Summary science plan for NERC's Marine and Freshwater Microbial Biodiversity research programme (revised July 2000)

Contents

1. Introduction

Microorganisms are the driving force of global biogeochemical cycles, performing a wide range of chemical transformations. Single-celled plants and animals, bacteria, archaea and other aquatic microbes have a major influence on the composition of the ocean, freshwater environments and the atmosphere. To understand the Earth system as a whole, it is therefore necessary to improve our knowledge of aquatic microbial biodiversity, the interactions among microbes, and their responses to human activities and climate change.

The taxonomy of many, perhaps most aquatic microbes is undescribed, greatly constraining research on their ecology. Classical classification approaches, using morphology and biochemical methods, can be inappropriate or impracticable. Even the species concept is of questionable validity for many of these organisms. However, molecular techniques and the application of functional gene probes now offer the opportunity for rapid gains in both evolutionary and ecological knowledge.

The programme focuses on the information which resides within microbial genomes (the basis for molecular taxonomy) and their interactions with the environment. It thereby provides the means to investigate microbial functions which define the sustainability of aquatic ecosystems, and also offers great potential for biotechnological exploitation. Microbial biodiversity in marine and freshwater habitats is relevant to industrial applications because of the novel biochemistry evolved in response to the range of natural environmental conditions, including extreme temperatures, high pressure, low oxygen and low nutrient environments (the latter requiring high affinity enzyme systems). There are also strong applied interests in horizontal gene exchange and chemical signalling systems at the molecular level, and the wide range of symbioses in which microbes are components.

A combined marine and freshwater programme provides the opportunity to bring together expertise in aquatic microbiology in a way that has not previously been achieved. Many natural microbial community structures, such as viral loops and syntrophic consortia, occur in both marine and freshwater ecosystems; in addition, there is strong commonality of many technological approaches, and complementarity for conceptual and dynamic models of microbial community structure and function. The limited water movement of standing freshwater bodies (and their smaller size, when compared to most marine systems) allows frequent sampling of the same microbial community. They also provide steep physical-chemical gradients, generating high microbial biodiversity per unit volume, and are amenable to manipulation experiments, for direct testing of ecological theory.

The scope of the M&FMB programme is potentially very wide, covering fundamental microbiology, molecular taxonomy, microbial and phytoplankton ecology, water chemistry, and biotechnology. However, the following five topics have been identified as of high priority for enhanced understanding of both marine and freshwater ecosystems, and for studies that compare the two environments:

- culturability;
- community structure;
- biogeochemistry;
- role of viruses;
- cell signalling.

Additional information on the programme's research scope is given in Section 5 below. Potential applicants should note that it is not expected that the programme will direct significant effort at the pathogenicity of commercially-important aquatic species (since this is already a relatively well-developed research field). Furthermore, it is expected that the freshwater component will focus on standing water bodies, rather than river or groundwater systems.

2. Research objectives

The scientific objectives of the programme are:

- to obtain improved descriptions of the diversity of marine and freshwater microbes (prokaryotes, eukaryotes and viruses) and their community interactions, for a range of marine and freshwater habitats, using new molecular techniques and approaches in combination with traditional techniques where appropriate
- to relate phylogenetic diversity to physiological diversity, ecological function and biogeochemical processes, including biogenic gas production and cycling
- to develop methods for the isolation and growth of, as yet, unculturable marine and freshwater microbes, including extremophiles, archaea, chemoautotrophs and anaerobic syntrophic consortia

- to access the phenotypes of marine and freshwater microbes and, through understanding of gene expression, to identify potential applications of novel enzyme systems and proteins in biodegradation, biocatalysis and the biomedical sector
- to improve our theoretical understanding of aquatic ecosystem structure and dynamics; evolutionary processes at the microbial level; and the relationship between microbial diversity and ecosystem functions (eg nutrient cycling, carbon fixation).

3. Programme deliverables

The performance targets of the programme are:

- to attain the scientific objectives stated above, through an integrated programme based on interdisciplinary collaborations, an international perspective and inter-Research Council linkages;
- to provide high quality training to develop national expertise in the ecology, physiology and biochemistry of marine and freshwater microbes;
- to develop fundamental research to a point at which industrial investment and exploitation is possible.

4. Programme funding and phasing

The M&FMB programme is funded by the Natural Environment Research Council (NERC) at £7.46m over c 5 years. An initial funding competition for marine work has resulted in a commitment of around £3.0m, plus associated support for fieldwork and other infrastructure costs, for projects starting in September-October 2000.

The current Announcement of Opportunity (with submission of outlines by 18 September 2000) is for freshwater proposals, and for comparative studies of freshwater and marine systems, to start from mid 2001. There will subsequently be a final funding phase, with emphasis on applications and exploitation for both marine and freshwater work. The Biotechnology and Biological Sciences Research Council (BBSRC) has assisted in developing the programme, and is willing to consider support for related project proposals through its responsive mode funding schemes.

5. Research scope

5.1 Background

Microbes are of fundamental importance for all major biogeochemical cycles in marine and freshwater environments. The activity of bacteria, archaea, algae, fungi, protozoa and viruses results in significant modification to water chemistry, and in the

production of volatile or gaseous compounds which influence atmospheric composition and climate.

The number of microbes in aquatic environments is immense. For example, there are typically 109 bacteria per litre in near-surface water (oceans, rivers and lakes) and even in abyssal sediments, 105 bacteria per g. However, it has only very recently been possible (through molecular ecology) to characterise aquatic microbial assemblages and to ascribe functionality. For example, functional gene probes have shown that nitrogen fixation in the sea is likely to be much more widespread than previously thought. Advances in data handling and analytical technologies (eg. pyrolysis mass spectrometry), now offer additional potential for rapid research progress.

Microbes are capable of growth under high pressure (300 - 1000 times atmospheric pressure at the deep sea floor); a very wide temperature range (from sub-zero in ice to the upper limit for protein structural integrity at hydrothermal vents); and chemical extremes (eg: anaerobic and reducing conditions in sediments; low nutrient levels in oligotrophic surface waters; high levels of potentially toxic metals and hydrocarbons). Survival across that range of environments requires 'non-standard' physiologies and biochemical processes. There is strong potential for biotechnological exploitation to replace or supplement existing industrial processes, particularly since many biogeochemical processes are similar to those carried out in chemical plants (often at great expense of energy and involving highly toxic and/or expensive catalysts).

It is not appropriate for this Science Plan to exactly specify the research to be carried out by component projects within the programme. Funds will be allocated according to the quality of individual proposals. However, the main effort (for both marine and freshwater work) is expected to be directed at the topics identified below, acknowledging that there are links between them and that one proposal may relate to more than one topic:

5.2 Culturability

Although existing UK culture collections are a valuable national resource, there is an urgent need for more aquatic microbes and consortia to be brought into culture. The choice of organisms has frequently been a random process, except for those groups for which selective media have been designed on the basis of comprehensive taxonomic databases. Molecular probes now offer a powerful tool to track specific organisms in enrichment culture and within aquatic habitats. This does not remove the difficulty of finding suitable culture conditions for, as yet, 'unculturable' organisms, but the effort can now be much more targeted at groups of ecological and biogeo-chemical importance, and at those with potential for biotechnological exploitation (see 6 below).

5.3 Community structure

New approaches also need to be developed for the analysis of natural microbial assemblages, to characterise their taxonomic and functional diversity, and to test theoretical models of community structure. For example, it has been suggested that the global abundance of microbes is so great that most species are ubiquitous - and that local microbial biodiversity never limits ecosystem functions (e.g. carbon fixation

or nitrogen cycling). Freshwater systems are generally more amenable to experimental manipulation than marine systems, allowing specific perturbations to be related to changes in community structure and function.

5.4 Biogeochemistry

Biogeochemical processes of particular interest to the programme include the microbiology of nitrogen cycling, halocarbon production and metabolism (eg bromomethane), the production of sulphur compounds (eg DMS), iron and other trace metals, and the cycling of methane, carbon dioxide and carbon monoxide. Marine and freshwater ecosystems are both sources and sinks of many biogenic gases of climatic importance, but the magnitude of natural fluxes is poorly scaled in comparison to anthropogenic inputs.

5.5 Role of viruses

The effect of viruses (the most abundant organic material in the sea and in freshwater) on microbial diversity and geochemical cycling is poorly understood. Viruses, through the specificity of their infection, may influence only part of a natural assemblage; hence they may have a strong stochastic effect on microbial biodiversity. Virus infection may also affect genetic diversity within host communities.

5.6 Cell signalling

The detection of novel signal molecules in aquatic microbes and their ability to elucidate alloinduction/alloinhibition is another exciting area of microbial ecological research. It is now known that even the simplest unicellular organism can interact with other individuals through cell signalling. However, the mechanisms of such signalling, and the potential for manipulation and exploitation, remain largely unknown.

6. Biotechnology

From the biotechnological perspective, additional phenotypic and genomic information is a high priority for barophilic, oligotrophic, chemolithotrophic, anaerobic, thermophilic and psychrophilic bacteria. Other groups of biotechnological interest, on account of their unique metabolic pathways and products, include archaea and haloarchaea (the latter tolerant of very high salinities); the uncharacterised microbes found in deep rock cores and in hydrocarbon reservoirs; and microbial symbionts (ranging from casual epibiosis to obligate symbiosis). The biotechnological potential of the programme will increase by embracing the diversity of micro-organisms that thrive in the range of natural freshwater environments, such as hot springs, snow fields and glaciers. Specific target groups include those that are theoretically possible but not yet discovered e.g. anaerobic photosynthetic ammonia oxidisers.

In the concluding 'applications and exploitation' phase of the programme, it is expected that emphasis will be given to potentially useful, novel or currently under-

exploited biotransformations, with scope both for new products and for replacing existing industrial processes with more efficient and environment-friendly technologies. For example: methanesulphonic acid oxygenases have potential for carbon-sulphur bond cleavage (of sulfonates, detergents and other sulphur containing organic molecules), and a wide range of anaerobic biotransformations are of increasing industrial importance. Processes which might be studied include novel reductions of ketones, enoates, carboxylic acids (including aryl carboxylic acids), aromatic ring reductions, aryl nitro group reductions and reductive dehalogenation. [NB: BBSRC responsive mode funding may be the most appropriate support mechanism for such research if it no longer includes aspects of direct environmental relevance].

The M&FMB programme is not intended to support the widespread screening of microbes in the hope that new enzymes or pharmaceutical compounds will be discovered. Instead, effort will be directed at understanding trophic processes and culturing requirements, hence making screening procedures (eg by industrial partners) more selective and efficient.

7. National and international collaborations

Joint proposals between research groups with complementary expertise (eg field- and laboratory-based), and with involvement of industry, are strongly encouraged within the programme, to stimulate technology transfer and linkages between fundamental and applied science. International linkages are also considered desirable, where scientifically appropriate.

8. Fieldwork

For marine projects requiring work at sea, shiptime will mostly be arranged centrally, using NERC research vessels. It is expected that this co-ordination will significantly enhance the complementarity of Phase 1 awards. However, either programme-wide or individual access to non-NERC research platforms is also possible, depending on the particular needs of the project. Detailed fieldwork plans for 2001 and 2002 are currently being developed through discussions with the Principal Investigators.

For freshwater fieldwork, there are also benefits if several studies focus on the same site. Whilst this is not obligatory, prospective applicants may wish to consider the potential advantages (in terms of background data and synergies with other projects) of working at Priest Pot, a one-hectare, 3.5 m deep pond in the English Lake District. This pond is considered typical of ca 750,000 other small water bodies in England and Wales. Access is easy, and fieldwork can be supported by modern laboratory facilities 5 km away. Intense physical stratification develops every summer, when the water column divides into superimposed layers ranging with increasing depth from oxygenated, to anoxic and sulphidic, each layer with a specific microbial community exploiting the local conditions. A review of existing ecological and microbial information for Priest Pot has recently been prepared by the Centre for Ecology and

Hydrology, Windermere in collaboration with the Freshwater Biological Association: copies are available from the M&FMB Science Co-ordinator (see 11 below for contact information).

If there is sufficient community interest in working at Priest Pot, NERC is willing to consider support for a study to characterise with high resolution the spatial and temporal variation in physical-chemical conditions and microbially-mediated ecosystem functions, in the water column and superficial sediment. This information would be made available to other M&FMB projects. Research groups wishing to carry out the characterisation study should submit an outline in a similar way to other project applications; if shortlisted, a full proposal (subject to peer review) would then be required.

9. Data management and culture deposition

It is NERC policy that research programmes ensure the longterm availability of data collected by award holders, to maximise the application and exploitation of their results. In most cases, NERC Designated Data Centres are used, both for quality-control and archiving, with costs covered from central programme funds. After a period of sole access by PIs for publication preparation, such data are made available to other programme participants and the wider community.

The British Oceanographic Data Centre (BODC) will have responsibility for the management of M&FMB datasets arising from the use of NERC research vessels. Arrangements for other datasets produced by the programme are currently under review.

Several studies within the M&FMB programme are likely to result in the description of new species or strains of microorganisms. Such novel organisms should be offered to the most appropriate UK culture collection (see www.ukncc.co.uk). If no UK collection is able to accept them, they should be offered to a recognised overseas collection. If not accepted by any collection, the award holders are expected to themselves make the organisms available to the scientific community for two years after publication of the description of the organisms.

10. Ownership and exploitation of Intellectual Property (IP)

Ownership of IP and IP Rights arising from NERC awards lies with the grant holding body (university, NERC Centre/Survey or other NERC-approved institution). Whilst all recipients of NERC funding must endeavour to ensure that the outcomes of their research are used to the advantage of the UK, NERC retains the right, for a limited period, to exploit IP in partnership with grant recipient organisations to the benefit of the UK and the organisations. This is to avoid circumstances where fragmentation of IP would reduce the likelihood of exploitation (eg for datasets collected by several research groups).

11. Programme management

The M&FMB Steering Committee is responsible for directing and integrating the programme science and for recommending the award of research funds. The Committee also has responsibilities for co-ordinating data management arrangements, and for promoting the transfer of the outcomes of the research to the user community. The MMB Steering Committee was appointed by NERC in June 1999 under the chairmanship of Professor D A Ritchie (Liverpool).

The M&FMB Programme Administrator has responsibility for all aspects of the day to day programme administration and for enquiries concerning the programme. Contact information: Lucy Parnall, NERC Polaris House, North Star Avenue, Swindon SN2 1EU; e-mail lcpa@nerc.ac.uk; tel 01793 411981; fax 01793 411545.

The M&FMB Science Co-ordinator assists the Steering Committee in the co-ordinated implementation of the programme's science objectives, through liaison with award holders and other bodies. Contact information for Science Coordinator: Dr Phil Williamson, School of Environmental Sciences, University of East Anglia, Norwich NR4 7TJ; e-mail p.williamson@uea.ac.uk; tel 01603 593111; fax 06103 507714.