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SCOTTISH
ASSOCIATION
for MARINE
SCIENCE

SAMS

MARINE BIOTECHNOLOGY IN SCOTLAND

RECENT ADVANCES IN AMNESIC SHELLFISH POISONING RESEARCH SEE PAGE 11

April | 05

SAMS COMMITMENT TO

Alasdair Munro, Top Country Development, Inverness

What is marine biotechnology? There are various definitions, but the most succinct is: *Marine biotechnology is the use of marine organisms to provide solutions, thereby benefiting society*. To achieve these solutions, it is often necessary to use other technologies such as electronics and advanced engineering; many would bring these applications into the marine biotechnology family.

But, why should Scotland be in the forefront of marine biotechnology? The most obvious reason is that Scotland is a maritime nation, with a coastline of over 11,500 miles. Some 63% of the UK continental shelf area is in Scottish waters and this area is five times greater than that of landward Scotland. There are huge, diverse resources in these seas.

HISTORY OF SCOTTISH MARINE BIOTECHNOLOGY

The first generation of marine biotechnology involved the burning of kelp to extract soda, potash and iodine. It started in 1698 and continued spasmodically for two centuries, mainly in the Outer Hebrides and Orkney. Thus it provided employment entirely in impoverished rural communities, using a renewable local resource.

The pioneering second generation started in a hut in Campbeltown in 1934.

Dr Grant Burgess, Heriot-Watt University

SYNCHRONISING BACTERIAL ACTIVITIES

They may be small and apparently simple in their construction, but bacteria are a highly successful group of organisms that communicate with each other. They do so by releasing small chemical messages, similar to pheromones, in a process known as ‘quorum sensing’. These chemical messages allow bacterial populations to coordinate the behaviour of large populations of cells. When this system evolved, it empowered them to behave like large, multi-celled organisms.

We now know that quorum sensing controls many aspects of bacterial physiology including biofilm formation and antibiotic production.

As bacteria communicate to coordinate the production of bioactive compounds, we investigate the use of chemical signals to control this production.

BIOFILM BUSTERS

But marine bacteria don’t only communicate and co-operate, they also compete with one another. And as they have been trying to kill each other for almost three billion years, they have developed some pretty sophisticated strategies. One of these is the recently discovered ability of some bacteria to destroy the biofilms of their competitors. Many bacteria rely on the formation of a biofilm in order to infect other cells – which is one of the causes for their ability to colonise humans. We are studying the mechanisms of biofilm busting, which may one day help develop effective ways of preventing infections.

ARE BACTERIA STIMULATING MICROALGAE?

The UK marine biotechnology sector has enormous potential, but is currently quite small, and so it is essential for those involved to work together to raise the profile of this new strategic sector and the commercial success stories that are currently emerging. The opening of the European Centre for Marine Biotechnology in Oban is a significant milestone in the emergence of a strong UK and European presence in marine biotechnology. There are currently joint projects being developed between my group at Heriot-Watt University and Dr Frithjoff Küpper’s group at SAMS to investigate whether bacteria play a role in the release of antibiotics and toxins by microalgae. ●

Dr Grant Burgess is a Reader in Marine Biotechnology at Heriot-Watt University and was a participant in the steering group which oversaw the establishment of ECMB. Further information can be found at www.esmb.org.

Marine bacteria found in the water column can be broken into two broad groups: photosynthetic cyanobacteria and heterotrophic bacteria. The heterotrophic bacteria are the ‘biological pump’ responsible for biogeochemical cycling of nutrients in the marine ecosystem. Our research at SAMS deals with heterotrophic bacteria, but instead of concentrating on the great majority of bacteria that live freely in the water column, we focus on bacteria that live associated with phytoplankton. Phytoplankton are at the base of the marine food chain, and bacteria that live with these primary producers are likely to represent the first stage in the global biogeochemical cycles that supply and re-supply the world's oceans with the nutrients that ensure continued biological production.

DIVERSITY OF PHYTOPLANKTON-ASSOCIATED BACTERIA

We are currently studying whether there are bacteria that only live with phytoplankton. For example, whether it is a random assemblage, a definable bacterial community that co-associates with one phytoplankton species, or a grouping common to algal genera or wider groups such as all diatoms or dinoflagellates.

We have found the bacterial diversity associated with several dinoflagellates, a group that includes species prone to forming harmful algal blooms, to be surprisingly conserved across both the species and the geographical divide. *Marinobacter algicola* (see image) for example was found to live with five different types of dinoflagellate from the UK, Spain, Canada, and Korea. We were puzzled to discover that assemblages living with coccolithophorids like *Emiliania huxleyi* display a remarkable similarity with those associated with dinoflagellates. This may be because dinoflagellates and coccolithophores both bloom during calmer weather periods and when nutrients become limiting, which may select for bacterial types that are adapted to these conditions.

What about bacterial consortia associated with the other main lineage of marine phytoplankton, the diatoms? We have so far only investigated one genus, the pennate diatom *Pseudo-nitzschia*, some species of which cause amnesic shellfish poisoning. While the same bacterial families are present, at the species level the bacteria are quite different from those found on dinoflagellates and coccolithophores.

These differences could reflect the environment, the physiology of the bacteria, the surface signals on the algal cell, or the algal excretory products. Alternatively it could be due to different evolutionary processes of different

EARLY YEARS

Ernst Georg Pringsheim was born on 26th October 1881 near Breslau in Silesia as one of four sons of a wealthy landowner. Like his brothers he certainly was not a good pupil and had to retake four school semesters. Nevertheless all but the youngest brother, who was killed in World War I, eventually became professors.

Initially the young Pringsheim was torn between painting and botany, and spent two years in Munich taking lessons in art. But he took against the bohemian lifestyle of artists, and increasingly delved into science, especially plant physiology. He moved to Leipzig to study botany under the great physiologist Wilhelm Pfeffer. There he found his vocation and focussed intensely on his botanical studies, culminating in receiving ‘summa cum laude’ (the top grade) for his doctorate thesis on water movement and turgor regulation in wilting plants after just three semesters.

DISCOVERING THE MICROCOSM

He then began work in Breslau, where he was introduced to working with microorganisms. He married Lily Chun, daughter of a Professor in Zoology, but this was to be an unsuccessful relationship. In 1909 he became lecturer in Halle, where he deepened his interest in microbiology, worked on plant nutrition, and developed the first axenic culture of Cyanobacteria. He was made professor just before the breakout of World War I, and worked on bacteriology prior to being called up at the age of 36. He hated all things military, and after his initial training served in an army hospital.

Soon after the war Pringsheim moved to the Institute for Plant Physiology at the University of Berlin in Dahlem. There, in 1920, he published an influential paper on the nutritional value of acetic acid for saprophytic flagellates. But these were difficult years that saw national insecurity, inflation, and hunger, and - for Pringsheim - divorce.

He thus happily accepted a professorship at the German part of Charles University in Prague leading an impoverished institute for plant physiology. He adored the city, appreciated his improved economic situation, and embarked for 15 years on a career focussed on improving techniques to cultivate microalgae and developing a sizeable collection. He also worked on the phylogeny of bacteria. He married a pharmacy student that had assisted him in the laboratory, Olga Zimmermann, who was to work with him for the next 30 years.

HITLER’S SHADOW

Pringsheim yearned to return to Germany, but when he was finally offered a prestigious professorship in Frankfurt in 1932, Hitler was rising, and Pringsheim, of Jewish origin (although Lutheran persuasion), could not be appointed. He thus remained in Prague until he was replaced by a Nazi late in 1938 just before

Drugs from sea squirts?

ISOLATING PATELLAMIDES ONBOARD AN AUSTRALIAN RESEARCH VESSEL

Professor Marcel Jaspars, University of Aberdeen

> Diving to collect our model organism certainly added enjoyment! © Anke Klüter

WHY BOTHER WITH SEA SQUIRTS?

Ascidians – more illustratively known as sea squirts – are an interesting class of animals. Their adult form is that of a simple sessile hermaphroditic filter feeder while the swimming juvenile has a tadpole-like appearance with a notochord and a dorsal nerve cord that define them as chordates. Being thus related to vertebrates like ourselves suggests that ascidian biochemistry may show significant similarities with ours, but as the relationship is not close, also novel biochemical compounds and processes can be expected. Ascidians have been identified as prolific producers of biologically active compounds some of which may prove to be valuable active agents for pharmaceuticals.

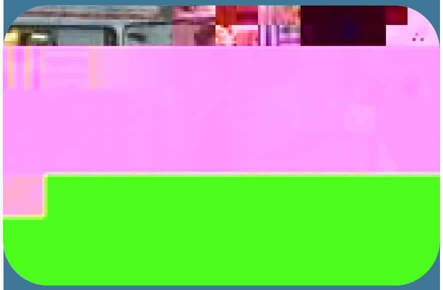
We have been particularly interested in a colonial species, *Lissoclinum patella*, which occurs on the Great Barrier Reef and looks like green candle wax dripping from coral reefs. The green colour originates from a photosynthetic symbiont, *Prochloron*, which inhabits the cloacal cavity of the sea squirt host. We are investigating *Lissoclinum* as model organisms mostly because they contain patellamides which may act as primitive enzymes. It appears that they are synthesised by *Prochloron*. Patellamides are compounds of great promise for medical developments as they are likely to find applications in the treatment of multiple-drug resistant cancers or inflammatory diseases.

MASS PRODUCTION OF COMPLEX BIOMOLECULES

One of the challenges that hampers the commercial exploitation of *Lissoclinum patella* as that of many other marine organisms is the difficulty of accessing significant numbers of them. Collection from the wild is ecologically dubious and could result in overexploitation. Other options to increase and facilitate their availability include aquaculture or tissue culture. In our case, however, *Prochloron* could not be cultured independently as it relies on its ascidian host for survival. Alternatively the bioactive compounds, once isolated and identified, could be synthesised chemically, or with molecular biological techniques. None of these methods is unproblematic. Aquaculture may be a solution where a supply of just one compound is needed. Growing single cells in tissue culture on the other hand is still very difficult because we know too little about the physiology of these simple chordates – we know more about growing complex human cells! Chemical synthesis of the more complex active sea squirt compounds would be ideal, but many of these molecules take more than 20 steps to synthesise which can be difficult to achieve on a larger scale. We are therefore using molecular biological tools to produce the compounds of interest.

We initially isolated large stretches of *Prochloron* DNA, and we then inserted these sections into the bacterium *E coli* using a ‘vector’. Now we are attempting to identify the particular colony of bacteria (from 1433!) that might contain the patellamide-coding DNA. To achieve this we are combining molecular biology and analytical chemistry techniques.

> Dr Paul Long from the London School of Pharmacy extracts DNA. © Marcel Jaspars



> The colonial sea squirt *Lissoclinum patella* is green due to an autotrophic symbiont, *Prochloron*, that is responsible for the production of patellamides. © Carsten Wolff

AND THE BEST: FIELDWORK ON THE GREAT BARRIER REEF

Most biotechnology research is conducted in clean laboratories in large cities, but it does not always have to be that way! As *Lissoclinum patella* occurs on the Great Barrier Reef, in the summer of 2004 my colleague Dr Paul Long, a molecular biologist from the London School of Pharmacy, and I teamed up with Drs Chris Battershill and Walt Dunlap from the Australian Institute of Marine Science to conduct this research aboard the AIMS Research Vessel *Lady Basten* on Davies Reef. This allowed us to double as SCUBA divers and collect our own organisms, which gave this work expedition a rather exciting edge! We were faced with some unusual challenges at sea, one of which was the need to transplant the guts of a chemistry and molecular biology lab to the rather limited space in a generic lab on a research vessel, a lab that rocked, yawed and pitched. Fortunately our Australian marine science colleagues - especially Walt - were veterans of many expeditions and knew exactly what to take. To us lab chemists it was unusual to say the least to heat our samples to 37°C in the cosy confines of a vessel’s engine room and to conduct such sophisticated work as chemical extractions, chromatography, enzymic digestions and DNA extractions in such an environment. Fortunately all this work can be achieved with a limited amount of robust and reliable equipment. ●

Meeting surFace to surFace

BIOSURFACTANTS BRING THE WORLD CLOSER TOGETHER

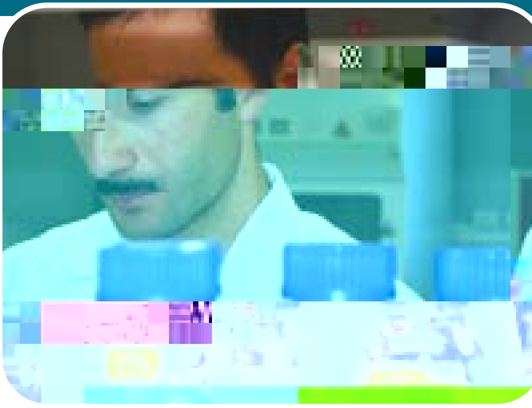
Drs Tony Gutierrez and David Green, SAMS

[illegible]

Detergents and soaps are essentially what scientists refer to as surfactants, substances that are able to interact with oils and water simultaneously and allow both to associate, so to speak. As the name suggests, surfactants act on surfaces, and it is here that they reduce the tension – i.e. surface tension – between two relatively non-mixable liquids. Lowering their surface tension at the interface between the two liquids allows the molecules of both parties to interact and mix together. But how or why does this happen? It all has to do with the intrinsic properties of the surfactant molecule: they are 'amphiphilic'. This means that they possess both water-loving and oil-loving components on the same molecule. With this, surfactants are able to grasp a hold of oil and water molecules simultaneously, acting as glue that binds two otherwise non-miscible liquids together.

With such a useful property, it is no wonder that surfactants find application in almost every sector of modern industry, from agrichemicals, to food ingredients, textiles, construction, healthcare, pharmaceuticals, and most importantly in our household washing-up liquid and bathroom soap. But there is a problem. Most surfactants are derived synthetically from petroleum, which brings to light environmental as well as health and safety concerns.

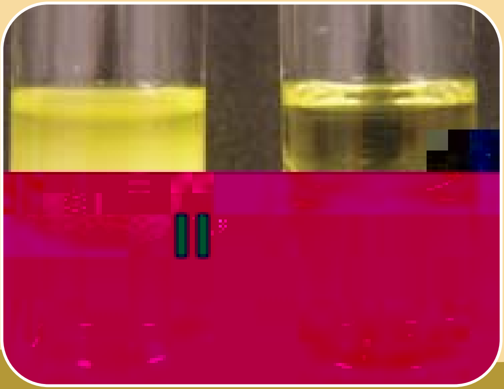
At SAMS we are investigating new types of surfactants that are produced naturally by biological processes, i.e. biosurfactants. Using the marine environment as our platform for discovery, we are targeting particular groups of bacteria that we have identified as likely producers of novel bioemulsifiers, a type of surfactant that can mix oils with water to form emulsions like ice-cream and mayonnaise. This research began due to our interest in the ecological roles of these compounds in the ocean, specifically with marine bacteria that degrade crude oil released by accident or from natural oil or gas seeps. Hydrocarbons are toxic to most organisms, and only relatively few species of bacteria have developed the knack to make a living from oil. By producing surfactants and emulsifiers, the oils can be made more soluble, so that the bacteria can enzymatically attack the oil and use it as a food source. It's one of nature's very own and effective clean-up processes. Furthermore, since surfactants sequester and act on surfaces, their accumulation at the ocean-atmosphere interface may influence the oceans' exchange of gases with the atmosphere.



> Dr Tony Gutierrez is developing biosurfactants from marine bacteria that one day may replace synthetic petroleum-derived emulsifiers for use in products such as ice cream and mayonnaise. (© Rory MacKinnon, SAMS)

> Surfactants reduce surface tension between different liquids, such as water and oil. This is measured using a tensiometer that can measure tension forces in milli-Newtons. (© David Green, SAMS)

> Emulsifiers are compounds that can interact both with polar water and apolar oil molecules, thereby producing emulsions. Shown is one of our emulsifiers mixing olive oil and water alongside its control without emulsifier. (© David Green, SAMS)



From an initial screen for novel biosurfactants, we have found four highly promising emulsifiers with properties similar to - or better than - existing commercial emulsifiers. The challenges ahead are significant because these emulsifiers don't only have to be every bit as good as existing commercial products (which we think they are) but it must also be possible to produce them more cheaply if they are to 'cut ice' as commercial products, let alone, make it into ice-cream and mayonnaise! ●