliquot of 10 mM OHHL (N-3-oxohexanoyl-L-homoserine lactone) on ice
- 1 x 350 µl aliquot of 10 mM furanones (4-Bromo-5-(bromomethylene)-2(5H)-furanone) on ice
- 1ml and 100 µl pipettes & sterile blue and yellow tips
- 1x P20ul pipette per lab group
- Hockey sticks, inoculation loops, flame alcohol.
- Shaker
- Sterile eppendorf tubes + racks - 1 beaker full per lab group
- Forceps
- Oven gloves for removing soft agar from hat water bath
- 2 x sterilised 100ml measuring cylinders
- General supply of sterile glass bottles for small cultures
- Spectrophotometric plate reader capable of measuring 96 well plates at Absorbance 600nm
- Tabletop Spectrophotometer and 1mL cuvettes
- Foil
Appendix 1: Culturing techniques in Microbiology

Plate Counts
Plate counts require the preparation of a set of serial dilutions (usually tenfold) calculated to provide 30 - 300 colonies within 100µl at some step in the series. Aliquots are spread over the surface of agar plates, the colonies counted after growth and this data used to work out the cell concentration in the original undiluted suspension. Precautions need to be taken to secure proper initial dispersion, retention of viability in the dilution fluid and a correct relationship between the successive dilutions.

Note:
- For most purposes, replication of counts is necessary. Obviously, a larger number of replicates will enable a more confidence in the results, but this must be weighed against equipment and time needed. Triplicate dilution series give adequate results for most purposes.
- It is important that plates are completely dried before spread or drop plating samples.

Spread plating
1) Determine the approximate concentration of cells/ml in your sample. Base this on information you know of the environment being sampled and turbidity.

2) Prepare 10-fold serial dilutions of your sample in appropriate diluent. Ensure that the samples/dilution is mixed well between dilution steps.

3) Select the dilution calculated to contain 30-300 cells in 100µl and the dilutions above and below this one.

3) Pipette 100µl of each suspension onto agar and spread evenly with a sterile hockey stick. Ensure plates are correctly labelled and incubate under appropriate conditions.

4) Calculate the mean of the three plates with ~ 30-300 colonies. Note the lack of precision for the dilution 1 log below the "most appropriate" dilution.

Drop plating- this method is a much more efficient us of agar plates. However it may be difficult to determine colony morphology or to isolate individual strains.

1) Carry out steps 1 and 2 as for spread plates.

2) Select the dilution calculated to contain 10-30 cells in 10µl (i.e. the same dilution as 30-300 cells in 100µl for the pour plates!) and the dilutions above and below this one.

3) Pipette 10µl of each suspension, in triplicate, onto quadrants of the agar (see diagram next page). Note: the plates must be carefully dried for this procedure to work, as otherwise the drop will disperse over the plate surface. Pre-dry the open plates for 1 hour in a 37°C incubator prior to use if in doubt.

4) Calculate the mean of the three drops from the quadrant with ~ 20 colonies. Note the lack of precision for the dilution 1 log below the "most appropriate" dilution.
Counting bacteria and expressing results

1) From each dilution series select and count ONE plate or drop with the appropriate number of colonies.

2) Multiply by the dilution factor and convert to cells/ml, i.e. a count of 60 colonies from 100µl on the 10⁶ dilution plate means that there were 6 x 10⁸ cells/ml in the original culture. Similarly, a count of 25 colonies in a 10µl drop from the 10⁶ dilution means that there were 2.5 x 10⁹ cells/ml in the original culture.

Gram Staining

1. Heat-fix a smear of a mixture of the bacterium as follows:
   a. Using the dropper bottle of distilled water, place a small drop of water on a clean slide by touching the dropper to the slide
   b. Aseptically remove a small amount bacterial isolate from the agar surface and mix it generously with the water. Flame the loop and let it cool.
   c. Using the loop, spread the mixture over the entire slide to form a thin film
   d. Allow this thin suspension to completely air dry
   e. Pass the slide (film-side up) through the flame of the bunsen burner 3 or 4 times to heat-fix

2. Stain with crystal violet for one minute. Gently wash with water. Shake off the excess water but do not blot dry between steps.

3. Stain with iodine solution for one minute and gently wash with water.

4. Decolorize by adding ethylalcohol/acetone drop by drop until the purple stops flowing. Wash immediately with water.

5. Stain with safranin for one minute and wash with water.

Appendix 2: Tips for designing scientific posters

Posters were introduced at scientific conferences simply because time constraints did not permit oral presentations by all participants. In fact, many conferences restrict talks to recognized experts, however this does not trivialise the poster concept. Scientists will often read a poster when they are unfamiliar with its subject area, whereas this is not the case with talks. For this reason posters need to be very clear and concise.

It is important to realise that any written medium can be read and re-read. This is perhaps the greatest downfall of oral presentations (unless they are videotaped and distributed!). The greater level of scrutiny, however, demands that posters be accurate in every respect. It is not surprising that posters are prepared in a format similar to that of published papers. They usually include an Introduction, Methods, Results, Discussion/Conclusion and References (an Abstract is normally prepared for the conference proceedings, but is not included on the poster). However since your poster assignment is in the form of a review of the literature this format is not expected of you.

Since the poster is to be effective as a visual medium, there are some important considerations that are essential for success. **Text must be kept to a minimum**, point form can be used where appropriate. Conference organisers usually specify the exact dimensions of the poster. As a guide, **the text must be easily read from one metre**. Unlike journals, posters may use colour freely. Do not forget the purpose of the poster. If the artwork detracts from the science, it has not achieved its purpose of a high-quality scientific communication. The biggest mistake made by a novice is **TOO MUCH TEXT**. The compromise between the omission of superfluous text and the risk of leaving out important information is one of the most difficult of all presentation skills.
Notes:
(you can use these pages for experimental planning etc)
Notes:
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