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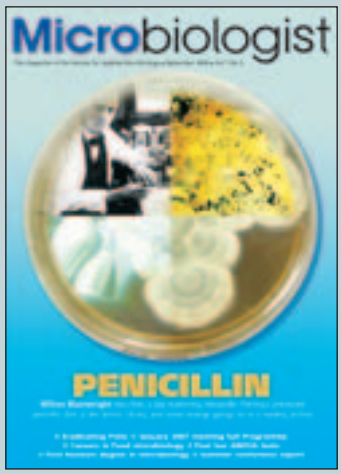
FEATURES

28 Cover story



Milton Wainwright describes a day examining Alexander Fleming's preserved penicillin dish at the British Library, and some strange goings on in a leading archive

WRITE FOR US!

The editor is always looking for enthusiastic writers who wish to contribute articles to *Microbiologist* on their chosen microbiological subject.

For further information please email the editor, Lucy Harper at: lucy@sfam.org.uk

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Editorial

MICRO-ORGANISMS HAVE formed the basis of life on earth for thousands of years and although many are pathogenic, sometimes life-threatening, we cannot deny that we owe our existence to them.

Bacteria existed long before human kind. The spread of disease by foreign bodies has been an established principle for many years. This wasn't always the case however. Aristotle taught the theory of abiogenesis or spontaneous generation, whereby living organisms could be created from decaying non-living matter. The first step in the refutation of this theory was demonstrated by Francesco Redi, who in 1668, proved that no maggots were bred in meat on which flies were prevented from laying their eggs. From the 17th century onwards it was gradually shown that, at least in the case of readily visible organisms, spontaneous generation did not occur, but that *omne vivum ex ovo*, every living organism comes from a pre-existing living thing.

The discovery of the microscope carried the refutation further. In 1683 Antonie van Leeuwenhoek viewed 'animalicules' for the first time, and it was soon found that however carefully organic matter might be protected by screens, or by being placed in stoppered receptacles, putrefaction set in, and was invariably accompanied by the appearance of a myriad bacteria. In 1768, Lazzaro Spallanzani, proved that microbes came from the air, and could be killed by boiling. His work paved the way for Louis Pasteur who disproved the occurrence of abiogenesis in the microscopic world as much as it had been disproved in the macroscopic world. He found that if organic matter were first sterilized and then prevented from contamination, putrefaction did not occur, and the matter remained free from microbes.

We are now over half way through our 75th anniversary year so to commemorate this occasion, it is an historical perspective of Microbiology which is the focus for this issue of *Microbiologist*.

The history of Microbiology is an interesting topic. Many world-changing events have had their roots in Microbiology — from Jenner's discovery that inoculation by a pustule of cowpox can protect people against smallpox in 1796, to the discovery of penicillin in 1928. Also, it may interest you to know that merely two years after the founding

of what was then called the Society of Agricultural Bacteriologists (SAB), 1933 saw the development of the first electron microscope by Ernst Ruska.

Our feature article for this issue discusses some less well-known events surrounding the discovery of penicillin. Fleming's Petri dish, which is held at the British Library comes under some detailed scrutiny on page 28, as do his laboratory notebooks...

As part of our 75th anniversary celebrations, a number of you entered our writing competition to describe your most significant microbiological event of the last 75 years. The winning two entries appear in this issue of *Microbiologist*. The first looks at the development of the polio vaccine (see page 31) and the second describes the first degree programme given the at-the-time-unusual title of 'Microbiology' in 1950 (see page 32). Remaining on the subject of history, this year sees the publication of a book describing the history of the Society. An interesting and amusing presentation given by professor Max Sussman, on this very theme kicked off this years 75th Anniversary Summer conference. Those of you who couldn't attend can read an entertaining and very informative report of the conference on page 18.

As well as looking back, it is also extremely important to look to the future and so we look forward to the next S*f*AM meeting — *Food and Health*, to be held in London on 11 January 2007. We have discounted rates for members of the Institute of Biomedical Sciences and the Chartered Institute of Environmental Sciences, so turn to page 24 for the full programme and booking form.

Finally, don't forget you can keep up to date with all the latest S*f*AM news and events by visiting our website.

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MEDIAwatch

MICROBIOLOGY IN THE NEWS

If you spot a story in the media which you think should feature in this column, then send it to the Editor at: lucy@sfam.org.uk. To keep up to date, don't forget to look at our 'News' section on the SfAM website: **www.sfam.org.uk/news.php**

2006 Summer Conference

The SfAM 75th anniversary conference proved an interesting meeting with excellent presentations from invited speakers, offered papers and students alike. We all enjoyed this packed scientific and social programme. We were extremely pleased to see the meeting covered extensively by the national press. Here are just a couple of the stories generated from the conference:
(All stories are copyright *The Scotsman*).

GM spray could keep dentist at bay

A SINGLE dental treatment that involves spraying genetically-modified bacteria into a patient's mouth could cut the risk of cavities by up to 90 per cent, according to new research. The spray, which could be on the market in as little as three years and should last a lifetime, contains bacteria similar to that found naturally in the mouth - with one crucial difference.

The natural bacteria, *Streptococcus mutans*, produces lactic acid that eats away at teeth, causing decay, whereas the GM bacteria does not.

Planting the new form inside the mouth means the natural kind cannot get a foothold, so only one application would ever be needed. For the full story visit: **http://news.scotsman.com/scitech.cfm?id=996272006**

Natural birth can give your baby better protection from disease

A NATURAL birth provides a baby with better protection against diseases and allergies than if it is born by Caesarean section, according to an expert on human nutrition. Researchers at Glasgow University discovered that the gut of babies who had been delivered normally contained higher levels of 'friendly' bacteria, such as bifidobacteria. These are particularly important, as they are the first bacteria to enter a child's digestive system and play a crucial role in developing the immune system. Professor Christine Edwards said researchers had found significant differences between the type and number of bacteria in the intestines of babies - those delivered by Caesarean could take a month to 'catch up.'

For the full story visit: **http://news.scotsman.com/scitech.cfm?id=990002006**

For those of you who couldn't attend the conference, see page 18 for a full report.

SfAM POLICY ON THE MEDIA

We will: ■ always do our best to provide facts, information and explanation. ■ if speculation is required, explain the rationale behind that speculation. ■ desist from hyping a story—whether it is the journalist or the scientist doing the hyping.

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Serving Biotechnologists in Europe and beyond

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Established by European scientists in 1978, the European Federation of Biotechnology (EFB) is the pan-European biotechnology federation for Public and Private Companies, National Biotech Associations, Agencies, Learned Societies, Universities, Scientific Institutes and NGOs with an active interest in biotechnology and a desire to play a central role and develop or maintain a high profile in the European biotech community.

The Aims of EFB are to advance the responsible use of the Life Sciences, to promote research at the cutting edge of Biotechnology, to provide a forum for interdisciplinary and international cooperation, to improve scientific education and to faciliate dialogue between scientists and the public.

The mission of the EFB is to promote the safe, sustainable and beneficial use of Nature's resources through the application of knowledge in the Life Sciences and Biotechnology.

Membership is open to all organisations and individuals involved in Biotechnology with an active interest in, and a desire to play a central role in the European collaboration between academia, research and industry.

The EFB aims to:

- Promote international and interdisciplinary cooperation throughout Europe and beyond by fostering collaboration between academia, research and industry.
- Stimulate innovation & technology transfer
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- Organize workshops, meetings and the biennial European Congress on Biotechnology
- Promote training & education
- Improve understanding of safety issues in biotechnology
- Promote public understanding of biotechnology
- Prepare position papers, white papers, briefings, reviews and reports
- Maintain good contacts with and serve in an advisory capacity to the European Commission
- Disseminate information on EC funding opportunities and science policy issues
- Promote and defend the beneficial use of biotechnology

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- Be informed of and participate in EFB projects, many of which are major European projects with an international focus and key partners around the world
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Dr Margaret Patterson reports on the 75th anniversary AGM of the Society

THE 75th ANNUAL GENERAL Meeting of the Society for Applied Microbiology was held on 5th July 2006. All AGMs are important as they allow members to air their views on how the Society is operating, but the 75th meeting was more important than most, certainly in recent times. The Committee and Custodian Trustees had proposed a resolution that the Society be dissolved and become established as a company limited by guarantee. This resolution was debated at the AGM and was agreed unanimously with no abstentions.

For those of you who were not able to attend the AGM or who would like further clarification, I would like to answer some of the members' most frequently asked questions.

Why does SfAM need to become a Company limited by guarantee?

The suggestion that SfAM become an incorporated body came initially from the Charity Commission, when it visited SfAM a few years ago. There were two main reasons for this recommendation:

(i) Currently the financial liability of the Society rests wholly on the Committee members (who are Trustees of the Charity) and the Custodian Trustees. Incorporation will mean that Committee will remain Trustees but will also form the Board of Directors of the company and individual financial liabilities will be restricted to £1. The Trustees will, however, still remain accountable for the activities of the Society.

(ii) An incorporated body can be more flexible and adaptable in the activities it undertakes on behalf of its members and its area of influence. For example, incorporation will enable more appropriate and beneficial banking to be arranged and implemented. Currently, as a charity, SfAM is not permitted even to have a credit card. This will change with incorporation.

Have other learned Societies gone down this route?

Yes. Other Societies, such as the Society for General Microbiology, became incorporated some time ago. In more recent years, the Federation of European Microbiological Societies (FEMS) and the Biosciences Federation have chosen this route.

Will SfAM still have charitable status?

Yes. SfAM will remain a charity, as well as being a company limited by guarantee.

Will the Committee structure stay the same?

Yes. The Committee structure will remain unchanged. There will still be a President, Honorary General Secretary, Honorary Treasurer, Honorary Meetings Secretary, Honorary Editors and nine Ordinary Committee members. However these individuals will also form the Board of Directors of the company, with the President as Chairman. Mr Philip Wheat, our CEO, will also have the title of Company Secretary.

What will the change mean for me as a member of SfAM?

There will be little or no obvious change in the day-to-day running of the Society as far as members are concerned. Members will still vote for new Committee members, just as they do now. Also the various grants and awards will all be available, just as they are now.

Will there be changes to the existing constitution?

Yes. The Constitution (governing document) will have to be rewritten to accommodate the fact that the Society will become a Company limited by guarantee. The last time the Constitution was substantially revised was in 1984. Therefore, an updated version is long overdue to reflect the objects of the Society as it exists today. We have looked at the governing documents of other learned Societies that have already become incorporated whilst remaining a charity, and will use these to form the basis of our new governing documents. We are also using professional legal advice to ensure that the process is carried out correctly and in full accordance with the law.

When will all these changes take place?

The process of becoming incorporated has to take place in stages and only when all the stages are complete will the present charity dissolve and become the new charitable company. One of the first stages is to have the agreement, by at least two-thirds of those present at the AGM, for the Society to dissolve and become established as a company limited by guarantee. This was achieved, unanimously, at the July 2006 AGM. Having got this agreement to proceed, the next stage is to prepare the new Constitution. We have already started work on this, in consultation with our solicitors, and plan to complete this stage within the next few months. The documents will then be lodged with the Charity Commission and Companies House for their approval. The approval process could take several months. Only when approval has been obtained will the Society become a company limited by guarantee and its funds and assets will be transferred to the new charitable company.

What are the disadvantages of becoming a Company limited by guarantee?

There will be some cost implications brought about by this change. The most obvious cost is for the solicitor's fees as we go through the process of altering the constitution and drawing up the new governing document. However, this is a one-off cost. Once we become incorporated we will have to prepare two sets of accounts, one for the Charity Commission and one for Companies House. The same information will be prepared but it has to be presented in different ways. Therefore, we will have a slight increase in accountancy fees.

We will keep members informed of our progress as we go through the various stages of the process. In the meantime, I hope I have answered any outstanding questions you may have about the Society becoming incorporated. However, if you have any further queries or comments, please do not hesitate to contact me or Phil Wheat, in the Society Office.

Dr Margaret Patterson
President of the Society

Philip Wheat reports on the latest developments within the Society

As the Honorary President has previously mentioned, members who attended the Annual General Meeting voted unanimously to accept the resolution that the current Society should dissolve and re-emerge still as a Charity but in addition be a Company Limited by guaranteed. The President has detailed some answers to possible questions that you as members might have concerning this issue. I would just like to re-emphasise that if you do have any further questions please do not hesitate to contact me (pfwheat@sfam.org.uk) and I will deal with your enquiry.

In my last column (June 2006) I highlighted that myself and the Officers would be attending several conferences and exhibitions in the coming months. I can report that all three exhibitions we attended (Biomedica , American Society for Microbiology and International Food Technology) were deemed successful and a number of new members were enrolled at each meeting. In addition, a large number of interested delegates asked for further details to be forwarded after the meetings. As well as making contact with new and prospective members, I thoroughly enjoyed meeting many existing members who called by the stand. Whilst on the subject of Society membership, can I just re-emphasise that member benefits (including grants and awards) are applicable to **all members** irrespective of whether they are based in United Kingdom (UK) or outside the UK. I spoke to several members who were based in the United States who did not realise that the grants and awards the Society offers were applicable to members who are not based in the UK.

This column is being written in the first few weeks of July. I can confirm that a five year lease for our new office premises is being prepared at this very moment. We have agreed a five year lease for 1000 square feet of office space at the following address:

Society for Applied Microbiology
Saturn Facilities Ltd, Bedford Heights
Manton Lane, Bedford, MK41 7PH

All email, telephone and fax contact details will remain the same. Look out for future issues of the *Microbiologist* for further details of the new office facilities.

I have also just returned from the Society's Summer Conference in Edinburgh. As well as being the Annual Summer Conference it also gave members an opportunity to celebrate the 75th year of the Society. The four day event was a resounding success with many favourable comments received on both the scientific and social aspects of the meeting. It was gratifying to see that the meeting was very well attended and in fact we did have to arrange extra accommodation because of the number of delegates attending.

A particularly pleasing feature of this years meeting was the number of young student members who took the opportunity to apply for Society studentship grant awards enabling them to attend. I spoke to a number of these students and all agreed that the conference had been a very worthwhile experience and they had thoroughly enjoyed their time in Edinburgh. These students also benefited from meeting and networking with other Microbiologists and hopefully they began professional relationships which will last well into their own careers in Microbiology.

Plans are finalised for the next Society meeting which is the one day Winter meeting (Thursday, 11 January 2007, Royal Society, London). The title of the meeting is ***Food and Health***. There will be two concurrent sessions one on Hospital Acquired Infections and the other on Food Microbiology**.** The full programme can be found on page 25 together with a booking form and studentship application. Alternatively, please visit the Society website at www.sfam.org.uk. Early registration is highly recommended as the meeting will appeal to a wide audience.

In addition to the 2007 Winter meeting we have nearly completed the planning for the 2007 Summer Conference (Park Plaza Hotel, Cardiff, 2 – 5 July 2007). The title of this meeting is ***Microbiology of Fresh Produce***. The general themes for the future Summer meetings have also been set. Water Microbiology and Zoonoses will be the themes for the 2008 and 2009 meetings respectively.

Philip Wheat
Chief Executive Officer

MED•VET•NET

Teresa Belcher reports on the challenges zoonotic pathogens pose for Food safety

MED-VET-NET IS A EUROPEAN Network of Excellence that aims to improve research on the prevention and control of zoonoses by integrating veterinary, medical and food science research. Comprising 16 European partners and over 300 scientists, **Med-Vet-Net** will enable these scientists to share and enhance their knowledge and skills, and develop collaborative research projects. **Med-Vet-Net** officially commenced on 1 September 2004, and is funded to the value of €14.4 million for five years.

The Press Conference at the Priority Setting Conference

THE INCIDENCE OF zoonotic pathogens and the risk of food contamination continue to be hot topics throughout the world. Zoonotic infections are those that can be acquired directly from animals, or through ingestion of contaminated food. The seriousness of these diseases in humans can vary from mild symptoms to life-threatening conditions. Infectious disease is the major cause of death worldwide (causing 14 million deaths worldwide (WHO). In the European Union alone, zoonoses of this kind generate costs well in excess of €6 billion per year. Of the 1,451 disease agents known to be pathogenic to humans, 61% are zoonotic. Each year, new infectious agents appear and the problem is no longer isolated in individual countries as increasing global travel and international livestock trade mean that these diseases can spread far and at a rapid rate.

At the beginning of 2006, bird flu was one of the main news items in the media. There were fears that the virus would mutate, enabling it to transfer from human to human. If this did indeed occur, then the disease would pose an extremely serious threat to health and have the potential to spread and cause a flu pandemic. Despite the scare and great deal of media attention, so far, these fears have proved unfounded.

Other zoonotic diseases, however, such as those caused by *Salmonella*, *Escherichia coli* and *Campylobacter*, currently have a much bigger impact on health and the national economy.

Priority setting of foodborne and zoonotic pathogens

Foodborne illness is a persistent and highly dynamic problem that requires continuous attention, and it is vital that there is global cooperation in this field. Safe food is a joint responsibility between government, industry and consumers who effectively need to share information to support a complex decision-making process.

These challenges have led to calls for a food safety system that is more science- and risk-based. This means developing a system that allocates its resources more in accordance with the distribution of risks and the opportunities to reduce risk across the food supply. The approach calls for ranking the public health impact of significant foodborne risks and then prioritising opportunities to reduce those risks. This task requires the right analytical and decision tools – organised ways of collecting and using relevant information to devise effective research, regulatory and educational interventions and make resource allocation decisions.

A move to begin dealing with this

challenge was taken up during a three-day meeting held at the Federal Institute for Risk Assessment (BfR) in Berlin, from 19 to 21 July 2006. Scientists, including risk researchers, epidemiologists and economists from **Med-Vet-Net** and the American Food Safety Research Consortium (FSRC*) met to discuss how to establish meaningful scientific priority setting for the control of zoonotic

pathogens. The conference also sought to elucidate key scientific questions, identify opportunities for the promotion of research and optimise global cooperation in this field.

The conference focussed on public health problems in developed countries, and the possible impact of imported foods on public health. While discussions did not aim to set the priorities, the further development of scientific methods to identify the main risks to health from zoonotic pathogens was highlighted. To this end, the available data must be collected, rated and any gaps identified. This includes data on the importance of the various routes of infection and sources of foodborne and zoonotic infections in humans, as well as other data on the incidence of human disease, quality of life and related costs. By integrating this information, it is possible to weigh the importance of these pathogens and the diseases caused by them. This scientific priority setting helps to make consumer health protection more effective. Besides the prevention of illness

and suffering in humans, the emphasis is also on the most effective use of financial resources. As these resources are limited, it is important to help contain the costs generated by zoonoses every year and find the most cost-effective ways of controlling them.

Further Information

■ For more information about **Met-Vet-Net**, visit our website at http://www.medvetnet.org/ or contact Teresa Belcher at the SfAM offices in Bedford on: **+44 (0)1234 271020**

Teresa Belcher
Med-Vet-Net Communications Director

Trends and sources of zoonoses in the EU

Each year, the EU collates and analyses information from the National zoonoses country reports. For the first time, the European Food Safety Authority (EFSA) has produced a *Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance*. This data collection currently covers 11 zoonotic diseases – zoonoses that are important for the public health in the whole European Community as well as zoonoses, which are relevant on the basis of the national epidemiological situation.

In 2004, 20 Member States and Norway reported 6,883 foodborne outbreaks affecting altogether more than 44,000 people. In the Member States, these outbreaks resulted in 4,361 hospitalisations and 13 deaths (in the UK the figures were 52 outbreaks, affecting 1903 people, with 102 hospitalisations and 1 death). The report reveals that by far the most frequently reported zoonotic diseases in humans are salmonellosis and campylobacteriosis. In 2004, 192,703 salmonellosis and 183,961 campylobacteriosis cases were recorded in the Member States. In the UK, 2004 figures were 14,476 salmonellosis cases and 49,233 campylobacteriosis cases.

Over the past 25 years, there has been general decreasing trend of the occurrence of salmonellosis cases. However, from 2003 to 2004, with the expansion of the EU to include ten new Member States, these figures increased again. In contrast, there has been a general increase in reported cases of campylobacteriosis over the last few years in the old Member States.

More information can be found at **http://www.efsa.europa.eu**

*The U.S.- based Food Safety Research Consortium (FSRC) is a vehicle for collaboration among researchers from diverse institutions and disciplines to improve how the U.S. food safety system works to reduce the burden of foodborne illness. The FSRC includes six universities and one non-profit think tank and works collaboratively with government agencies and the private sector across the United States.

New Members

We would like to warmly welcome the following **new members** and hope that you will participate fully in the activities of the Society.

Australia

Dr M Lepesteur-Thompson

Denmark

Dr T Wassenaar

Ireland

Ms H Barry; Ms L Burke; Mr J McEniry; Ms M Walsh

Malaysia

Prof M Wan

Nigeria

Mr S A Ajani; Dr M O Ilori; Mrs O F Obidi

Spain

Miss E M Martin

United Kingdom

Dr R Allen; Dr K Bellamy; Miss M Bouzid; Ms J Brown; Mr P Davenport; Miss S Dunn; Mrs A M Field; Mr D P Green; Mr A J Hall; Miss T L Hook; Miss D J Martin; Miss M Mehmi; Prof D Naughton; Miss F Pinto; Miss S K Sandiford; Dr H Schäfer; Mr R M Thorn; Dr D Wareing; Mr G J Williams

USA

Prof L Baillie; Dr L Ellis; Dr T A Else; Dr G Gearner; Dr K Kesterson; Dr C Marques; Dr R Moyes; Prof S Pillai; Dr A L Reysenbach

NOT A MEMBER?

Did you know that the Society has many generous grants and prizes available to members? To find out more turn to page 54 or visit the website at:

www.sfam.org.uk

Call for Society Journals - Digitisation of *Sf*AM Journal Archive

Blackwell publishing are in the process of digitising the Society Journals for an archive, which, when complete will be **accessible free of charge to all *Sf*AM members.** However, there are a number of volumes missing from their collection. To make the archive complete, Blackwells are looking for hard copies of the *Journal of Applied Bacteriology/ Microbiology* from Volumes 1 – 29. We are assured that all copies will be returned to their owners after use.

So if you, or a friend are in possession of any of these journals, please contact Adam Finch, Journal Publishing Assistant, at Blackwell publishing on: email: adam.finch@oxon.blackwellpublishing.com or telephone: **+44(0)1865 476245**

Attention Postgraduate Members

The Society is establishing a forum for postgraduate members to discuss Society issues relevant to them and a delegate of the forum will report to main committee.

If you are interested in joining this forum and would like to learn more, please email Anthony Hilton (a.c.hilton@aston.ac.uk) by **20 October 2006 at the latest.**

Impact Factor increase for Society Journal

We are extremely excited to be able to inform members that the impact factor for *Environmental Microbiology*, the journal which SfAM runs in partnership with Blackwell publishing, rose from 3.995 in 2004 to 4.559 in 2005. Among the review journals from the microbiology journal ranking, this places *Environmental Microbiology* in fourth place, behind *Clinical Infectious Diseases*, *Cell Microbiology*, and *Molecular Microbiology*, all journals specializing in, or containing a fair amount of medical microbiology.

Congratulations to the Editors, reviewers, authors and all who have contributed to this fantastic result.

Sponsor a new Member of the Society and win a £50 Book Token!

Could you be the next winner of the 'SfAM Sponsor of the Year' Award?

If you feel you could be our next winner for 2005, and would like some promotional material to help you recruit new members please contact Julie Wright, Membership Co-ordinator on 01234 326661 or email julie@sfam.org.uk.

Erratum

In the June 2006 issue of *Microbiologist*, the Overseas Development award report by Dr Keith Jones of Lancaster University was rather heavily edited by the production team resulting in a number of sentences becoming slightly incongruous. Our sincere apologies go to Dr Jones and his colleagues in Ghana for this error.

W H Pierce Prize Winner

On 5 July 2006 Dr David Post presented the W H Pierce prize for young microbiologists who have made a substantial contribution to the science, to Dr Roy Sleator of University College, Cork. Here he introduces himself and gives an overview of his full and accomplished career.

I graduated top of my class with a first class honours degree in microbiology from University College Cork (UCC) in 1997.

Following graduation I accepted a PhD fellowship with Prof. Colin Hill at the National Food Biotechnology Centre, BioResearch Ireland, focusing on the osmotic stress response of the halotolerant intracellular pathogen *Listeria monocytogenes*. The main focus of this work was to identify the genetic mechanisms used by the bacterium to cope with salty conditions encountered both in foods prior to ingestion and subsequently within the animal host. I completed my PhD in 2001 with seven first author publications.

In 2002 I spent an extended period in the laboratory of Prof. Janet Wood at the University of Guelph, Ontario, Canada, studying the biochemistry of the listerial osmotic stress response, thus complementing the earlier genetic analysis completed during my PhD studies.

Upon returning home I was awarded a prestigious Government of Ireland Embark Postdoctoral Fellowship jointly with Prof. Hill at the Department of Microbiology, UCC and Prof. Fergus Shanahan, Department of Medicine. This study allowed me to extend my interest in the listerial stress response to include stresses encountered by the bacterium during the gastrointestinal phase of its infectious life cycle.

This was a very productive period and resulted in the discovery of two novel virulence factors in *Listeria*; specifically BilE a bile exclusion system which helps protect the bacterium against the detrimental effects of the biological detergent bile which, in addition to solubilising fats during digestion, also forms an important part of the bodies physiochemical defence system, degrading the bacterial cell wall. OpuC, the other gastrointestinal specific virulence factor facilitates the accumulation of carnitine, a compound which protects *Listeria* against the elevated osmolarity of the gastrointestinal tract, thus promoting gastrointestinal persistence and subsequent systemic infection.

In 2004, in recognition of my work on *Listeria* stress sensing, I was awarded the inaugural ESCMID-FEMS Fellowship; presented each year to the top young European in the fields of clinical microbiology and infectious diseases. This fellowship is awarded jointly by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the Federation of European Microbiological Societies (FEMS).

Currently I am Principal Investigator on a Health Research Board (Ireland) grant focusing on a novel area for which we have coined the term Patho-biotechnology. This concept promotes knowledge transfer from the well developed area of Pathogen research to the growing area of probiotics in medicine; and involves the manipulation of probiotic bacteria for enhanced stress tolerance, thus generating improved vaccine and drug delivery platforms.

Winning the Pierce Prize and thus gaining international recognition of my research represents the highest point in my career to date and for that I am extremely indebted to the Society for Applied Microbiology.

Roy Sleator
University College Cork

2006 SfAM AGM

The 75th annual general meeting of the Society for Applied Microbiology was held on Wednesday 5 July at 4.30 pm at the Apex International Hotel, in Edinburgh. The Honorary President, Dr Margaret Patterson, was in the chair

Present

Margaret Patterson, Anthony Hilton, Jane Sutherland, Karen Stanley, John Rigarlsford, Peter Setlow, Christine Dodd, Will Waites, S. C. U. Nwachukwu, Martin Adams, Tim Aldsworth, Jim Lynch, Sally Cutler, Basil Jarvis, Hilary Dodson, Claire Cassar, Mark Fielder, Peter Lambert, Tony Worthington, Andrew Sails, Louise Fielding, Shona Nelson, Charlotte Lindhardt, Don Whitley, Anthony Hardy, Susannah Walsh, John Coote, Carol Phillips, Robert Madden, David McCleery, Peter Silley, Arthur Gilmour, Sue Lindsay, Peter Green, Alan Godfree, Stewart Pettit, Max Sussman, David Post, Fred Skinner, Valerie Edwards-Jones.

1. Apologies for absence

Apologies were received from Peter Watson, Colin Harwood, J. F. Lowe, C. W. Simmons, R. G. Board, Maurice Moss, David Owens, Eveline Bartowsky

2. 74th Annual Meeting

The minutes of the 74th Annual Meeting held in Brighton, 2005 were approvedand accepted as correct by those present.
Proposed: Stuart Pettit
Seconded: John Rigarlsford

3. Matters arising

Mr Philip Wheat reported that value added tax was not charged for Society meetings. This was in response to a question which had been raised at last years AGM.

4. Report of the Trustees

of the Society for the year 2005. *Copies of the report for the year 2005 were previously distributed to all members attending the meeting.*

This was accepted with additional comments from Peter Walker. The Society is paid for through its membership and journals resulting in an income surplus. This has resulted in the ability of the society to staff an office with professional people to administer the Societies business. However, with the introduction of the freedom of information act and open access publishing, the Society should be watching carefully where it is investing its portfolio. Is there a list of investments available to the membership?

The treasurer, Dr Valerie Edwards-Jones responded, explaining that the Society does utilise a very reputable firm of stockbrokers to manage the Societies investment affairs. The Society has also allocated designated funds in case there were a reduction in the income the Society received.

Dr Peter Silley also added that the number of downloads from Society journals outweighs the impact of the impact factor. This is assumed to be due to the applied nature of the Societies journals.

5. Adoption of the Annual Report 2005

Dr Patterson asked for the report to be officially adopted by those present. Basil Jarvis proposed and Peter Silley seconded.

6. Election of new Committee members

Dr Anthony Hilton reported that this year there were three committee vacancies as Professor Diane Newell, Dr Shona Nelson and Dr David McCleery were retiring by rotation. Dr Hilton thanked these three people for their contributions and hard work during their terms in office.

Dr Hilton then stated that there had been four nominations to committee resulting in a postal ballot. He then announced the results of this ballot and the new members of committee for 2006-2009: Professor Carol Phillips (proposed by Dr Anthony Hilton and seconded by Professor Martin Adams), Dr Mark Fielder (proposed by Professor Diane Newell and seconded by Dr Margaret Patterson) and Dr Joanna Verran (proposed by Dr Valerie Edwards-Jones and seconded by Dr Shona Nelson. All these names were unanimously accepted.

7. Election of new members

A list of names of applicants for ordinary membership was tabled and all were accepted.

In addition, Anthony Hilton stated that five new honorary members had been appointed. These are: Professor Brian Duerden
Professor Colin Dennis
Professor Peter Boriello
Professor Peter Setlow
Professor Duncan Stewart-Tull

8. Dissolution of the Society

and establishment of SfAM as a company limited by guarantee and without a share capital.

Dr Margaret Patterson commented that the Committee and the Custodian Trustees were proposing a resolution to dissolve the current Society and become a company limited by guarantee whilst still retaining charitable status. She then went on to comment that for this change to happen required a two thirds majority of members attending the AGM. Dr Margaret Patterson then presented the benefits and limitation of the proposal. Dr Margaret Patterson then offered the members an opportunity to air their opinions concerning the issue. The resolution was then debated.

Anthony Hilton then highlighted that all members had in advance of the meeting been circulated with the following resolution:
"The trustees wish for the Society to become a company limited by guarantee whilst still retaining charitable status. In accordance with the current constitution I (as a member of the Society) agree with this action which involves dissolving the present charity and transferring all its funds and assets to the new charitable company".

Dr Patterson then asked for a show of hands in favour, against and in abstention. The resolution was voted for unanimously with no abstentions.

9. Any other business

Prof.Will Waites pointed out that adverts in the conference handbook for other symposia appear as if SfAM are endorsing the product. It was suggested that in future handbooks adverts are clearly labelled as such.

10. Date of next meeting

The next ordinary AGM will be on 5th July 2007 in Cardiff.

BIOSCIENCES FEDERATION

Biosciences Federation – a message from the Chief Executive

HOW DOES ONE GET THE money to support a really big project? Programme grants are difficult to get and very many research scientists think that their chance of a project grant submission being successful will be diminished if the amount sought is much out of the ordinary.

Funders deny that this is the case but are not really believed. So the active researcher wanting to develop a largish team and research programme resorts to writing a portfolio of grant applications. In a sense the major project is "salami sliced" into smaller projects that fit better with the system.

However these tactics are not helpful if one seeks to develop a really big project. If this had been the tactic for mapping the human genome I wonder how much would have been achieved today. And, of course, there are other big projects to be undertaken across the biosciences.

Currently the Karolinska is leading a major attempt to raise antibodies to all human proteins. The main sponsors of this work are the Wellcome Trust and the Wallenberg Foundation, although industry is also making an important contribution to the overall cost. This arrangement, whereby major funders come together uniquely for a particular project, is a very interesting approach to finding support for a big project. Indeed in some cases it may be the only way to obtain large sums of money.

Hitherto these arrangements have been rather *ad hoc:* there has been no platform for making introductions. The new Eurobiofund (a dreadful name because it is not a fund at all!) has the potential to change the landscape and will certainly provide the necessary platform to introduce exciting large scale science to consortia of funders. The Eurobiofund is essentially a forum for scientists to interest public and private funders in large scale projects. The first forum will be in Helsinki in December and is supported by the European Commission. In January, *Nature* (439, p244) stated that the Eurobiofund "may end up being just a small step towards the ideal, but it is the biggest single step that we have seen for some time. European scientists should give it their full support." The call for expressions of interest closes on September 4 2006 — but there will be another one next year. No matter what area of the biosciences you are in, if you have a grand idea and can muster persuasive arguments in its support, you might find the Eurobiofund to be of interest.

Closer to home, the BSF is beginning to think about how it can make a distinctive and effective contribution to discussions about the Research Assessment Exercise (RAE). Clearly there will be another RAE and equally clearly metrics will play an important role in the outcome. The key question concerns the metrics used and how they are interpreted. There is a possibility that different metrics will be used for different areas. For example the metrics for the Humanities do need to be different from the metrics for the Biosciences. However, one can also argue that different metrics could be applied usefully to different biosciences — for example, is it sensible to compare numbers of spin out companies in ecology with the numbers arising in biochemical areas? "Certainly not," many would say but all the biosciences will have to cope with a single array of metrics. A strength of some of the metrics is that they arise from the peer review process. This obviously applies to grants and refereed publications but it also applies elsewhere — for example to funds obtained from venture capitalists or seed funds. However this is not true for some important activities that should be included in the analyses. For example, assessment of quality is much more problematic when one considers outreach activities. I would certainly welcome individual views on how to tackle this question.

This is an 'interesting' time for enquiries that will impact upon the research landscape. The BSF has already responded to a Parliamentary enquiry about Research Council Institutes and we are currently developing our views about the coalescence of MRC funds with the research funds of the Department of Health. The real difficulty in all of this is to understand the detail – and where there is no detail, to predict what it might eventually be. Consider the merging of MRC and DoH research money. At one level it seems a sensible idea to have a single fund of public money for research in this area. However the idea immediately seems less attractive to many if a consequence of change is altered accountability.

All of our responses to enquiries require effort and research. Currently there is too much for our small team to tackle. However thanks to increased support from some Member Organisations we are now able to strengthen our team. I hope to introduce the new members to you in the next issue of *Microbiologist.*

Richard Dyer,
Chief Executive, Biosciences Federation

BIOSCIENCES FEDERATION

Science and education policy June 2006

Digest of reports in science journals and newspapers on UK Science Policy

THE DEPARTMENT FOR Environment, Food and Rural Affairs science and innovation strategy for 2005-08, due out in July, has been heavily criticised by the department's own science advisory council (SAC) (*Guardian*, 30 May).

The advisory council has warned that funding for some areas of research have been squeezed so badly that the department may not be able to carry out the science it needs to underpin the government's policy goals. The SAC believes that a £20m to £30m increase in Defra's research budget is needed to provide science to underpin its policy work. Researchers heavily dependent on Defra for funding raised similar concern in their responses to the strategy consultation. An analysis of responses published by Defra says: "There was a general consensus among respondents that greater clarity and improvements in mechanisms for collaborating with other funders would be beneficial" (and Annexes). The SAC also warned that the strategy's focus on Defra's short-term needs means it would have difficulty responding to unexpected future developments.

In a letter to the *Times*, three UK professors suggest that the merging of funding for the Medical Research Council (MRC) and the Department of Health's research and development arm could improve opportunities for clinical research by enabling clinical academics to conduct research while being free of clinical duties (*Times*, 2 June). The authors claim that the current job structure is putting many students off pursuing clinical academic careers, which will impact on the UK's ability to translate research in basic sciences to patient care. In light of the proposed new health research fund, the MRC is working to identify areas of research for coordinated bids with the Department of Health under the 2007 spending review (*Res Ft*, 7 June). The MRC has updated its delivery plan to include funding of £9 million to establish research centres in translational medicine and a call for proposals in biomarkers research worth £12 million. David Cooksey's review of the health research fund is expected to propose separate ring-fenced pots for clinical and basic research, administered via a peer review mechanism based on the MRC model (*Res Ft*, 21 June). Early indications suggest that an umbrella body will be set up initially, with further changes to the administrative structure in the longer term.

The Conservative party proposed a series of amendments to the company law reform bill currently going through parliament which would increase the protection of investors from animal rights activists by sparing businesses from having to file shareholder registers at Companies House (*FT*, 22 June). They argued that government plans in the legislation to protect investors from intimidation were "wholly inadequate" and want individual shareholders to have the right to ensure their names and addresses are not disclosed, rather than having to rely on the company to block access.

In a trial which will be monitored by researchers and the scientific publishing industry worldwide, the Royal Society is to launch an "open access" journal allowing people to read its new scientific papers free of charge (*FT*, 21 June; *THES*, 23 June). Costs of publication will fall to the authors or their research

sponsors, if they choose to make their papers immediately available online. The accounts of PLoS (the Public Library of Science) have revealed that the open access publisher faces financial difficulties (*Nature*, 22 June). Critics of the author-pays model have argued that this is further evidence that this system is not viable. New guidelines produced by the Research Councils stipulate that research grant holders from the Biotechnology and Biological Sciences Research Council, the Medical Research Council and the Economics and Social Research Council will have to deposit their research findings in open access repositories from October 2006 (*THES*, 30 June).

Science Policy: International

After receiving hundreds of complaints from researchers the US Department of Commerce announced that it was withdrawing a proposed rule change that would have required scientists and students from "countries of concern" — including Pakistan, India, Russia and China — to be licensed before using certain pieces of laboratory equipment that could be used to strengthen a nation's military capabilities (*Nature*, 8 June). Science advocates warned the proposal would create a mountain of paperwork and restrict access to laboratories. Instead the department is forming a 12-person committee, which will include experts in academia, industry and security, to examine the issue of laboratory security.

According to a report from the Centre for European Reform, European universities are failing to meet the challenge of helping build a knowledge-based economy (CER press release, 5 June). The authors argue that Europe needs to devote more resources to research, improve its teaching record, build up centres of excellence, strengthen links between education and business, and give its universities more autonomy. The authors also examine the role that the EU can play in improving higher education, for example by establishing the European Research Council.

Twelve national science academies issued two joint statements to the leaders of the G8 countries, prior to their annual summit in Russia this month (*Nature*, 15 June). One endorses a reinvention of the world's disease surveillance system; the other calls for a major expansion of energy research to address a looming global crisis in energy supplies. The statements were announced on 14 June by the academies of the G8 countries plus Brazil, China, India and South Africa. The academies argue that the size of global efforts in both infectious diseases and energy sourcing are out of touch with the scale of the problems, and describe the current systems of national and international disease surveillance as "multi-component and uncoordinated." They also argue that the threat of avian flu should be a catalyst for investment in a more tightly coordinated global system with animal and human health experts working more closely together.

The Vatican announced that it will excommunicate from the Catholic Church any scientists who carry out research on embryonic stem cells, and any politicians who pass laws which would support the research (*Daily Telegraph*, 30 June).

Higher Education

The Government published a consultation document outlining proposals for an overhaul of the Research Assessment Exercise (RAE) with a view to cutting down on bureaucracy and increasing efficiency (DfES press release, 13 June). A working group, jointly chaired by Professor David Eastwood, Vice Chancellor of the University of East Anglia and Sir Alan Wilson, Director General for Higher Education at the DfES, was established after the Budget in March, to come forward with proposals to the existing system, which would encourage, identify and reward research excellence in higher education. The group recommends that the 2008 RAE should proceed as planned, but the panels responsible for assessing individual subjects should be able to make greater use of metrics alongside or instead of peer review where they think this is appropriate. It is intended that this will make it unnecessary to collect any additional information from institutions. The group also recommends that a shadow metrics exercise covering all subjects should be run in parallel with the RAE to allow combined, predominantly metrics-based, research assessment and funding system to be phased in from 2009/10 in England. The consultation proposals include a range of models that might be used for subjects like science, technology, engineering, maths and medicine. These models, based largely on external research income, and are already facing heavy criticism (*THES*, 16 June). Assessment and funding metrics for other subjects will be developed from a project currently being taken forward by the Higher Education Funding Council for England and the Arts and Humanities Research Council and are likely to contain an element of peer review.

In an open letter to finance directors at universities and other research organisations, David Harman, chairman of the Research Administration Convergence Group of Research Councils UK, warned that they will have to provide more justification for their claims for funding from the research councils in future (http://www.pparc.ac.uk/jes/FEC2505006.pdf). Research Councils have continued to monitor research applications submitted under Full Economic Costing (FEC) arrangements since September 2005. In the letter RCUK says information provided in the justification of resources, including investigators time has not been satisfactory. Most councils will now require an attachment to applications in the form of a one page statement justifying the resources sought. The letter also reminds research organisations that they do not need to justify estates and indirect costs, and that peer reviewers are instructed not to consider these costs. The letter states that such costs are "fundamental to sustainability" and should not be reduced or omitted to keep bids competitive.

In his first appearance before the Commons Education Select Committee, David Bell, Permanent Secretary at the DfES, warned that future spending on education would be "tight", confirming fears that universities stand to gain little from the next Comprehensive Spending Review (THES, 16 June). He stressed that there would be major future capital investment in schools but the introduction of variable fees was designed to provide support to higher education beyond Government funding.

Secondary education

Education experts have expressed concern that the Government's target for all pupils to have the opportunity to take triple science at GCSE by 2008 will not be realised, because of the shortage of science teachers with appropriate qualifications (*Res Ft*, 21 June). Concerns were also raised over the large number of teachers reaching retirement age, poor careers advice and the perception that science is difficult.

75th Anniversary Summer Conference report

Andrew McBain and **Peter Gilbert** report

Living together: polymicrobial communities

Apex International Hotel, Edinburgh, UK
Monday 3 to Thursday 6 July 2006

AS UNDERGRADUATE microbiologists in the early 1990s and late 1960s respectively, we were welcomed to the subject by opening lectures that included such delights as elegant drawings of van Leewenhoek's "animalicules", lessons on the importance of isolating and growing such organisms in pure culture and of the omnipotence of Koch's postulates.

Practical classes allowed us to experiment with flame, loop and agar to determine whether our separation technique was going to be of the "Round and Round the Garden" school or involve serious "Cross-streaking". Hot loop meeting agar surface excited all of the senses as we branded our primary rub-ups with scalding platinum and the smell of burnt media pervaded our nostrils. Decades later whilst those lessons have not been lost, we recognise that mixed communities of bacteria, rather than individual species, are the subject of environmental selection pressures, that such communities might not be comprised solely of culturable bacteria, and that it is often mixed communites of bacteria that are responsible for our continued health or disease. Fittingly, the scientific programme this year addressed all that was missing in those early experiences of microbiology dealing with mixed bacterial species as functional, polymicrobial communities. Held at the Apex International Hotel, Edinburgh, the conference was a landmark for S*f*AM, née *'The Society for Agricultural Bacteriologists'*, marking seventy five years of existence. Whilst microbiology has progressed considerably in terms of the available technologies and in popularity since 1931, the need, even in this age of electronic communication for like-minded scientists to meet face-to-face both formally and socially, is as strong as ever.

Professor **Max Sussman** opened the celebrations by delivering the Lewis B Perry Memorial Lecture. This lecture, given in the shadows of Edinburgh Castle at the Royal Museum of Scotland on the Monday evening, delved into the archives of the Society and reviewed its first 75 years. The talk, entitled, "Out of the Dusty Archive, from *SAB* to S*f*AM, the First 75 years" was an amusing and informative

overview of the origins of S*f*AM. The presentation included formal vintage photographs from summer conferences up to 60 years ago and past conference delegates that included such luminaries as Sir. Alexander Fleming, and the mysterious *Dark Lady*. Later, in true S*f*AM/*SAB* fashion, a drinks reception was held inside the museum, enabling participants to catch up with old friends and other vintage artefacts.

The first scientific session of the conference addressed the physiology and functionality of polymicrobial communities. **Miguel Cámara** gave an excellent overview of Interspecies signalling and communication. Whilst cell-cell signalling is now widely accepted, much of the current understanding has naturally focussed on cross talk between individuals of the same or closely related species. Clearly, if polymicrobial communities represent functional, evolutionary consortia then cross-talk between phylogenetically unrelated species is important. Miguel presented data on signalling between pseudomonads and staphylococci often found together in the lungs of cystic fibrosis patients. Over time, *P. aeruginosa* are normally able to displace *S. aureus* and become a dominant coloniser by a mechanism that is presumably more complex than the simple selection pressure of antibiotic therapy. Miguel explained that *P. aeruginosa* HSL and quinolone signalling facilitate inter-species communication. He demonstrated such communication to be genuine and to have profound clinical implications. This was an excellent overview with some hints about the mechanisms that may underlie signalling in true polymicrobial communities.

Soren Molin considered adaptation and evolution in a two species structured community. The work used acinetobacter and *P. putida* as a paradigm community, and examined both metabolic and physical interactions. The acinetobacter produces benzoate from benzyl alcohol that would normally enable *P. putida* to cross feed, but the benzoate degradation produces a localised anaerobiosis and associated inhibition of the pseudomonad. Over time, pseudomonas regulatory mutants emerged which were not strongly inhibited by low oxygen concentrations, and which were able to overcome the ecological barrier. Highlights of the talk were the beautiful confocal micrographs that made this a thought-provoking talk and an elegant demonstration of the use of a simple approach to better understand some very complex ecological and physiological phenomena that occur when bacteria live together in fine balance between competition and cooperation.

Andrew Whitely discussed coordination and competition in microbial communities that colonise phenol degrading bioreactors used for the bioremedioration of industrial wastewater. He used $13^{o}C$ –labelled phenol to isolate DNA from the functional phenol degraders present within highly complex polymicrobial communities. Microarray analyses of the active fractions showed that different communities had evolved separately within two different bioreactors. This work gave insight into the biodegradation process and of microbial communities as units of evolution in their own right. This theme was extended by **Neil Gray** who gave further insight into the current thinking of mechanisms that underlie the composition of microbial communities in the environment. The talk, entitled, "The Role of Niche Differentiation in the Community Assembly and Coexistence of Uncultured Bacteria from the Genus Achromatium", used the large sulphur bacterium achromatium as an example. He concluded that everything is everywhere and that the environment is generally the dictator of community composition. Whilst there is a need to study more organisms and more environments this type of seminal work is important to microbial ecology where pragmatic studies often dominate.

Holger Daims, talked of the genomics, ecophysiology and interactions of as yet uncultured nitrifying bacteria. This was another presentation that considered fundamental ecological mechanisms, using the competition between nitrospira and nitrobacter, together with Fluorescence *in situ* hybridisation (FISH) — micro autoradiography to link identity to activity. The hypothesis was that high diversity increases functional stability and that intermediate disturbances can increase nitrifier diversity in a full-scale wastewater treatment plant. Deliberate shifts in conditions could improve

functional community-stability. Chlorite dismutase is an ancient and well conserved enzyme. Holger questioned why it had evolved since the substrate, chlorite, is normally sourced from environmental pollution. Since the enzyme also has mild catalase activity it might have evolved to enable survival following, for example macrophage engulfment. This was a thought provoking presentation with broad implications.

The final talk in the opening session, "Living together while being eaten" was presented by **Carey Lambert** and discussed the bacterium, *bdellovibrio*. We learnt that these hold the Guiness record as the fastest moving bacteria and whilst normally obligate parasites, can survive as heterotrophic mutants. Carey used microarray technology to study responses to the *bdellovibrio* of a prey community of *E. coli*. The data suggest that the prey are aware of being predated, and upregulate a general stress response, but that they cannot resist or readily evolve resistance mechanisms. Carey drew attention to the potential use of bdellovibro as living antibiotics.

The afternoon session, "Influencing Polymicrobial Resistance" commenced with a talk by **Jeremy Webb** entitled 'Combating Polymicrobial Communities: Learning from Nature'. Jeremy's presentation focused on the importance of signal agonists which have evolved in marine plants such as *Delisea pulchr* as an anti-fouling strategy. It was apparent that antisignals, including the furanones can influence population dynamics in natural microbial communities, and that such compounds have potential as commercial antifouling agents. Jeremy also discussed the possible use of nitric oxide as an initiator of biofilm dispersal. The adaptation of natural biofilm modulation mechanisms for control rather than the elimination of adherent bacterial communities looks to be a promising, highly effective approach that is coincidentally environmentally friendly.

Jeffrey Hillman followed and discussed the possibility of probiotic modulation of the oral flora. This approach attempts to eradicate *Streptococcus mutans*, which is, according to many, a primary cariogenic bacterium in the human mouth. Jeffrey and his co-workers have developed a genetically engineered *S. mutans* that lacks the lactate dehydrogenase genes and synthesises a lantibiotic that confers an ecological advantage. This looks to be a promising technology. The outcomes of current clinical trials will be the acid-test. **Stig Bengmark** followed with his talk, "Using synbiotics to address major gut problems". Stig presented convincing human volunteer data for the health benefits of synbiotics (a combination of probiotic bacteria and prebiotic oligosaccharides).

Peter Gilbert discussed the effects of biocides on biofilm communities and considered the possibility that these could select for antibiotic resistance. He presented data from a range of long-term microcosm studies that suggested that although biocides select for naturally resistant communities of bacteria this is through clonal expansion of pre-existing strains rather than the resistance selection and transfer that is normally associated with antibiotic misuse.

Peter Silley gave a useful update on new regulations regarding antibiotic residues in human food. Much effort has gone into establishing a microbiologically acceptable daily intake and harmonising regulation between countries. Peter explained the rationale of the regulations their implications.

Wednesday morning concerned the gut microflora, and reminded each of us that we carry our own individualised complex microbial ecosystem, that considerably outnumbers our own cells, from cradle to grave, and that these organisms significantly influence health and disease.

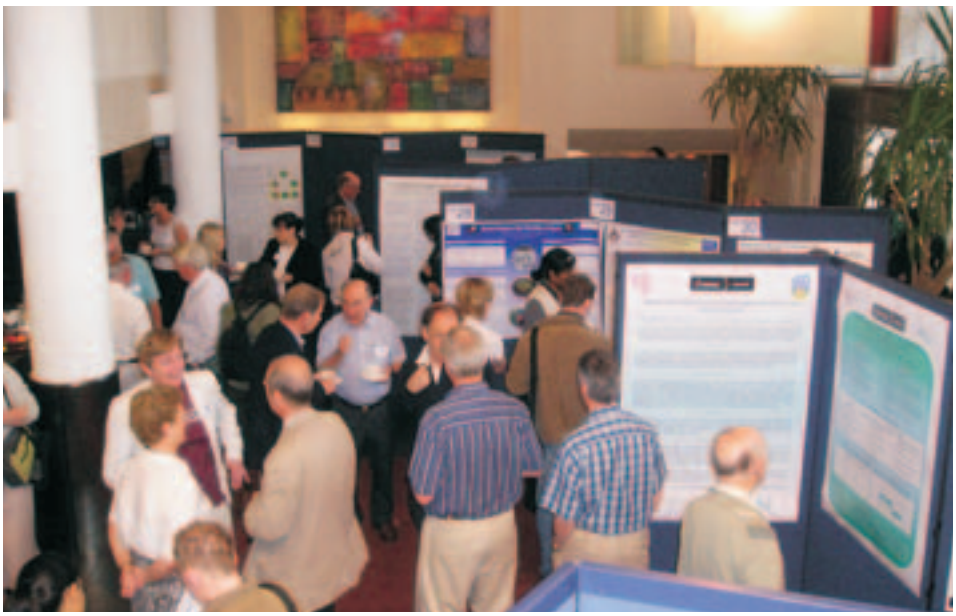
Sandra Macfarlane talked about upper and lower gut microbiology with a strong medical theme, and described a condition known as Barrett's oesophagus (BO). This involves cellular changes in the epithelium of the oesophagus, leading to adenocarcinoma, which has a very high associated mortality. Sandra has studied BO patients and identified a high incidence of unusual nitrate-reducing campylobacters in BO patients, which could have genotoxic effects through the production of direct acting carcinogens such as nitrosamines. A second study used synbiotics to produce clinically significant reductions in inflammation in a clinical trial with ulcerative colitis patients, and underpinned several of the talks given the previous day. **Harry Flint**

continued the theme with a presentation entitled, 'Understanding the effects of diet on bacterial metabolism in the large intestine'. Elucidation of the composition of the large intestinal microbiota has progressed significantly in the last few years. Harry presented data for *Roseburia* and *Eubacterium rectale*, which have been identified as major producers of butyrate, a substrate for the colonic epithelium. It was also interesting to see data on the effect of the Atkins diet. Not surprisingly, this markedly lowered butyrate production and significantly influenced microbial community composition.

Christine Edwards spoke about the with those associated with antibiotic use. She went on to show very positive data for the use of synbiotics in the elderly. Such interactions are of particular significance when one considers their impact on immune-development. **Elizabeth Furrie** spoke of microbial interactions with the gut immune system, an area of vital importance to health, but one that has received little research. We learnt of structure and function of intestinal tight junctions, acting as selective microbial barriers and of the distribution of Toll-like receptors, stimulation of which can lead to inflammatory cytokine production.

Offered papers were presented in the **Roy Sleator** (see page 13). Roy delivered an excellent lecture entitled, "Exploring a Pathogen's 'Gut Feelings': The Gastrointestinal Phase of Listeria." This talk distilled a decade's work into the molecular mechanisms underlying survival of the various harsh conditions encountered in the intestine and was an excellent and interesting presentation of world-class science.

The conference and 75th Anniversary dinner took place that evening at the Hub in the Royal Mile. It was highly enjoyable, with great food and drink, but also the chance to have informal discussions that in many a case, in true S*f*AM tradition, continued into the early hours of the



gut microflora in early life and considered the microbial succession that occurs during the colonisation of this initially sterile environment. The flora is acquired from both the mother and the environment. Christine went on to explain the differences in evolution of the colonic flora in breast versus bottle-feeding and discussed some implications of the hygiene hypothesis.

As we age, the healthy climax gut flora that we acquire from nurturing mothers and the nursery also ages with respect to its composition and metabolic activity. Since the population is ageing, this is an increasingly important area in medicine and physiology. **Emma Woodmansey** described experiments in which real-time polymerase chain reaction (PCR), fluorescence in-situ hybridisation (FISH) and culture were used to characterise age-related changes in gut flora together

afternoon, where we learnt about the use of ion-flow mass spectrometry for the rapid diagnosis of bacteraemia (**Randall Allardyce**), the effect of ozone and 'open air factor' on micrococcus biofilms (**Louise Fielding**), and substrate specificity of the human colonic flora (**Carol Leich**). A number of student offered papers followed, and demonstrated the strength and vitality of S*f*AM, the next generation. Talks were given on molecular analysis of human skin (**Michael Barton**), poultry probiotics (**Alun Carter**) and the impact of neoplastic disease on gastrointestinal flora (**Alex Glancey**). **Christopher Ibenegbu** discussed the use of solid substrates for xylanase production and **Andrew Hall**, spoke of the microbial population dynamics on vending machines.

This year's **Pierce Prize** winner was

morning.

The final sessions covered Bioremediation beginning with an excellent talk from **Geoffrey Gadd** on bacterial and fungal transformations of metals and minerals. Geoffrey discussed the influence of various environmental and microbial variables on outcomes of *in situ* microbial activities that have obvious significance in the bioremediation of mineral and metal-contaminated land. Natural microbial communities normally include protozoa and metazoan as well as bacteria and fungi so it was fitting that **Hauke Harms** spoke about the roles of protists and fungi as drivers of contaminant degradation in bioremediation consortia. Previous research has focused mainly on metabolic activities of bacteria, in some circumstances overlooking the fungi and protists that often function in concert

with bacteria. This was as an elegant demonstration of the functionality of microbial communities as they most often exist in the environment, spanning kingdoms and synergising activities. Regardless of the microbial diversity present in a bioremediation site, an important driver of the effectiveness of the process is the composition and availability of substrate.

Kirk Semple spoke about improving the methods used for determining the bioavailability of organic contaminants by comparing cyclodextrin extractability of organic contaminants with microbial degradation in laboratory and field contaminated soils. Importantly, actual degradation data correlated with extractability data. This work suggests that the extraction method can be used to predict substrate availability, thus giving a good indication of bioremediation potential.

Assessing the theoretical catabolic potential of microorganisms present in a given site can be achieved following isolation or preferably using the microcosm approach, but as **Mike Larkin** explained, accurately assessing in situ catabolism can be challenging. Mike discussed the use of molecular and conventional techniques to assess *in situ* catabolism rates. As with other areas of microbial ecology, every technique has inherent strengths and weaknesses that need to be understood in order to properly interpret derived data.

We are all aware of the potential environmental damage that can be caused by major oil spills. **Ian Head** spoke about the key species involved in the remediation of spilled oil. Apparently, naturally-derived hydrocarbons cover the oceans in a layer of oil 20 molecules thick and the microbial world has evolved considerable biodegradative capacity. Ian discussed community adaptation to the hydrocarbon influx that occurs during a marine oil spill. **Roger Pickup** finished the session with a practical look at the potential for natural attenuation in two highly contaminated aquifers, differing in both geology and composition of organic compounds. Data suggested that the potential for natural attenuation of the site could be inferred by examining multi-level samplers.

In conclusion, the conference undermined everything learnt in those early lectures that were so preoccupied with monoculture biology. It emphasised that microbiology has come of age and now engages with whole, interactive ecologies. It would be nice to be a fly on the wall at the 150th Anniversary, but then perhaps not. The *Dark Lady* will no doubt be there in spirit and will be suitably impressed.

Next year...

Microbiology of Fresh Produce
2 – 5 July 2007
Park Plaza Hotel, Cardiff, UK
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www.sfam.org.uk/sumconf.php

Andrew McBain and Peter Gilbert
University of Manchester

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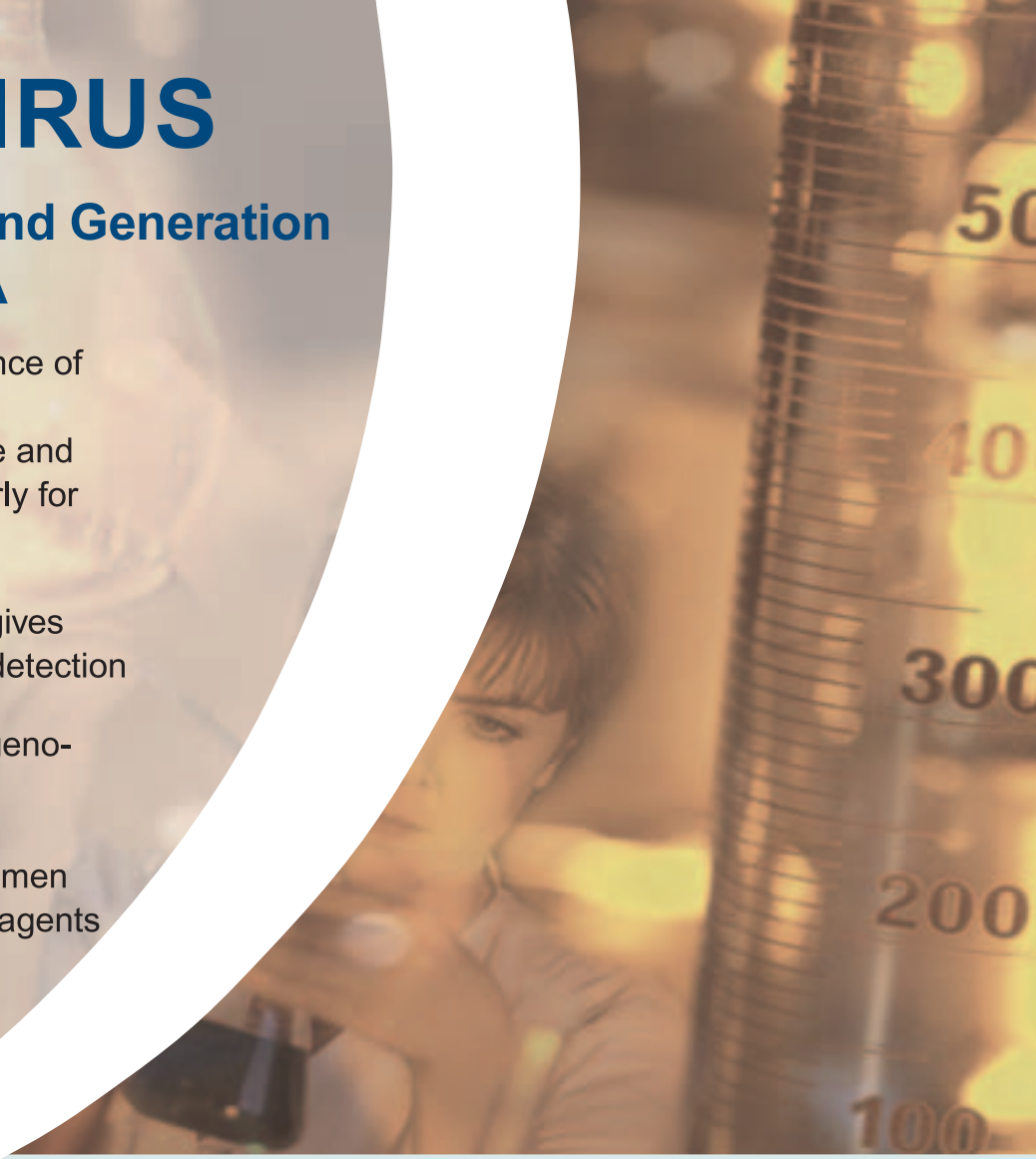

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QUADRATECH

a one day meeting on

Food and Health:

The Royal Society, Carlton House Terrace, London
Thursday 11 January 2007

CPD ACCREDITATION

Six credits awarded. Further credits have been applied for

Including:

The Denver Russell Memorial Lecture:
'Naturally Occurring Microorganisms and their Resistance to Physical and Chemical Agents' given *by Martin Favero, Advanced Sterilisation Products, Johnson & Johnson, USA.*

A one day meeting with sessions on:

- Hospital Acquired Infections and
- Food Microbiology (in collaboration with the Chartered Institute for Environmental Health)

Topics to be covered will include:

Hospital Acquired Infections:
- the government perspective;
- infection control team's perspective;
- clinical microbiologist's perspective;
- *C.difficile*
- MRSA
- *Acinetobacter*

Simmering Questions in Microbiological Food Safety
- Is there a scientific basis for safe eating practice?
- How and why do enteropathogens make you ill?
- Norovirus and Hepatitus A — an important cause of foodborne illness?
- How do foodborne pathogens emerge?

Please note that the meeting programme was correct at the time of going to press but may be subject to change.

For the latest information, please visit us online at:
www.sfam.org.uk

Programme

10.00-10.30 **Arrival/Coffee/ Registration**

10.30-11.15 **The Denver Russell Memorial Lecture 'Naturally Occurring Microorganisms and their Resistance to Physical and Chemical Agents'**
Martin Favero, Advanced Sterilisation Products, Johnson & Johnson, USA.

11.15- 11.45 **What role can Government play in controlling hospital acquired infection?**
Prof Brian Duerden

11.45-12.15 **Food poisoning – what are the real risks?**
Bob Adak, CDSC, Health Protection Agency.

12.15-13.15 **Lunch**

Afternoon: two parallel sessions of 5 talks

Session A. Hospital Acquired Infections

13.15-13.45 **'Infection Control Teams - friend or foe?'**
Martin Kiernan, Southport and Ormskirk NHS Trust

13.45-14.15 **Hospital acquired infections: a clinical microbiologist's Perspective.**
Kathleen Bamford (Hammersmith Hospital)

14.15-14.35 **Tea and Coffee**

14.35–15.05 ***Clostridium difficile:* current situation and prospects for the future.**
Jon Brazier, NPHS microbiology, Cardiff

15.05–15.35 ***Acinetobacter* outbreaks: how long before they are unmanageable?**
Kevin Towner , Nottingham University Hospitals NHS Trust

15.35–16.05 **MRSA: do we have the situation under control?**
Barry Cookson, Health Protection Agency

Session B. Simmering issues in food safety

13.15-13.45 **The Foodborne Disease Risk Matrix: have we got it right about the public health risk of foodborne disease?**
Joyce Brown & colleague, Operational Research, ASSRPD, Food Standards Agency

13.45-14.15 **Transfer and persistence of *Campylobacter* and *Salmonella* in food preparation environments**
Dr Frieda Jorgensen, University of Bristol

14.15-14.35 **Tea and Coffee**

14.35-15.05 **Norovirus and Hepatitis A – important causes of foodborne illness?**
Mike Carter, School of Biomedical and Molecular Sciences, University of Surrey

15.05-15.35 **Microbiological criteria for foods**
Linden Jack, Microbiological Safety Division, FSA

15.35-16.05 **Emerging problems: new foods or new pathogens?**
Jim McLauchlin, Food Safety Microbiology Laboratory Centre for Infections, HPA

16.10 **Meeting closes**

Please note that the meeting programme was correct at the time of going to press but may be subject to change.

For the latest information, please visit us online at:
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BOOKING FORM and INVOICE

SFAM JANUARY MEETING THURSDAY 11 JANUARY 2007

Food and Health

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If you are a Student (S), Honorary (H), Associate (A), or Retired Member (R), please enter the applicable letter (S, H, A or R) into the appropriate FEES BOX below:

FEES

Whole Conference Rate: inc. of registration fee, coffee breaks and lunch. **Please tick applicable box:**	**Full Members**	**Student, Honorary, Associate & Retired Members**	**Student Non - Members**	**Non - Members**	**CIEH/IBMS Members**
	£50.00 ☐	£30.00 ☐	£60.00 ☐	£100.00 ☐	£75.00 ☐

YOUR INTERESTS

Please indicate which of the two parallel sessions of 5 talks you wish to attend. Each session begins immediately after lunch at 13.15

Session A: Hospital Acquired Infections ☐ **Session B:** Simmering issues in food safety ☐

YOUR COSTS

Charges - please tick the applicable box(es)	Amount
☐ **Whole Conference Rate:**	£
☐ **LATE BOOKING FEE** Payable for all bookings made after Friday 22 December 2006	**£30.00**
TOTAL AMOUNT REMITTED:	£

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Please indicate any special dietary or other requirements (such as disabled access):_____

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YOUR PAYMENT

● **For all participants: The Society DOES NOT INVOICE for conference fees. Please treat your completed booking form as an invoice. Cheques must be in £ STERLING ONLY and made payable to 'The Society for Applied Microbiology'. Foreign cheques/ drafts MUST be negotiable for the full amount due. Please note that AMERICAN EXPRESS and DINERS CARDS are NOT ACCEPTED.** However the following credit and debit cards are acceptable: VISA, Mastercard, Eurocard, Delta, Electron, JCB, Maestro and Solo.

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Why do you wish to attend this meeting?

Please give your reasons:_____

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Your signature:_____ **Date:**_____

(If you need more space for your answer please continue on a separate sheet)

Will you be contributing to the meeting by offering a Poster or presenting a paper? ☐ **Offering a Poster** ☐ **Presenting a Paper**

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This section **MUST** be completed by your Supervisior or Tutor. Applications which are not supported by your Supervisor will be automatically rejected. **Please give your reasons why the applicant should receive a studentship:**

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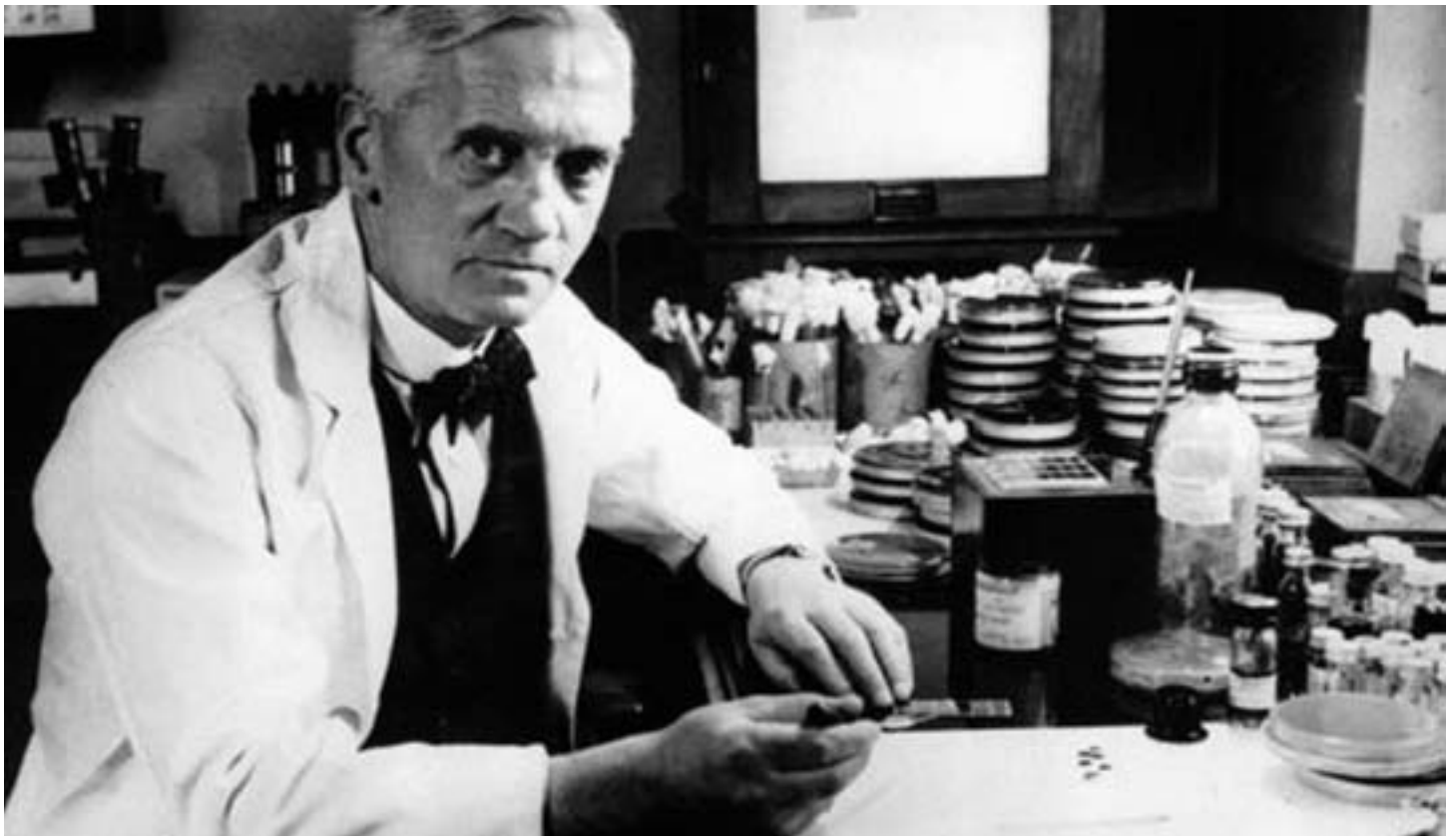
Milton Wainwright describes a day examining Alexander Fleming's preserved penicillin dish at the British Library, and some strange goings on in a leading archive

PENICILLIN



A Day with Fleming's Famous Petri Dish and the Case of the Missing Page

THE PETRI DISH that led Alexander Fleming to penicillin is one of the most famous icons in science.

The plate has languished in a store room in the British Library in London since it was donated (on Fleming's death) by his second wife, where it is kept together with an amazing replica, which turns out to be exactly that — an artist's model made of wax and paint.

Although the Fleming plate looks simple (Fig.1) the phenomenon it contains is surprisingly complex and has often been misunderstood (Wainwright, 1988, 2002, 2005). For some twenty or so years workers in my laboratory have tried, intermittently, and without success, to replicate the famous plate. We have used a meat-extract medium commonly used in the 1920s and Fleming's original isolate as the fungal inoculum. We have also altered the order in which the fungus and bacterium are grown, and have exposed the inoculated plates to a wide variety of variations in temperature and period of incubation. Despite all this effort, we simply cannot replicate the famous plate. Fleming did not deposit his culture of *Staphylococcus* (which he often isolated from pus taken directly from patients) in a culture collection, so we have had to use a stock culture; our lack of success may therefore simply result from this culture being resistant to lysis. So, while it is easy to show that Fleming's fungus kills *Staphylococcus*, we, and others, have found it impossible to repeat the bacterial lysis occurring on Fleming's plate.

This leads to an inescapable conclusion; could it be that the fungus on the famous plate is not the penicillin-producing fungus (*P. notatum*)? Maybe the fungus on the plate produced bacterial lysis, but not penicillin. Perhaps Fleming later inadvertently swapped this mould for one that produced penicillin, but not lysis. Fleming's laboratory notebooks support this possibility since he refers to the fact that he checked a range of mould cultures and found a *Penicillium* isolate that was as good as the

original at killing bacteria. This culture-collection isolate would then have come down to us as *P. notatum-chrysogenum*, the penicillin-producing mould. This theory suggests that the bacteriolytic fungus, originally isolated by Fleming, was not *P. notatum*, or even any species of *Penicillium*. If correct, this theory would explain why we have been unable to induce the bacteriolysis that caught Fleming's attention.

Another possibility is that the fungus, seen on the original plate, was *Penicillium rubrum*, the name C.J. La Touché, the mycologist at St Mary's, originally gave to Fleming's mould. La Touché later apologised to Fleming for this miss-identification and has been criticised for this apparent blunder ever since (Wainwright, 1993). But maybe Fleming's original mould was *P. rubrum*, and it was this mould that produced the lysis (the obvious way to prove this would be to see if *P. rubrum*, lyses *Staphylococcus* without producing penicillin).

Any suggestion that the lysis–inducing fungus on Fleming's plate was not *P. notatum*, or that two fungi were present on the plate, does however, run into a number of problems, notably in relation to the sequence of events given in Fleming's notebooks. Might there however, be a way of proving this suggestion? The obvious answer is yes, by examining and trying to identify the fungus *in situ* on the original, preserved dish.

Initially, I thought that the authorities of the British Library might let me see the iconic dish, but never examine it with a microscope. However, after some discussions, and after I had met all of their strict requirements, regarding the safe handling of the dish, I was given permission to take an inverted microscope into the subterranean world of the British Library and examine the famous Fleming plate.

Any examination of Fleming's original dish is beset with problems. Firstly, the dish must remain sealed and no samples can be removed from it. It must also be kept upright and obviously has to be handled with extreme care. This means that the mould colony cannot be viewed from above, using a microscope, in the normal fashion. The only alternative was to use an inverted microscope and examine the colony from below, through the thick glass of the petri dish. Fortunately, after more than 75 years, the agar in the dish has dried to a brown sliver, thin enough to allow the mould to be viewed through the bottom of the dish. However, the x10 objective was the only one I could use, to ensure that the petri dish was not damaged whilst racking up and down; earlier tests, using fungi growing in glass petri dishes, showed that this was just sufficient to see the spore-producing bodies of most fungal species, including species of *Penicillium*.

Fleming preserved the contents of the famous dish using formalin vapour, and its contents have aged for 75 years, so it seemed unlikely that anything of interest would remain to enable the mould to be identified. Nevertheless,

Figure 1. *What appear to be two types of fungi on Fleming's plate; fine hyphal filaments can be seen and what appears to be a single, thick, pigmented hypha (x10 objective).*

helped by British Library staff (Dr Jeremy John, Dr Barry Knight and Laura Fielder); in December of 2005, I undertook the first *in situ* investigation of Fleming's mould in over 75 years.

Surprisingly, the whole process went remarkably smoothly and the underside of the mould, and adjacent bacterial colonies, could be clearly seen using the inverted microscope, and photographed. So, what did we find? Firstly, fungal hyphae of about the size typical of Hyphomycetes (e.g. species of *Penicillium*) could be clearly seen (Fig 1). There were however, no signs of spores or spore-bearing structures, and certainly no phialides or spore chains, typical of *Penicillium*. There was however, what appeared to be the pigmented, branching hyphae of another fungus (Fig 2). This fitted the hypothesis that two fungi were present on the plate; one, probably a species of *Penicillium*, and another that may, perhaps, have produced the all-important bacterial lysis. This second "fungus" looked like a pigmented, wood-rotting *Basidiomycte*; but how could such a fungus have found its way onto Fleming's plate? Then, I wondered what other fungus-like material might have found its way into the agar on Fleming's plate. A piece of cotton thread from a lab coat looked similar to the "other fungus" when viewed under the microscope, but certainly not identical; then another possibility came to mind. I took another common laboratory material and examined it, against the background of a piece of dried agar, using the identical magnification conditions used to examine the famous plate. The "other fungus", disappointingly, turned out to be nothing more than cotton wool! Fleming frequently used cotton wool to plug his tubes and flasks. Presumably, as he poured the medium into the famous plate, traces of cotton wool were transferred from the neck of his flask and into the dish, where they settled to the bottom of the agar; here they have remained for over seventy five years, ready in waiting to trick the unwary.

So what did the day achieve? Well, we now know that a fungus is still in evidence on Fleming's plate. It is the same thickness as a *Penicillium* species, although frustratingly, there are no signs of phialides and spores.

Perhaps the fungus was a *Penicillium*, but was too immature to have sporulated; more probably perhaps, any typical *Penicillium* spore-bearing structures may have decayed over the 75 years of the plate's existence. Of course, the possibility still exists that the fungus on Fleming's plate is not a species of *Penicillium*. The obvious way to confirm this would be to remove a sample and perform some kind of DNA/RNA based analysis. Currently, permission for such an invasive approach seems unlikely to be given; hopefully in the future, non-invasive means of such analysis might be available and used to identify the fungus. For the moment, however, the enigma of the fungus on Fleming's famous plate remains.

There was however, one last productive outcome of the visit. Laura Fielder, who is currently indexing Fleming's notebooks, was able to provide me with details of the medium used by Fleming. This medium is markedly different from the meat medium that was standard in 1928, and which we have been using to replicate the famous plate. Armed with this new recipe, we can again repeat the experiments in the hope of finding out if it was *P. notatum*, or some other fungus that caused the staphylococcal lysis that so excited Alexander Fleming on that fateful September day in 1928.

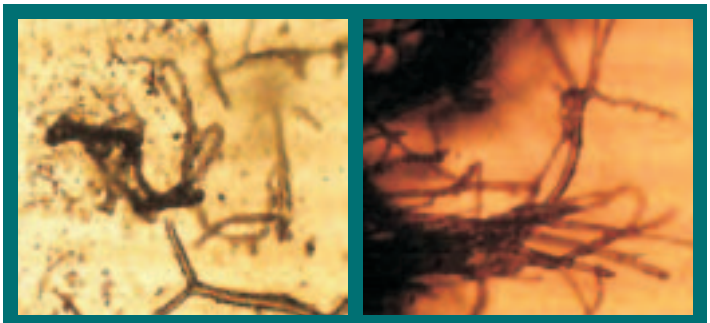


Figure 2. *A group of what appear to be thick, pigmented fungal hyphae on Flemings plate (left) and (right) cotton wool against a background of dried agar at the same magnification (x10 objective.)*

Penicillin-The Case of the Missing Page

The afternoon of my visit to London, was devoted to a search of the Archives of the Medical Research Council to see if Fleming ever applied for grants to support his early penicillin work. Despite the fact that Fleming continued working with penicillin right up until Florey and Chain took an interest (Wainwright, 2002), he has been criticised for not actively pursuing its therapeutic potential. For example, had he obtained grants to employ a competent chemist, then the all-important purification step might have been achieved in the early, rather than late 1930s. Until now, there has been no suggestion that Fleming, or his collaborators, ever applied for grants to pursue the penicillin work. However, when I investigated the records of the Medical Research Council grant applications, for 1930, I found an intriguing reference to a possible penicillin grant application. In the index of the volume of grant requests for 1930 we find reference to the following grant application:

16th May, 1930.
Dr S. Craddock.
St Mary's.
Application Denied.

However, when I turned to the page in question, I found that it was missing! Is this fact of no consequence, or are darker forces at work here? The S. (Stuart, R.) Craddock in question was a young Research Scholar who assisted Fleming in early work on penicillin. Together with another assistant, Frederick Ridley, Craddock did sterling work during 1929, discovering as much as he could about the physical-chemical properties of penicillin, with a view to purifying it. Craddock is often referred to in the penicillin story because Fleming used some penicillin-rich filtrate to cure him of a nose infection; the young man also drank mould filtrate, describing it as tasting like Stilton cheese. Later in 1930, Craddock left St Mary's to take up a post at the Wellcome Research Laboratories at Beckenham.

There seems little doubt that had Stuart Craddock applied to the MRC for a grant in 1929-30 it would have been for funds to continue this penicillin work. Of course it could be argued that the grant was not related to work on penicillin. In that case, why was the page, detailing the grant, removed? Maybe it is purely coincidental that this, of all pages, was excised. However, there remains the obvious possibility that, once penicillin had become famous, someone removed the relevant page to save embarrassment to themselves, or the MRC.

The perpetrator presumably felt guilty because had the grant been awarded, penicillin might have saved countless lives during the early 1930s. However, it is unlikely that large amounts of penicillin could have been produced during the early 1930s. Indeed, had it been made available, the Axis powers would have shared its benefits during the Second World War.

In hindsight then, by denying the grant the people involved almost certainly helped the Allies win the War. Whoever removed the page should perhaps have relaxed and left the record as it stood!

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Milton Wainwright

Eradicating Polio

Bill Newson reviews Polio vaccination

POLIO vaccination must be one of the biggest micro-biological breakthroughs of the Society's lifetime — 40,000 cases of paralytic polio occurred in the US as late as 1952.

Epidemics can be viewed as a downside of hygiene. Originally, babies were infected when protected by their mothers' antibodies. However, improved sanitation prevented infection and a population of susceptible children emerged. I had febrile illness during an epidemic in the 1940s and our school was closed after two boys died. It was a frightening time.

The most famous sufferer was Franklin D Roosevelt. When he became President he initiated the 'March of Dimes' charity and raised so much money that a grateful Congress put his head on the dime coin. The money was administered by the 'National Foundation for Infantile Paralysis' (NFIP) – used mainly for rehabilitation, but also for research.

Karl Lansteiner (Nobel Prize for blood groups) isolated the virus in monkeys in 1908. Attempts at vaccine production in the 1930s by 'attenuating' the virus, or inactivating it with formalin, failed. This meant that so many people died, the word 'vaccination' became a dirty word. However, Enders' team (more Nobel prizes) at Boston Childrens Hospital grew the virus in tissue culture in 1949 – almost by accident while testing their tissue culture system with some poliovirus from the fridge. The third Nobel prize-winner involved was Macfarlane Burnet who showed that there were three different antigenic types of virus that did not cross-protect. With this finding and the *in vitro* culture methods available, the pace of research accelerated. Jonas Salk used strictly controlled formalin inactivation to produce a product that was immunogenic, but free from viable virus. Even before he was ready the NFIP mounted probably the largest controlled clinical trial ever, involving two million school children, the 'polio pioneers' and their parents. When the results (60-80% protection) were announced, Salk became famous and he was invited to the White House. When asked about the patent he said 'it belongs to the people – you might was well patent the sun'. Sadly, two weeks later the 'cutter' vaccine, which had been improperly prepared killed eleven children. As a reflection of the time - Shades of the 1930s – it still contained live virus. Nonetheless the killed virus became a great success.

Albert Sabin and Hilary Koprowski were experienced virologists (they regarded Salk as a rather 'pushy' youngster) who were aiming at producing an attenuated virus vaccine. NFIP no longer had funds for trials in the US, where the Salk vaccine was working well so Sabin tried his vaccines in Russia and Koprowski in the Congo. Both vaccines worked but it was Sabins that was adopted by WHO as it was slightly safer and only required a drop on a sugar lump.

Polio re-appeared in my life in 1960 when I demonstrated the 'Van Slyke' machine to measure blood bicarbonate in the Army Laboratory in Singapore. In the excitement I swallowed some blood from a lady who then died of polio. I had a very worrying few weeks. Then in 1962 I had the unenviable job of autopsying two polio victims during an epidemic in Hong Kong. I looked like a moon man. Perhaps this explains my later role as Chairman of the BSI Committee on Microbiological Safety cabinets.

The Sabin vaccine was amazingly efficient. Everyone in Hong Kong was vaccinated in early 1963 and the epidemic ceased. Only 40 cases have occurred in the UK in the last twenty years, either vaccine-associated or imported. Salk has the last laugh, because his vaccine is safer, gives long lasting immunity and is now being used again here.

However, the story is not quite ended – polio is still around in Africa. A persuasive theory suggested that HIV spread from chimpanzees to humans via the vaccine programme in the Congo. This has been discounted by the CDC among others, and todays vaccines are definitely free from HIV. Sadly however Northern Nigeria banned vaccination – 'a Western plot to make us impotent, or worse still – give us AIDS'. WHO are making strenuous efforts to target the last few affected areas and declare the world free from polio – something that would never have been expected by our founders.

Dr S W B Newson
Cambridge

Birth of BSc

Dr Murial Rhodes-Roberts recalls the first honours degree in microbiology

Reading University

THERE WERE MANY PEAKS OF microbiological significance during the past 75 years, but I would nominate the very first Honours degree courses in Microbiology in the UK.

These were inaugurated at the University of Reading in 1950 with my appointment as an assistant lecturer in Microbiology, a new word and the butt of many jokes. Six months later, Professor B.C.J.G. Knight was appointed to a unique chair of Microbiology and together with Dr P.M. Frances Shattock (with research experience at the nearby National Insitute for Research in Dairying, NIRD, at Shinfield), and Dr Audrey Jones (with a Reading degree in Dairying), we designed the new courses using a combination of our particular skills, in the absence of any relevant reference syllabuses from elsewhere. I myself had an honours degree in Botany with Accessory Zoology, a prelude to the one-year postgraduate diploma course in Bacteriology, which had been in existence in Reading for several years thanks to the enterprise of the late Dr Tal Richards. Our new Honours courses evolved from an extension of this Dip. Bact. Course, together with the undergraduate courses in bacteriology and mycology given to the dairying students.

I well remember staff meetings to discuss the choice, content and length of the courses, e.g. how much mycology, phycology, protozoology and virology should be included. Fortunately a two year Biochemistry course and a one year Chemistry course were provided by other departments. Our meetings were often memorably fiery; Professor Knight was no taxonomist, but some of us were anxious to widen the expertise of the *E.Coli* biochemists.

Flogging out possible practicals was an extremely time-consuming element. None of us however anticipated the ultimate difficulties of determining standards for degree categories, or indeed finding suitable external examiners. In retrospect we were probably stingy assessors, but one was bedeviled by the unknown, *viz:* what attainments might one subsequently encounter in future students? It was indeed a challenging time. As the only biologist I devised courses in mycology, phycology, protozoology as well as coping with the 'Exotica', as Prof. Knight dismissively clumped the actinomycetes, sulphur bacteria, stalked, sheathed and slime bacteria *et al.*

Meaningful practicals with these organisms were not easy, but I remember acquiring a whole ox head on a dustbin lid to isolate the lumpy jaw *Actinomycete*, and cycling to the NIRD with a dipper and vacuum flask to obtain a rumen sample from their fistulated cow. We were all transfixed by the unbelievable millions of mobile rumen protozoa; Prof Knight even emerged from his office tasks to join in this visual excitement, and the Flatters and Garnett prepared slides of amoebae, paramecia *et al*, were consigned to the museum.

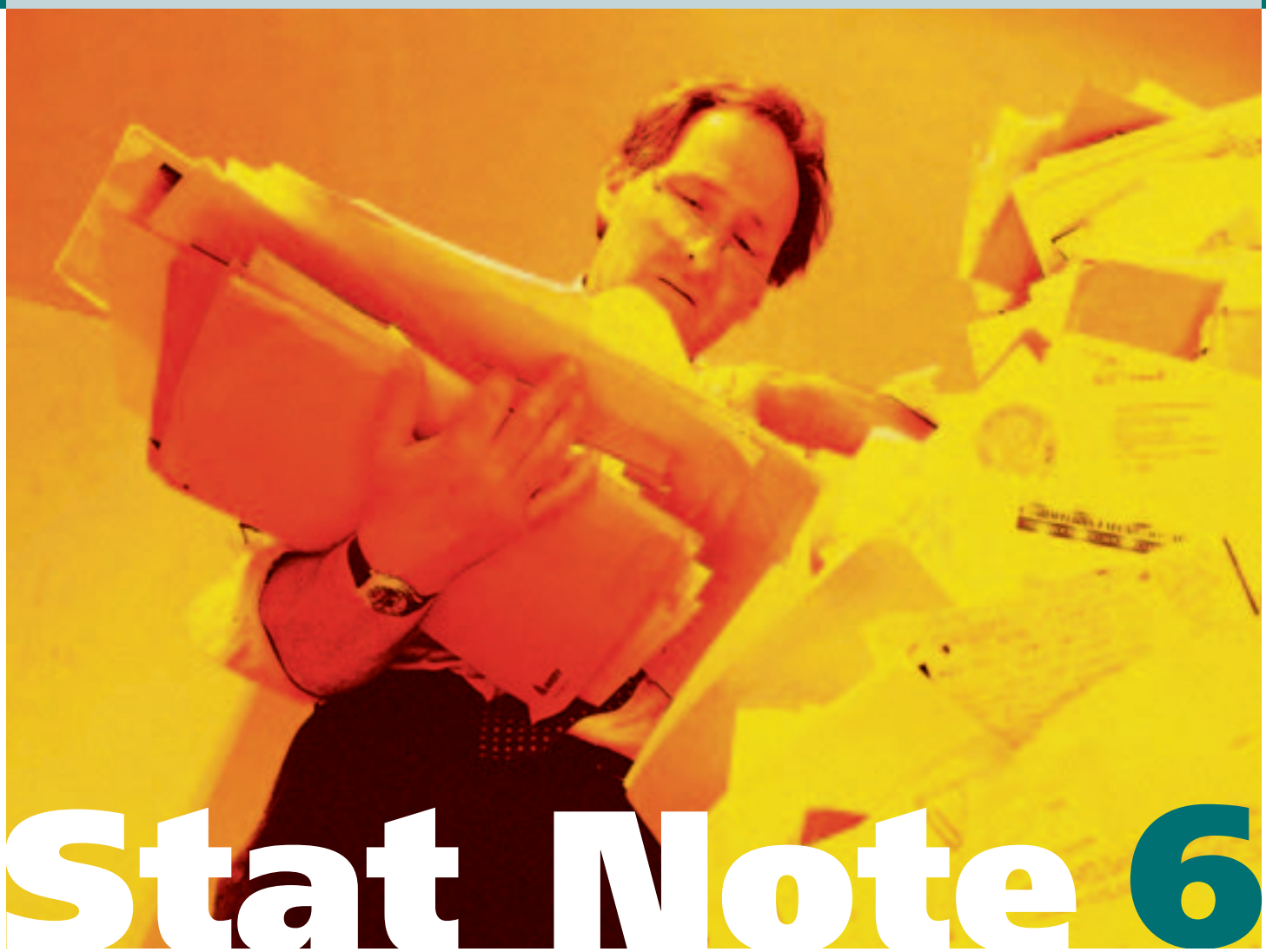
I was also dragooned into 'doing' the *Clostridium* practicals. This involved a 6.00 am start to generate sufficient hydrogen in a Kipps apparatus for a few desicators as 'anaerobic jars'.

Therefore my second nomination as a significant event is the availability of affordable anaerobic jars and gas cylinders with simple devices to manipulate gas mixtures, thus revealing myriads of new micro-organisms with unimagined specific gaseous requirements. In retrospect the above experience perhaps influenced my decision to opt for the aerobic pseudomonads as a research topic!

I have numerous memories of those truly pioneering days in Reading, training enterprising students, many of whom today are as 'leaders in their field' of microbiological specialties.

The passing of my close colleagues, *viz;* Dr Tal Richards, Prof. B.C.J.G. Knight, and Dr Frances Shattock and our unflappable technician Mr W Pendery inevitably invoke sad memories, but I am grateful that I shared so much with them during 1950-61.

Muriel E. Rhodes-Roberts



Stat Note 6

In the sixth of a series of articles about statistics for biologists, **Anthony Hilton** and **Richard Armstrong** discuss:

post hoc ANOVA tests

IN A PREVIOUS article in *Microbiologist* (Armstrong & Hilton, 2004), we described the application of analysis of variance (ANOVA) to various experimental designs in Microbiology.

ANOVA is a data analysis method of great elegance, utility and flexibility and is the most effective method available for analysing experimental data in which several treatments or factors are represented. In the simplest case of a one-way ANOVA, in which the experiment consists of a number of independent treatments or groups, the first stage of the analysis is to carry out a variance ratio test (F-test) to determine whether all group means are the same. If treatment groups are few, say three or four, a non-significant F-test would indicate no meaningful differences among the means and no further analysis would be required. However, a significant F-test suggests real differences among the treatment means and the next stage of the analysis would involve a more detailed examination of these differences.

There are various options available depending on the objectives of the experiment. Specific comparisons may have been planned before the experiment was carried out, decided after the data have been collected, or comparisons between all possible combinations of the treatment means may be envisaged. This Statnote provides a more detailed discussion of these questions than was possible in our original article (Armstrong & Hilton, 2004).

The scenario

An experiment was designed to investigate the efficacy of two commercial plasmid-prep kits compared to a standard alkaline-SDS lysis protocol. A 5 ml overnight recombinant *E. coli* culture containing a high copy plasmid was harvested by centrifugation and the pellet resuspended in 100 μl of lysis buffer. Plasmid DNA was subsequently extracted from the cell suspension using a standard SDS-lysis protocol or a commercially available kit following the manufacturer's instructions. In total, ten independent cultures were processed using each of the three extraction methods under investigation. Following extraction the purified plasmid DNA pellet was dissolved in 50 μl of water and the concentration determined spectrophotometrically at 260 nm. The yield of plasmid DNA using each preparation method is detailed in Table 1.

Planned comparisons between the means

The experiment may have been designed to test specific ('planned') differences between the treatment means. Planned comparisons are hypotheses specified *before* the analysis commences whereas *'post-hoc'* tests are for further explanation after a significant effect has been found.

How are the tests done?

The basic strategy for planned comparisons is to divide up the treatments sums

of squares among the various hypotheses, called 'contrasts', which are then analysed separately either by an F-test or a t-test. If this procedure was carried out for all possible comparisons between the means, then the sums of squares for all contrasts would be greater than the treatments sums of squares as a whole since the comparisons overlap and based on the same sources of variance. Strictly, such comparisons cannot be made independently of each other. As a result, comparisons must be constructed so that they are not overlapping, i.e., they have to be *'orthogonal.'* Essentially, orthogonal comparisons have no common variance and their coefficients sum to zero. Hence, the sums of squares can be calculated for each contrast and a test of significance made on each. The number of possible contrasts is equivalent to the number of degrees of freedom (DF) of the treatment groups in the experiment. Hence, if an experiment employs three groups, as in our scenario, then two contrasts can be validly tested. This approach has two advantages. First, there is no problem as to the validity of the individual comparisons, a problem present to some extent with all conventional *post-hoc* tests. Second, the comparisons provide direct tests of the hypotheses of interest. Most commercially available software will allow for valid contrasts to be tested for a range of experimental designs.

An illustrative example

An example of this approach is shown in Table 1. In our scenario, we compared two commercial plasmid-prep kits with a standard alkaline-SDS lysis protocol. Two valid contrasts are possible using this experimental design. First, a comparison of the mean of the two-commercial prep kits with the standard method, *viz.*, do the commercial kits on average improve plasmid yield (contrast 1)? Second, a comparison of the two commercial prep kits themselves (contrast 2). Contrast 1 is highly significant ($t = 5.11$, $P < 0.001$) indicating the superiority of the commercial kits over the standard method but contrast 2 is not significant ($t = 0.54$, $P > 0.05$) showing that the two commercial kits did not differ in their efficacy.

Table 1. Comparison of two commercial plasmid-prep kits (plasmid yield mg) compared to a standard alkaline-SDS lysis protocol using planned comparisons and *post-hoc* tests.

Culture	Alkaline-SDS lysis	Commercial kit A	Commercial kit B
1	1.7	3.1	4.7
2	2	2.2	3.5
3	1.2	2.8	2.6
4	0.5	4.8	4.3
5	0.9	5	3.8
6	1	1.9	4.5
7	1.4	2	4
8	2.7	3.6	1.9
9	3.2	4.1	2.8
10	0.7	4.7	4.6

ANOVA

Source of variation	Sums of squares	DF	Mean square	F
Treatments	27.3807	2	13.690	13.28
(P<0.001)				
Error	27.998	27	1.0370	

Planned comparisons

Contrast	Estimate	Std. error (SE)	't'
1. Std. v (Kit A + Kit B)/2	4.03	0.79	5.109
(P<0.001)			
2.Kit A v Kit B)	0.25	0.45	0.54
(P>0.05)			

***Post-hoc* tests**

Test	Std. v Kit A	Std. v Kit B	Kit A v Kit B
Fisher PLSD	P<0.001	P<0.001	P<0.05
Tukey-Kramer HSD	P<0.001	P<0.001	P<0.05
SNK	P<0.001	P<0.001	P<0.05
Scheffé	P<0.001	P<0.001	P<0.05

Post-hoc tests

There may be circumstances in which tests between the treatment means are carried out *post hoc* or where multiple comparisons between the treatment means may be required. A variety of methods exist for making post-hoc tests. The most common tests included in commercially available statistical software are listed in Table 2 (Abacus Concepts, 1993; Armstrong *et al.*, 2001). These tests determine the critical differences that have to be exceeded by a pair of treatment means to be significant. However, the individual tests vary in how effectively they address a particular statistical problem and their sensitivity to violations of the assumptions of ANOVA. The most critical problem is the possibility of making a Type 1 error, i.e., rejecting the null hypothesis when it is true. By contrast, a Type 2 error is accepting the null hypothesis when a real difference is present. The *post-hoc* tests listed in Table 2 give varying degrees of protection against making a Type 1 error.

Discussion of the tests

Fisher's protected least significant difference (Fisher's PLSD) is the most 'liberal' of the methods discussed and therefore the most likely to result in a Type 1 error. All possible pairwise comparisons are evaluated and the method uses Student's 't' to determine the critical value to be exceeded for any pair of means based on the maximum number of steps between the smallest and largest mean. The Tukey-Kramer honestly significant difference (Tukey-Kramer HSD) is similar to the Fisher PLSD but is less liable to result in a Type 1 error. In addition, the method uses the more conservative 'Studentised range' rather than Student's 't' to determine a single critical value that all comparisons must exceed for significance. This method can be used for experiments that have equal numbers of observations (N) in each group or in cases where 'N' varies significantly between groups. However, with modest variations in N, the Spjotvoll-Stoline modification of the above method can be used. The Student-Newman-Keuls (SNK) method makes all pairwise comparisons of the means ordered from the smallest to the largest using a stepwise procedure. First, the means furthest apart, i.e., 'a' steps apart in the range, are tested. If this mean difference is significant, the means a-2, a-3, etc., steps apart are tested

until a test produces a non-significant mean difference, after which the analysis is terminated. The SNK test is more liable to make a Type 2 rather than a Type 1 error.

By contrast, the Tukey compromise method employs the average of the HSD and SNK critical values. Duncan's multiple range test is very similar to the SNK method, but is more liberal than SNK, the probability of making a Type 1 error increasing with the number of means analysed. One of the most popular methods is Scheffé's 'S' test. This method makes all pairwise comparisons between the means and is a very robust procedure to violations of the assumptions associated with ANOVA (Armstrong & Hilton, 2004). It is also the most conservative of the methods discussed giving maximum protection against making a Type 1 error. The Games-Howell method is one of the most robust of the newer methods. It can be used in circumstances where 'N' varies between groups, with heterogeneous variances (see Statnote 5), and when normality cannot be assumed. This method defines a different critical value for each pairwise comparison and this is determined by the variances and numbers of observations in each group under comparison. Dunnett's test is used when several treatment means are each compared to a control mean. Equal or unequal 'N' can be analysed and the method is not sensitive to heterogenous variances. An alternative to this test is the Bonferroni/Dunn method that can also be employed to test multiple comparisons between treatment means especially when a large number of treatments is present.

Which test to use?

In many circumstances, different *post-hoc* tests may lead to the same conclusions and which of the above tests is actually used is often a matter of fashion or personal taste. However, each test addresses the statistical problems in a unique way. A good way of deciding which test to use is to consider the purpose of the experimental investigation. If the purpose is to decide which of a group of treatments is likely to have an effect, then it is better to use a more liberal test such as Fisher's PLSD. In this scenario it is better not to miss a possible effect. By contrast, if the objective is to be as certain as possible that a particular treatment does have an effect then a more conservative test such as the Scheffé's test would be appropriate. Tukey's HSD and the compromise method fall between the two extremes and the Student-Newman-Keuls (SNK) method is also a good choice. We would also recommend the use of Dunnett's method when several treatments are being compared with a control mean. However, none of these methods is an effective substitute for an experiment designed specifically to make planned comparisons between the treatment means.

An illustrative example

As an example, we analysed data from our scenario using four different post-hoc tests, viz., Fishers PLSD, Tukey-Kramer HSD, the SNK procedure and by Scheffé's test (Table 1). In this example, the results are clear cut and all four tests lead to the same conclusion, i.e., both commercial kits are superior to the standard method but there is no difference between commercial kits A and B thus confirming the results of the planned comparisons.

Conclusion

If data are analysed using ANOVA, and a significant F value obtained, a more detailed analysis of the differences between the treatment means will be required. The best option is to plan specific comparisons among the treatment means before the experiment is carried out and test them using 'contrasts'. In some circumstances, *post-hoc* tests may be necessary and experimenters should think carefully which of the many tests available should be used. Different tests can lead to different conclusions and careful consideration as to the appropriate test should be given in each circumstance.

Table 2. Features of the most commonly used post-hoc tests (modified from Abacus Concepts 1993 and Armstrong *et al*., 2000)

Method	Equal N F	Normality	Use	Error control	Protection
Fisher PLSD	Yes	Yes	Yes	All	Most sensitive to Type 1
Tukey-Kramer HSD	No	Yes	Yes	All	Less sensitive to Type 1 than Fisher PLSD
Spjotvoll-Stoline	No	Yes	Yes	All	As Tukey-Kramer
Student-Newman Keuls (SNK)	Yes	Yes	Yes	All	Sensitive to Type 2
Tukey-Compromise	No	Yes	Yes	All	Average of Tukey and SNK
Duncan's Multiple Range	No	Yes	Yes	All	More sensitive to Type 1 than SNK
Scheffé's S	Yes	No	No	All	Most conservative
Games/Howell	Yes	No	No	All	More conservative than majority
Dunnett's test	No	No	No	T/C	More conservative than majority
Bonferroni	No	Yes	Yes	All, TC	Conservative

Abbreviations: PLSD = Protected least significant difference, HSD = Honestly significant difference.
T = treatment groups, C = Control group, Column 2 indicates whether equal numbers of replicates (N) in each treatment group are required or whether the method can be applied to cases with unequal 'N'. Column 3 indicates whether a significant between treatments F ratio is required before post-hoc tests can be applied and columns 4 and 5 whether the method assumes equal variances in the different treatments and normality of errors respectively. The final column indicates the relative degree of protection against type 1 and type 2 errors.

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Dr. Rosario G Monsalud of the Philippine National Collection of Microorganisms (PNCM) reports on the preservation of an important culture collection

2006 Endangered Culture Collection Grant report

Further Information
The *Sf*AM **Endangered Culture Collection Fund** is intended to help Society members to assist an endangered culture collection whose existence is under threat.

To apply for this award, please contact the Society Office or visit the website at:
■ **www.sfam.org.uk/prizes.php**

THE PHILIPPINE COLLECTION of Microorganisms (PNCM) evolved from the Microbial Culture Collection (MCC) of the National Institute of Molecular Biology and Biotechnology (BIOTECH), the centre of excellence in biotechnology research and development of the University of the Philippines Los Baños (UPLB).

The BIOTECH-MCC was established in 1981, a year after the establishment of the Institute, as an in-house culture collection with the primary function of preserving the microbial strains which have been isolated and acquired through the activities of the Institute. The Collection developed into a service laboratory in response to requests for cultures particularly from the academe, local communities and the industry, and became the BIOTECH-Microbial Culture Collection and Services Laboratory (MCCSL) in 1987.

In 1995, the Department of Science and Technology (DOST) through the Philippine Council for Advanced Science and Technology Research and Development (PCASTRD), provided a three-year grant to upgrade the BIOTECH-MCCSL and assume the role of national repository for microorganisms. The BIOTECH-MCCSL was then renamed the Philippine National Collection of Microorganisms (PNCM) to reflect its national repository status. To date it has a collection of about 4,000 strains of various species of bacteria, molds, yeasts, and a few algae.

The PNCM organized the Philippine Network of Microbial Culture Collections (PNMCC) in 1996 with the PNCM as the headquarters. At present there are seven members of the network, namely: PNCM, Museum of Natural History Culture Collection (MCC-MNH) also of UPLB, the University of Philippines Natural Science Research Institute Culture Collection

(UPCC) of University of Philippines, Diliman, The Industrial Technology Development Institute Microbial Culture Collection (ITDI-MCC) of the Department of Science and Technology, the Ecosystems Research and Development

Bureau (ERDB) Endomycorrhizal Germplasm Bank of the Department of Environment and Natural Resources, the University of St. Tomas Research Center for Natural Sciences - Collection of Microbial Strains (UST-CMS) and very recently the United Laboratories (UNILAB) Clinical Culture Collection.

The PNCM is a member of the World Federation of Culture Collections (WFCC) and is registered with the World Data Center for Microorganisms (WDCM), collection number 620. It has published three catalogues of strains, the most recent of which is the Directory of Culture Collections in the Philippines that contains the culture holdings of the members of the network.

The *Sf*AM Grant

The S*f*AM Grant will primarily go towards the proper preservation of the recent marine bacterial collection of PNCM which now totals 1,464 strains. The preservation method so far has been limited to freezing at -70°C in glycerol. However, due to frequent power failures and the lack of suitable and dependable alternative power source, the need to use another method of preservation, particularly L-drying is necessary.

A few isolates have been L-dried, but only at three ampoules per strain because of the scarcity of ampoules. Moreover, as our freeze dryers are already nine years old, the ampoule adaptors have become brittle and a considerable number have already cracked and can no longer be used. We also do not have filters to prevent cross contamination of cultures. These materials are not only difficult to import but in the case of PNCM which now has a very low budget because of the economic situation of the country, their costs have become prohibitive. For instance, two years ago the cost of one imported ampoule from Singapore (the nearest source to the Philippines) was US $2.00 and one ampoule adapter for US$ 12.50 while the PNCM only gets an equivalent of US$ 160.00 in annual budget from the university.

The grant will therefore be used to purchase ampoules, ampoule adaptors, filters and if possible, some reference books on taxonomy. Part of the grant will also go to UPCC who also needs to buy ampoule adaptors for their rehabilitated freeze-dryer.

Dr. Peter Green's visit

Dr. Peter Green, who is currently chair of the World Federation of Culture Collections (WFCC) and Executive Board member, visited the Philippines from May 7-13, 2006 to make an assessment of the PNCM and how he can help properly preserve its bacterial collection. Upon his arrival at the University, he briefed the Vice Chancellor for Research and Extension, Dr. Rico Supangco, about the generosity of the S*f*AM grant for the rescue of the marine bacterial collection of PNCM and the seminar that he presented at BIOTECH where the PNCM is affiliated. Dr. Supangco was very thankful for the S*f*AM grant and Dr. Green's visit and help to the University.

In the seminar at BIOTECH, Dr. Green tackled two very important aspects about culture collection work, namely: "Culture Collection Management" and "Culture Preservation". He also presented a video from WFCC. The seminar was attended by all the PNCM staff, other BIOTECH personnel and the Executive Board

members of the Philippine Network of Microbial Culture Collection (PNMCC) who came all the way from parts of Metro Manila. The seminar was a success, not only in terms of attendance, but also because of the valuable information that was passed on to us by Dr. Green. Prior to the seminar, Dr. Green toured the PNCM laboratory and saw its meagre facilities. He gave us some important tips for the L-drying process. He was then briefed on the other research undertakings of the Institute by the BIOTECH Director, Dr. Teresita M. Espino.

Dr. Green's visit coincided with the 5th Asia Pacific Biotechnology Congress and 35th Annual Convention of the Philippine Society for Microbiology (PSM) so we also took this opportunity to invite him to be one of the plenary speakers which he graciously accepted. He presented two papers, one was on bacterial vaccines for the plenary session and "CBD: Getting the Balance Right" for the Special Session for Bio-resource Centers. Aside from these paper presentations, he was also tapped to judge the Best Paper Competition.

The University of the Philippines Los Baños, and the PNCM in particular, together with the PNMCC and the PSM sincerely thank Dr. Peter Green for all his support. We are also very grateful to S*f*AM for making his visit possible, and most of all for the financial support to rescue the marine bacterial collection of PNCM and UPCC.

Dr. Rosario G. Monsalud
Head PNCM & Chair PNMCC

CAREERS

Food Microbiology

Roy Betts explores the work of a Food microbiologist

Photo by the kind permission of CCFRA

OVER RECENT YEARS' FOOD microbiology has moved from being a lesser known part of food science, to an area of major public interest. The tabloid press have ensured that organisms such as *Listeria*, *Salmonella*, and avian influenza in food products are major headlines.

I now find it not uncommon to switch on breakfast TV to find fellow food microbiologists being interviewed about the latest food 'scare' to which the public must be aware. Of course food microbiology is a fascinating part of our science, any food product is simply seen as an environmental niche to a micro-organism. It is the job of the food microbiologist to understand the different niches presented by the wide range of foods on the market, to know which microbial groups could take advantage of those niches, either to spoil the food or pose a health risk to the consumer, and finally to develop strategies to destroy or inhibit the growth of those organisms. By doing this we should have a variety of safe and stable foods on the shelves of our shops. Fail to do it and foods will spoil before the end of their shelf life, or worse consumers may contract food poisoning.

So how did I become a Food Microbiologist? It was certainly not a career path that I carefully picked from my early days in education. At school, science was the area I liked, with a definite leaning towards biology. This lead to a Biological Sciences Degree course, at what was then The Polytechnic at Wolverhampton (now of course the University of Wolverhampton). As I entered my third year of the course, thoughts turned to the future, and as I enjoyed the microbiologically orientated parts of the course, a research degree in microbiology seemed an interesting path. So it was, that I found myself at the University of Sheffield undertaking research for a Ph.D. on carbon metabolism by methylotrophic organisms supervised by Prof. Rod Quayle. Again in my third year of research, thoughts turned to future employment, and a small advert in *New Scientist* caught my eye. It noted that the Food Research Association at Chipping Campden were looking for a Microbiologist. Some pre-interview research into Food Microbiology whetted my appetite to this area of microbiology, whilst my previous knowledge of the geography of the Cotwolds, indicated that this area would be a beautiful place to live.

So it was a little over 21 years ago that I joined the Microbiology Department at Campden & Chorleywood Food Research Association (CCFRA). Now much can happen over a 21-year time period, and enjoyment and persistence in one area can lead into job roles you could never expect early in your career. My early work was as a junior researcher working in areas of the cleaning of food processing equipment and environments. Some management changes allowed me to move, to research rapid detection and enumeration methods for foodborne micro-organisms. This area was one I enjoyed greatly; I managed a range of UK Government, internal and confidential projects on test methods, and built up a research team of up to 12 staff. As time moved on, more changes at Campden opened up the position of Head of Department. I applied and successfully obtained this position. I rapidly realised the amount I still had to learn, not just about the science of food microbiology, but about the management of a Department of well over 45 staff with a large budget and numerous clients all of whom needed our help as quickly as possible. That was nearly ten years ago, and although I've learned much over that time, I'm still learning. One of the major positives about food microbiology is that you get to know a large number of people that work in the area quite quickly. Whilst these people may change jobs, they will usually stay in the field of food microbiology making them not only good working colleagues, but also friends that

can be called upon for advice.

Campden & Chorleywood Food Research Association, is a membership-based organisation serving the food and associated industries. At present we have around 1700 member companies world-wide, who are members in order to make use of our technical knowledge and expertise in food production. CCFRA expertise covers the whole food chain from primary agriculture to the end point consumer, and that means the Microbiology Department must maintain up to date knowledge and information on the microbiology of the complete food chain, in order to provide members with the advice and information they require.

My working day is very varied. I may be in the office, in which case I will check through emails phone messages and mail, in order to answer questions raised by members. A look through my notebook indicates the sort of things that require an answer, usually within a very short timescale. One call was asking when results from microbiological tests sent into our testing laboratory were due, I passed this onto our testing laboratory with a request for an urgent answer. A second call was questioning what typical microbiological criteria should be applied to a chilled ready meal. An email asked if I could visit a production site to observe the making and processing of a new product and to comment on potential microbiological problems that could occur and provide possible solutions. Finally there was a message from a company that wanted to know more about test methods for *Listeria*, particularly the possible application of validated rapid methods that would give fast results to tests done by that company. These are just some of the enquiries that the Microbiology Department at CCFRA gets from its clients on a daily basis. Answering them promptly with good advice is a major part of the service we offer, but it is also a challenge, and in the busiest months we can get over 400 individual and varied enquiries.

After the early morning questions are answered, it's into research project reviews. The Microbiology Department currently manages 19 research projects; these may be funded by our own research funds and have been requested by our members, or by external organisations such as the Food Standards Agency, LINK or the European Union. All of our research projects are carefully managed and must be reviewed by the Project Manager, Head of Department and a Director at least twice per year, with interim written reports on a quarterly basis. Whilst the project reviews do consume time, they are an excellent and essential way of managing and directing research and providing guidance to project mangers. Today's reviews cover varied projects including rapid microbiological test methods, the survival and growth potential of *Listeria*, the development of a guide for setting microbiological criteria and specifications for foods, and the development and use of predictive microbiology for shelf life estimation of food products.

It's now mid-afternoon and I have two visitors to see before the end of the day. The first is a member company who has been doing confidential work with us to evaluate the shelf life of a new product. We need to meet to discuss the microbiological results we have obtained and plan a route forward, so that the shelf life quoted on the product ensures that it remains safe and stable. This is not an easy task; the product is chilled, the reduced temperature being the major hurdle that controls bacterial growth. Whilst the temperature control during production, distribution and retail display is known to be good, we also know that once it is in the hands of the consumer, control is impossible to maintain. We must therefore build in safeguards to ensure bacterial growth cannot occur to a level that compromises product safety or quality. This discussion ends in an understanding that we will have to do more research on the product, particularly with the microbiology during the possible temperature abuse that could occur in the hands of the consumer. Finally, I meet up with a manufacturer of pathogen test kits for the food industry. This company needs to understand how their method should be validated to show that it gives comparable results to an International Reference method (ISO Standard) for the pathogen concerned. This may require some considerable testing within our laboratories that are expert method validation labs recognised by the AOAC Research Institute in the USA, AFNOR in France and MicroVal throughout Europe.

The days in the office are balanced by working away. Days out may be to audit the microbiology laboratory of a food producer, to study particular microbiological issues in a food production plant, or to present some of our research work at a scientific meeting. Any of these meetings may be in the UK, or aboard and whilst attendance at scientific meetings are planned many months in advance, troubleshooting visits to laboratories or production plants may have to take place at a moments notice. Travel is just part of the job, it may sound excellent to travel abroad on business, but more often than not it involves a large amount of waiting (at airports, taxi ranks, railway stations), and good views of the inside of airports, taxis & various hotel rooms. What is interesting is to see the various approaches to assuring food safety operated in different countries, together with factory and microbiology laboratory operation. There are many very difficult issues faced by laboratories in some parts of the world, with supply, operation and repair of very basic equipment. This puts into perspective the relatively minor problems faced by laboratories in the UK. It is, however always a pleasure to talk with food microbiologists the world over and realise we do all speak the same microbiological language and strive to work at the same high standards.

Food microbiology is a challenging but very enjoyable part of our science, its role is always to work to give food producers the advice needed to develop and market a range of products, whilst ensuring that these are of high quality, and ultimately are safe. CCFRA are always looking for microbiologists with a interest and maybe some previous experience in food microbiology, who may want to take up the fascinating challenges of the job. Anyone entering food microbiology can be assured of an exciting and challenging career with good colleagues around them, although their after-dinner conversations about work, may not get them an invitation to every dinner party!

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Roy Betts, Head of Microbiology, Campden & Chorleywood Food Research Association (CCFRA).

Am I eligible - can I apply?

Grants can be made available to ANY FULL member who is able to offer a suitable undergraduate student a work placement for a period of up to 10 weeks during summer. The grant is £160 per week for the student for a maximum of 10 weeks and up to £50 per week for lab costs for a maximum of 10 weeks. To apply, visit **www.sfam.org.uk/members/prizes.php**

GUIDELINES

1. Any full member of the Society who can offer an undergraduate student, or a recent graduate (within 6 months of graduation) a work placement is eligible to apply for this grant. The placement can last up to a maximum of 10 weeks, normally during the summer vacation.

2. The Grant will normally provide support at the rate of £160 per week for the student and up to £50 per week for lab costs. The monies will usually be paid to the Department in which the student/graduate works unless a specific request is made for an alternative method of payment.

3. Applications should be made by the supervisor using the PDF form provided on the website or the paper form obtainable from the Society Office.

4. Successful applicants and their students/graduate must write a report on the placement within 4 weeks of completing their placement which will be published in *Microbiologist*. Photographs of the applicant and/or the work done during the placement are desirable. These should be supplied as (a) digital images at a size of not less than 4 inches square at a resolution of not less than 300 pixels per inch, or (b) original photographic prints which will be scanned and promptly returned.

5. Normally a member may not apply for a further grant until a period of two years has elapsed.

6.There is no closing date for this Grant and applications can be made any time during the year. Applicants must apply at least 6 weeks before the proposed start date.

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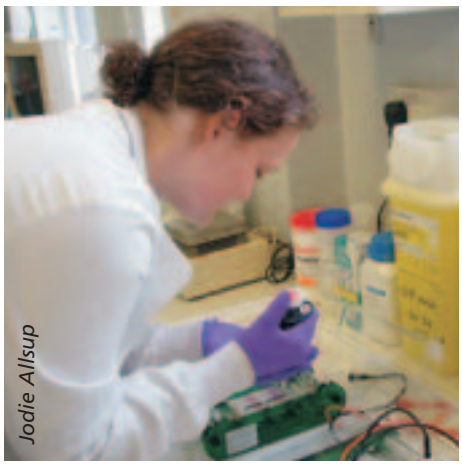
Metal Ion Homeostasis and Bacterial Pathogenicity

Jodie Allsup reports on her project

IN THE SUMMER BREAK before entering the final year of my course at the University of Manchester, I was the grateful recipient of a SfAM Student into Work award.

This allowed me to carry out a ten week project placement under the supervision of Dr Clare Taylor in Dr Jennifer Cavet's laboratory in the Faculty of Life Sciences. Research in Dr Cavet's lab is concerned with characterising bacterial metal homeostatic proteins and examining their role in pathogenicity. My project contributed to ongoing studies involved in determining the metal stresses encountered by a pathogen (*Salmonella enterica serovar* Typhimurium) during infection of host cells.


Jodie Allsup

Salmonella species cause a variety of diseases from localised gastroenteritis to systemic typhoid fever. *S. typhimurium* is a major cause of food poisoning in humans; the main characteristics being diarrhoea, nausea and abdominal pains, although bacteremia and focal systemic infections are also observed in the young, elderly and immunocompromised. In mice, *S. typhimurium* causes a severe systemic disease similar to human typhoid fever and hence the *S. typhimurium* murine infection model is often used to study systemic *Salmonella* infection. Once ingested, *Salmonella* travels through the stomach to the intestine and penetrates the intestinal epithelium. After invasion, the bacteria survive and propagate within the Peyer's patches and spread to target organs, such as the liver and spleen. The ability of *Salmonella* to survive in macrophage phagosomes is critical during the systemic stages of disease. Within the macrophage phagosome, *Salmonella* must sense and respond rapidly to changing environmental conditions inflicted by host defence mechanisms.

Metals (such as iron, copper, zinc, cobalt etc) are essential for bacteria as they are required as cofactors for large numbers of fundamental proteins; including proteins involved in macromolecular synthesis, energy metabolism and oxidative stress responses. However, these metal ions can also be toxic in excess and redox active metals (eg iron) can catalyse Haber Weiss and Fenton reactions resulting in the production of highly toxic hydroxyl radicals. Within the macrophage phagosome *Salmonella* must therefore effectively compete for essential metal ions whilst avoiding metal-mediated toxicity. The nature of the metal stresses encountered by *Salmonella* within the macrophage phagosome are largely unknown. To determine the nature of these stresses, studies in Dr Cavet's lab are using reporter gene fusions within *Salmonella* to report the phagosomal levels of metals sensed by *Salmonella* during infection.

The aim of my summer project was to generate a construct containing a reporter gene (*lacZ*) fused to a cobalt-responsive promoter to detect changes in cobalt levels within the *Salmonella* cytosol. A gene encoding a previously characterised cyanobacterial cobalt responsive regulator, and its target promoter DNA, were amplified by PCR, purified, ligated into a PCR cloning vector and introduced into *E. coli* cells. Insertion of the PCR product within the vector was then confirmed by plasmid purification and DNA sequencing. The regulator gene and promoter DNA were then released by restriction digestion, purified and fused to lacZ in a reporter gene vector. Following confirmation of the fusion to lacZ, the construct was introduced into *S. typhimurium* and beta-galactosidase activity was measured following exposure of cells to increasing concentrations of cobalt. Beta-galactosidase activity was substantially increased in response to elevated cobalt

levels, the maximum activity being detected at maximum permissive cobalt concentrations. Furthermore, this response was subsequently shown to be specific to cobalt and no increase in beta-galactosidase activity was detected following exposure of cells to a range of other metal ions or oxidative stress.

These results are extremely encouraging and have demonstrated that the cyanobacterial cobalt-responsive regulator and its promoter can be used to report cobalt levels within *Salmonella*. Furthermore, the specificity for cobalt means that this is an ideal system to use to report the cobalt levels encountered by *Salmonella* within macrophage phagosomes.

My SfAM work placement has allowed me to acquire a number of valuable laboratory skills (including Polymerase Chain Reaction, culturing of bacteria, gel electrophoresis, DNA purification, DNA cloning techniques, and reporter gene assays) that will be extremely helpful for me to carry out my final year honours project and hopefully to pursue a future career in research. Prior to this placement, the only laboratory experience I had was from laboratory practical classes within the university as part of my degree course. This award has given me the chance to gain experience of working in a professional research environment and has encouraged me to apply to study for a Ph.D. upon completion of my degree course. I would like to thank SfAM, Dr Clare Taylor and Dr Jen Cavet for giving me this wonderful opportunity.

Jodie Allsup
University of Manchester

The detection of *Burkholderia* bacteria associated with cord-forming fungi using culture-independent methods **Daniel Brown** reports

The genus *Burkholderia* contains over 30 species (nine of which are a closely related group named the *Burkholderia cepacia* complex [Bcc]); these include species of particular ecological, biotechnological and pathogenic interest.

The majority of species in this genus are widely known as soil bacteria, which exhibit different types of non-pathogenic interactions with plants. Several species of the Bcc have been seen to develop intimate beneficial interactions with plant roots, stems and leaves, especially promoting plant growth and crop production. However, several Burkholderia species are plant pathogens; for example *Burkholderia caryophylli* is a pathogen of carnations and also causes 'sour skin' rot in onions, and *Burkholderia glumae* causes rot of rice grains and seedlings. Other species include the opportunistic and primary human pathogens. Cystic Fibrosis patients are particularly susceptible to Bcc lung infection from nosocomially or environmentally acquired pathogenic strains.

Mycelial cords are aggregations of predominantly parallel, longitudinally aligned hyphae. Mycelial cords exhibit a range of forms, from true rhizomorphs which show a high degree of apical dominance, to initially diffuse fans of mycelium which become consolidated into cords behind the growing mycelial front. Cord-formers are predominantly of the wood-decaying basidiomycotes, and produce cords to search for new nutrient source for the main body to exploit and utilise. These may be extensive long-lived systems which are particularly abundant on the floor of deciduous woodlands, found in the interface between the leaf litter and the soil.

There is a growing body of evidence that bacteria may form close associations with fungi and in some cases even live as endosymbionts within them. The aim of this project was to determine via culture-independent methods (Polymerase Chain Reaction [PCR]-based methods) if there is an interaction between *Burkholderia* bacteria and the fungal cords. The host laboratory for my project at Cardiff University have developed molecular methods to examine *Burkholderia* species diversity using the recA gene and the development of PCR primer sets BUR 1 & 2 and BUR 3 & 4. PCR-based methods for differentiation of Burkholderia species include: *recA* gene amplification using the latter *recA* specific primers, the creation of *recA* gene clone libraries, Restriction Fragment Length Polymorphisms (RFLP) analysis of the recA gene, and the creation of phylogenetic trees from recA sequence data. The 16S rRNA gene is not highly discriminatory for speciation of *Burkholderia* bacteria, however, to reveal diversity beyond the *Burkholderia*, this is the most useful gene to target and was also examined as part of the project. Preliminary analysis had shown that *Burkholderia* species *recA* genes were associated with mycelial cords and my summer project aimed to examine this finding in greater depth.

Cord samples were collected from Tintern woods, a deciduous mixed woodland near Chepstow. In total, 34 cord samples were collected from four locations. Each cord sample was washed thoroughly in sterile distilled water to remove most of the surface associated bacteria. After washing, a section of cord material was subjected to DNA extraction using the Fast DNA® spin kit (Qbiogene, Cambridge, UK), a commercial system developed for DNA extraction from soil. The resulting DNA was then used as a template for bacterial identification using the PCR method based on the *recA* and 16S rRNA genes. As well as examining cord material from the natural environment, 17 pure cultures of cord forming fungi were subjected to DNA extraction as above, followed by PCR for both the *recA* and 16S rRNA genes. Neither PCR produced positive results, indicating that no bacterial DNA was present in the pure lab cultures and suggesting that obligate endosymbiotic interactions between these fungal cords and *Burkholderia*/other bacteria do not occur.

To further examine the bacterial diversity associated with fungal cords recovered from woodland, firstly I amplified the *recA* gene using the BUR 1 & 2 primers which enable *Burkholderia* several other α-proteobacterial *recA*

genes to be amplified. From the 34 samples that underwent the PCR (58°C annealing temperature), 13 were BUR 1 & 2 positive. These were then diluted 1:1000 with sterile distilled water for the second round nested PCR (55°C annealing temperature) with the *Burkholderia* specific primers BUR 3 & 4. Despite several repetitions, the second round PCR was not successful, suggesting that no or insufficient *Burkholderia* DNA was present in the samples. To aid detection, a *recA* gene clone library of the BUR 1 & 2 positive PCR products was constructed from sample 30, DNA that had been extracted from fungal cord associated with the roots of nettles. To facilitate cloning, the BUR 1 & 2 PCR was repeated with a 20 minute extension period at the end of the final cycle; during this extension, Taq polymerase adds adenine bases to the terminals of the PCR products and allows the insertion of such DNA in to plasmid vectors carrying thymine overhangs at their cloning sites.

The plasmid chosen was the p-GEM T easy-vector (Promega UK, Southampton), a dual selection vector with a multiple cloning site. PCR products were ligated into the vector and the resulting recombinant plasmids transformed into competent *Escherichia coli* JM109 cells via a heat-shock mediated transformation. The transformants were then detected by growth on agar seeded with antibiotics for selection of the plasmid and chromogenic substrates (X-Gal and IPTG); strains carrying recombinant plasmids were detected as white colonies (blue colonies had closed vectors which had re-ligated with no insert within the cloning site). By carrying out cloning of the PCR products, I observed first hand how insertional inactivation of the betagalactosidase gene can be used as a screen to isolate recombinant genetic constructs. Individual clones from this library (a total of 384) were picked, grown and stored frozen for further analysis.

The next stage of the investigation was to perform a rapid screen of the recA gene clone library using the BUR 3 & 4 PCR primers and with an aim to detect *Burkholderia* specific *recA* genes. After revival, the *E. coli* clones from the library were added to a lysis buffer and boiled in a thermal cycler in order to inactivate DNAse's present within the bacteria. A BUR 3 & 4 PCR mixture was then added to each well and a thermal cycle run with an annealing temperature of 55°C. The PCR products were analysed by agarose gel electrophoresis and 15 of the 384 samples were determined to be positive for the 385 bp *Burkholderia*-specific PCR product. A plasmid preparation was then performed on these individual *E. coli* clones using the Wizard SV Fast prep DNA extraction procedure (Promega UK, Southampton). This alkaline lysis based method of plasmid preparation had been discussed in the second year of my course and it was great to actually apply it. The recA gene inserts within each plasmid were then subjected to DNA sequencing using the automated PCR-based sequencing facility in the department.

The resulting sequences demonstrated that four of the 15 *recA* genes analysed clustered phylogenetically as *Burkholderia* species. The remaining eleven *recA* genes fell outside of this group and were not closely related to any other specific group of bacteria. Of the four that did fall within the *Burkholderia* species, two were in a novel species grouping which had also been identified in the maize rhizosphere, while the other two aligned closely with *recA* genes from previously cultured *Burkholderia*: sample 10 with *B. caldonica* and *B. xenovorans*, and sample 14 with *B. glathei*. Overall, examination of the fungal cord material associated with nettle roots using the *recA*-based methods demonstrated that while *Burkholderia* species *recA* genes were present, they were low in numbers within the clone library, suggesting that *Burkholderia* species were not the dominant bacterial species associated with the cord sample.

To gain a more complete understanding of the diversity of the recA gene clone library as a whole and ascertain which DNAs were the dominant in the sample, RFLP analysis was performed on a random selection of clones that were negative for PCR by BUR 3 & 4. Rather than perform a plasmid extraction on each *E. coli* clone, a fast DNA preparation method was applied to freshly revived culture material from agar plates. The method involved two cycles of boiling and freezing a suspension of bacteria in the presence of 5% CHELEX resin. The resin removes divalent cations and other enzyme cofactors that can facilitate degradation of DNA and the boiling inactivates DNAse's. The resulting DNA was amplified using a BUR 1 & 2 PCR and the PCR products digested with the restriction endonuclease *HaeIII*. The RFLP patterns were visualised after agarose gel electrophoresis and from the 64 samples, three common banding patterns emerged with one being dominant in the library and representing 21 of 64 (33%) samples. Ten clones representing these common *recA* genes were chosen for DNA sequence analysis.

Because the *recA* gene has not been widely used as a tool of identification of bacteria, analysis of the 16S rRNA gene was performed in parallel with the *recA*-based strategy outlined above. A 16S rRNA gene clone library was constructed after PCR of the nettle-cord DNA with the primers 63f & 1387r. A total of 76 random clones from this library were analysed by RFLP using restriction endonuclease *DdeI*; a greater diversity of RFLP patterns were observed in this library. Ten clones were chosen for sequence analysis to be representative of the dominant RFLP types found in the 76 clones analysed. DNA sequence analysis of the dominant *recA* and 16S rRNA genes associated with nettle-associated cord DNA demonstrated that several unusual bacterial species were present. In particular, several of the sequences aligned with a novel species of bacterium originally identified in the gut of Antlion larvae, the larva stage of insects such as dragon flies and lacewings.

The following conclusions were drawn at the end of the ten week project: Although Burkholderia species *recA* genes were present in the fungal cord samples, they were not dominant suggesting that other bacterial species are associated in greater numbers with these mycelial projections. Novel classes of bacterial *recA* and 16S rRNA genes are dominant in gene libraries made from PCR products amplified from total fungal-cord associated DNA, suggesting that

novel bacteria are present in high numbers.

The whole experience of this summer project has been worthwhile and I am very grateful for having the opportunity to gain lab experience and perform techniques that I had previously only knowledge of theoretically from my lectures. I would highly recommend the Students into work placements to other undergraduates as the experience was enjoyable and I learnt a great deal about the many scientific techniques, including culture-independent analysis of bacterial diversity. The experience of working in a lab will help me now going into my final year and having to carry out a lab-based research project. The placement has also strengthened my desire to pursue a postgraduate career in microbiology research.

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Daniel Brown
Cardiff University

Ability of *Listeria monocytogenes* to survive the stresses encountered during gastrointestinal transit

Tanya Clifford reports on her project

Tanya Clifford

I AM PRESENTLY undertaking a biological science degree in University College, Cork (UCC) majoring in Microbiology. Last summer I was keen to obtain experience in a research laboratory before my fourth and final year, both to gain experience with some practical laboratory techniques and to explore the world of research science. The *Sf*AM student into work scheme was therefore a great opportunity for me.

One of the areas that interested me a great deal throughout the course of my studies was medical microbiology and in particular food borne pathogens. With this in mind I approached Dr. Roy Sleator of the food pathogen laboratory of UCC and together we come up with a research project focusing on *Listeria monocytogenes*.

L. monocytogenes is a Gram-positive non spore-forming rod and is emerging as an important food borne pathogen in recent times. In addition to being the causative agent of the invasive systemic disease of listeriosis, the symptoms of which include septicaemia, meningitis and spontaneous abortion, it is also the causative agent of febrile gastroenteritis. It was responsible for 71% of all recalls of food products in the USA between 1993 and 1998 and is thus a significant financial as well as a clinical burden.

While the majority of published work to date has centred on the intracellular phase of *Listeria monocytogenes* infection, the food pathogen labatory in UCC is one of the first labs to investigate the gastereointestinal phase of listerial infection.

My project was to focus on the ability of *Listeria monocytogenes* to survive the stresses encountered during gastrointestinal transit. These stresses include the low pH of the stomach, bile and water activity (aW) stress associated with the small intestine. The underlying genetic mechanisms governing the listerial stress responses in the gastrointestinal transit have come to light through a number of recent research efforts. While many *L. monocytogenes* virulence genes are under the regulation of the Positive regulatory factor A (PrfA) the alternative stress transcription factor SigB has recently been shown to regulate a number of genes (namely Gad, BilE, Bsh and OpuC) involved in gastrointestinal transit and thus may be as important as PrfA in regulating listerial virulence and pathogenesis – at least in the context of the gastrointestinal tract.

Throughout my project *in vitro* experiments were performed using both wild type and σB negative mutant strains of *L. monocytogenes* to investigate the contribution of σB to the stresses most often encountered during the gastro-intestinal phase of infection, namely acid, bile and elevated osmolarity. Significantly, preliminary results suggest an important role for σB in combating the stresses mimicking the gastrointestinal tract. We suggest that while PrfA is the major determinant of virulence potential during intracellular infection, σB plays the dominant role during gastrointestinal transit.

Tanya Clifford
University College Cork

ARE YOU ELIGIBLE?

A Students into Work Grant can be made available to ANY FULL member who is able to offer a suitable undergraduate student a work placement for a period of up to 10 weeks during summer. The grant is £160 per week for the student for a maximum of 10 weeks and up to £50 per week for lab costs for a maximum of 10 weeks. To apply, visit **www.sfam.org.uk/members/prizes.php**

Am I eligible - can I apply?

The President's Fund provides limited grants to ALL members to assist them to attend scientific meetings or workshops related to their area of work. Awards are made at the sole discretion of the Honorary President. Please note that this Fund is open to members of all ages! Why not apply to the Fund? The maximum grant available is normally £1,000.

To apply, visit **www.sfam.org.uk/members/prizes.php**

TERMS & CONDITIONS

1. The applicant must have been a member for at least a full subscription year before the event to be attended and must be a fully paid-up member at the time of application.

2. A successful applicant cannot re-apply to the Fund for three years from the date of the award.

3. Preference will be given to applicants who are contributing to the meeting they wish to attend and/or are unable to obtain funds elsewhere.

4. Completed applications must include an abstract of any intended contribution to be made at the meeting and must be received by the Society Office not less than six weeks before the date of the event.

5. Student member applications must be supported by their supervisor and include the contact telephone number(s) and email address(es) of the supervisor or head of department who is supporting their application.

6. The maximum grant available is normally £1,000.

7. Under exceptional circumstances this maximum may be exceeded.

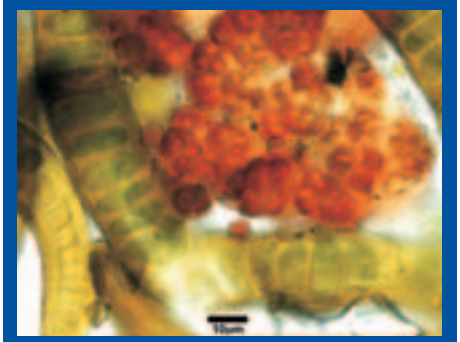
9. The award of this grant is at the sole discretion of the Hon President of the Society.

10. The applicant must write a short article of between 400 - 600 words within 4 weeks of the meeting, the content of which will be agreed with the Editor of sfam *Microbiologist* and will be published in the magazine. Photographs of the applicant and/or the subject of the article are desirable. These should be supplied as (a) digital files in TIFF or JPEG format at a size of not less than 4 inches square at a resolution of not less than 300 pixels per inch, or (b) original photographic prints which will be scanned and promptly returned to the applicant.

Microorganisms and our Cultural Heritage

ENEMIES OF OUR CULTURAL heritage, needing only light and water to grow and destroy, phototrophic biofilms form as primary colonising layers on the internal and external surfaces of historic and cultural buildings all over the world: from the wooden huts of the first Antarctic explorers, to the stone buildings of ancient civilizations in Europe and the tropics.

Fig 1.



Variously coloured biofilms on 13th century temple at Kompong Cham, Cambodia.

Consisting predominantly, on stone, of cyanobacteria (Fig.1), these biofilms lead to discolouration and degradation of the surface, in addition to providing retained water and organic nutrients for other deteriogenic organisms – heterotrophic bacteria, fungi and, at the end of the succession, higher plants. The most frequent microorganisms in well-developed biofilms on stone monuments in the tropics are the coccoid cyanobacteria of subsections I and II, although the eukaryotic algae are sometimes predominant in Europe (Gaylarde & Gaylarde, 2005). Filamentous fungi, often considered more damaging, are common early colonisers of more nutritious substrates, such as wood and painted surfaces, while non-photosynthetic autotrophic bacteria utilising atmospheric nitrogen or sulfur pollutants can be important in cities and industrial areas.

Everyone is aware of the destructive activity of higher plants, whose root systems can break up concrete walls and mosaic floors. Microorganisms are more discrete, acting through the production of acid or alkaline metabolites, the reduction (mobilisation) of stone components such as Fe and Mn, with subsequent re-oxidation and deposition at the surface as the so-called patina, and through the proven ability of some microbial groups to penetrate and grow within stone structures as endoliths. Dark-coloured fungi and cyanobacteria produce pigments that cause green, red, brown and black discolouration on buildings (Fig. 2). Although the pigments are useful to the microbial cells, protecting them against various types of stress, they add yet another weapon to the microbial arsenal against historic monuments. Differential heating of the multicoloured surfaces when exposed to sunlight leads to tensions between the layers, which can result in spalling and the exposure of a new surface for colonisation (Gaylarde *et al.*, 2003).

In order to combat this microbial attack on our cultural properties, we need to recognise the organisms responsible, and recent work in this area has included the use of new surface analytical and molecular biological techniques. Highly sensitive methods, such as atomic force microscopy, synchrotron-based spectroscopic analyses and X-ray microprobe studies, allow changes in the substrate, induced by the microorganisms, to be determined (Schreiner *et al.*, 2004). Genomic studies such as Polymerase Chain Reaction-Single Strand Conformational Polymorphism (PCR-SSCP), Microautoradiography-Fluorescence In Situ Hybridisation (MAR-FISH), Denaturing Gradient Gel Electrophoresis (DGGE), together with DNA sequencing, allow more detailed analysis of the microorganisms present in the biofilms and, when used with Reverse Transcription PCR (RT-PCR), enable the active organisms to be determined. A combination of these techniques should lead to a better understanding of which organisms are really important in the deterioration and degradation of historic properties.

Molecular techniques using 16S and 23S rRNA sequence analyses have demonstrated the previously unsuspected presence of halophilic bacteria on deteriorated church wall paintings. The same methods have detected members of a new phylum of bacteria, the Acidobacteria, on paleolithic wall

paintings in Europe (Schabereiter-Gurtner *et al.*, 2002). The phylogenetic depth of this group is as wide as the entire Proteobacteria phylum. Since few representatives have been cultured, there is no knowledge of the bacterial activities that might relate to the degradation of historic buildings and the direction that future research must take is obvious. The final aim of all these studies is to control the growth and activity of deteriogenic

Fig 2.

Dark coloured coccoid and filamentous cyanobacteria in biofilms on a historic church in Ouro Preto, Brazil.

microorganisms and to facilitate the development of new methods for the conservation and restoration of historic monuments. Novel treatments are already being researched in Scotland, along with other countries of the EU. Interesting possibilities include inhibitors of pigment production and of the excreted polymeric material (EPS) that enhances stone degradation. Alternative interventions for restoration of damaged buildings include bioremediation, where carbonate-depositing bacteria or biologically produced calcite are used to consolidate weakened limestone monuments. All of these studies are being aided by European Community grants. No mention has been made of the microbial problems faced by museum and document conservators. These are, perhaps, even more acute, as many of the materials making up museum artefacts and all of those composing written documents are nutrient sources for microorganisms. Protective mechanisms must be constantly maintained (Allsopp & Gaylarde, 2004). However, this complex subject requires a separate article.

Christine C. Gaylarde, Federal University of Rio Grande do Sul, Brazil.

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Regulation of antibiotics for veterinary use

ANTIMICROBIAL AGENTS (antibiotics) are widely used in human and veterinary medicine to treat and prevent infectious disease. Marketing and usage of all medicinal products, including antibiotics, are thoroughly regulated.

Principal regulatory bodies are the European Medicines Agency (EMEA) for the EU and the Food and Drugs Administration (FDA) for the USA. In 1996 the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH), a trilateral EU-USA-Japan programme, was initiated to harmonize requirements for veterinary product approval.

This article will briefly review some of the regulatory guidelines that require microbiological studies. Many companies that market veterinary antibiotics choose to outsource these studies to contract research organizations. Don Whitley Scientific Limited (DWS) is one such organisation that provides these laboratory services and readers of *Microbiologist* may be interested to learn about the type of work that keeps us busy.

VICH Guideline 36, "Studies to evaluate the safety of residues of veterinary drugs in human food: general approach to establish a microbiological ADI", applies to antimicrobial agents that are destined for use in food-producing animals. In order to market such antibiotics it is necessary to establish an Acceptable Daily Intake (ADI), defined as the quantity of drug that can be ingested daily by a human without risk to health. It is necessary to determine toxicological, pharmacological and microbiological ADIs, but only the latter will be discussed here.

The intestinal bacteria of humans play an important role in maintaining and protecting health, through functions such as metabolising dietary components and protecting against colonisation by pathogenic organisms, but ingested antibiotics may alter the ecology of the intestinal flora. Guideline 36 describes laboratory methods for studying this issue. The usual first step is to determine the Minimum Inhibitory Concentration (MIC) of the antibiotic against the major groups of human intestinal bacteria, currently designated as *Escherichia coli*, *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Enterococcus*, *Eubacterium*, *Fusobacterium*, *Lactobacillus* and Gram-positive anaerobic cocci (Peptostreptococcus, etc.).

The ADI is calculated from MIC results against the most susceptible bacterial groups, but the calculation can be modified to account for the fraction of an orally administered antibiotic dose that is available for interaction with intestinal bacteria. In our laboratory, this issue is investigated by conducting MIC-type assays in a standard culture medium with the addition of human faecal material. Differences in antibiotic activity with and without faeces allow the degree of binding to faecal material, and thus the fraction available to interact with gut bacteria, to be calculated.

Laboratory studies to investigate the issue of antibiotic resistance are set out in VICH Guideline 27, "Guidance on pre-approval information for registration of new veterinary medicinal products for food producing animals with respect to antimicrobial resistance". Characterization of an antibiotic's spectrum of activity is usually required, and can be provided by determining the MIC against a panel of bacteria including target animal pathogens, potential food-borne pathogens associated with the animal species and commensal organisms, which may be associated with transfer of antibiotic resistance between animals and humans. The Guideline also

requires information on the prevalence and molecular genetic basis of resistance to the antibiotic. This information is sometimes obtainable from existing literature but can also require specially designed studies. The occurrence of cross-resistance and co-resistance between the antimicrobial agent in question and other antimicrobial agents must also be considered. It was formerly sufficient to provide only phenotypic data (*e.g.* MICs) but it is increasingly necessary to provide a genotypic description of multiply resistant strains.

A guideline specific to the EU is EMEA/CVMP/627/01, "Guideline for the demonstration of efficacy for veterinary medicinal products containing antimicrobial substances". There is considerable cross-over between this and VICH Guideline 27 with regard to spectrum of activity, resistance mechanisms, *etc.*, but in addition EMEA guideline 627 includes some more interesting *in vitro* studies. For example, a useful adjunct to the basic (but essential) MIC data is Minimum Bactericidal Concentration (MBC), defined as the lowest concentration of an antimicrobial agent that reduces an initial bacterial inoculum by 99.9%.

This assay differentiates purely bacteriostatic agents from those that kill bacteria. Antibacterial activity can be further characterized by determining the kinetics of bacterial killing: a standardized inoculum of bacteria is exposed to selected antibiotic concentrations and surviving bacterial cells are enumerated at intervals during incubation: a time-kill curve is thus constructed. A more complex refinement is the determination of Post Antibiotic Effect (PAE): this describes suppression of bacterial growth that persists after brief exposure of bacteria to an antimicrobial agent, which is then removed from the culture. PAE is increasingly recognized as a very relevant measure of antibacterial activity because of the concentration profile to which target bacteria are exposed *in vivo*. PAE determinations facilitate prediction of in vivo activity and optimization of dosage intervals.

A somewhat different EU guideline of interest to microbiologists is EMEA/CVMP/276/99, "Note for guidance for the assessment of the effect of antimicrobial substances on dairy starter cultures". Safety evaluation of an antibiotic used in milk-producing animals and excreted in the milk must include assessment of its potential effects on microorganisms used in the manufacture of dairy products (cheese and yoghurt). Antibiotics in milk may cause substantial problems in dairy processing by inhibiting the activity of "starter cultures", the consequences of which may include reduced acid production, incorrect ripening of cheese, modification of flavour, etc.

Initial assessment of such antibiotics includes MIC determinations against bacterial strains relevant to dairy processing, including *Streptococcus*, *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Lactobacillus* and *Bifidobacterium* and against mixtures of these species. The effect of milk on MIC values is also determined. A further requirement of CVMP Guideline 276 is to determine the maximum concentration of antibiotic that will not effect acid production by starter cultures found to be susceptible in the initial MIC test. Laboratory methods for determining this concentration include measurement of the time between initiation of incubation and most rapid period of acidification, acidification rate and terminal pH value. In our laboratory, these measurements are achieved using special equipment to continuously record the pH in incubated milk inoculated with the cultures of interest.

For acceptance by the regulatory bodies, laboratory studies have to be conducted at a facility that complies with Good Laboratory Practice (GLP) — an internationally accepted standard, which DWS have maintained since 1994. Arguably more challenging is the maintenance of an appropriate culture collection, which includes human intestinal bacteria, pathogens of animals and commensal organisms from each species. Our collection comprises approximately 14,000 strains and allows us to conduct the variety of studies outlined here: it is undoubtedly one of our greatest assets.

Andrew Pridmore, Head of Microbiology, Don Whitely Scientific Ltd

Actinobacillus pleuropneumoniae

IT IS STRANGE TO THINK that an organism such as *Actinobacillus pleuropneumoniae* (APL) is unheard of considering the devastation it can cause to swine all over the world.

In fact, the effects of APL are not only related to the pigs themselves but APL has a global economic impact on the swine industry, as farmers are unable to yield enough meat to sell, and in some cases there is no meat at all. It takes around 8 weeks for a piglet to mature into a pig, of which 90% of the pig is used for consumption; farmers sell the pig based upon its weight. However, APL infection can radically alter this by either killing the pig or ensuring the pig has poor weight gain. Both these consequences have severe economic impacts within the swine industry, and farmers do all they can to prevent exposure to this organism. To farmers APL is their ultimate nightmare, so what exactly is APL and how can it be controlled? APL is a Gram-negative cocobacilli, with strict host specificity to swine, and is sub-divided into two biotypes: biotype 1 is nicotinamide adenine dinucleaotide (NAD) dependent whereas biotype 2 is not. Currently there are 15 known serotypes and these are based on the antigenic diversity of the capsular polysaccharide and lipopolysaccharide. The serotypes vary in their degree of virulence, serotypes 1, 5, 9 and 11 are particularly virulent, although all serotypes can cause severe disease and death. APL causes porcine pleuropneumoniae, a severe fatal respiratory disease where disease prognosis ranges from pre-acute to chronic. The pre-acute form of the disease shows a dramatic fall in illness with the onset of vomiting, diarrhoea, a discharge of blood via the nostrils and mouth, death ensues within 24-48 hours. Acute cases also include an increase in temperature along with lethargy and lameness. Anorexia, depression, respiratory difficulties and circulatory failure are also seen, however the acute form of the disease results in either the complete recovery of the animal or death, where the initial four days of survival is critical in determining the complete recovery from illness. The chronic form of pleuropneumonia exhibits sub-clinical forms of the disease usually manifested by chronic lesions in the lungs. Here, little or no fever is detected along with a decrease in weight gain caused by a decrease in appetite. Studies in slaughterhouses revealed the only evidence for infection are the formations of unresolved lung lesions.

All age groups are susceptible but the disease is most prevalent in fattening and growing pigs at approximately three months of age. Infected breeding herd piglets seem to be protected against infection through maternally derived immunity, but in endemic infected fattening herds, APL causes decreased weight gain. Other external factors can also attribute to the susceptibility as well as the severity of the disease, for example, moving pigs between pens, overcrowding and adverse climate conditions.

Transmission of APL predominantly occurs through direct pig-pig contact, as APL is incapable of surviving within the environment for long periods. Transmission via aerosol has been suggested as colonisation of tonsillar and nasal tissue is frequently reported with infection. Currently the most important cause in the spread of the pathogen between herds is through sub-clinically infected carrier pigs, an area in desperate need of attention to curtail the spread of infection to non-infected pigs. Control and prevention of APL in swine involves several methods and in order to employ the most desirable method certain factors need to be taken into consideration. For example, cost and risk of a disease outbreak. Current methods include; culling seropositive carriers to reduce the risk of introducing APL into a herd; purchasing seronegative stock and quarantining new stock. The most common route of transmission is sub-clinically infected carriers and although the ideal method of control is to eradicate the herd, this seems impractical but can prevent further colonisation. Some farmers choose to allow pigs with sub-clinical symptoms of less virulent strains; which can reduce the risk of an acute outbreak in herds not previously exposed to the organism.

Treatment is the only possible solution upon evidence of clinical symptoms but available antibiotics fail to clear the infection entirely, thus allowing APL to continually shed, not only this but antibiotic treatment can only be administered once symptoms have developed, which seems futile as the organism could have spread through the entire herd. Thus with increased reports of a rise in antibiotic resistance, antibiotic therapy is only effective in the treatment of clinical outbreaks, achieved through an intermittent or continuous course of antimicrobial therapy.

Swine previously infected with APL are immune to infections with the homologous serotype and in some cases at least partially resistant to heterologous challenge. Immunoglobulins (Ig) G are the likely mediators of such immunity, local antibodies have also been detected but their exact role is yet to be elucidated. First generation vaccines were heat-killed or chemically inactivated preparations. However, immunisation with killed bacteria induced only partial protection against the homologous serotype and offered no protection against heterologous serotypes. Second generation vaccines are acellular multivalent, subunit vaccines containing various combinations of serotypes and were found to provide some degree of protection, whereby reducing mortality and the occurrence of clinical manifestations. Thus it seems that future control and preventative measures desperately need to be addressed. With the marked rise in antibiotic resistance, vaccines seem the most likely candidate in preventing APL infection. However, as discussed there are no commercially available cross-protective vaccines for APL. Generating vaccines without cross-protective capabilities seems pointless, and thus efforts need to be directed towards identifying new OMPs that are antigenic and found across all strains. Research into the identification and characterisation of such proteins is currently underway. Proteins of particular interest are those involved in the initial attachment to host cells, as adhesion is the primary step in establishing disease. Intercepting this pathway could be pivotal however; this has been difficult as the mechanisms involved in establishing an APL infection are poorly understood. The urgent need for the development of a vaccine is further intensified by the farmers, whose livelihoods are dependent on the quality of their livestock which could be drastically affected by an APL outbreak.

In summary, APL is a highly infectious organism responsible for a huge number of deaths amongst swine. APL is also responsible for the economic devastation caused within the pork industry. There is a desperate need to prevent APL infection in pigs, as the existing control measures are not adequate enough. Current forms of treatment are only administered once clinical symptoms have been exposed, and these forms of treatment cannot prevent the further spread of infection. Vaccines once available are cheap, easy to administer and are capable of producing a humoural immune response. However development of such a vaccine has been plagued by a poor understanding of the APL organism, thus further research into APL pathogenesis is required to allow a complete insight into the mechanisms of attachment and colonisation, which would allow scientists to then elucidate proteins to act as potential vaccine candidates for the development of a cross-protective vaccine. However, until this step is reached APL will continue to destroy pigs, devastate farmers and contribute to the economic problems seen in the pork industry, as the current methods of control and prevention can do little to stop APL.

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Stressed out *Bacillus subtilis*

BACTERIAL SPORES ARE extremely long lived (Gould, 2006) and resistant to wet and dry heat, pressure, x-ray and UV irradiation, chemicals, antibiotics, and extremes of pH (Setlow, 2006).

Most of these resistances are probably produced as a result of the same structures which are responsible for longevity, since the effect of heat treatments, such as 121°C for 30 min, is unlikely to occur sufficiently often under natural conditions to have been significant in the evolutionary development of spores. Recently, new disinfectants such as a Sterilox® have been shown to kill spores readily, probably by oxidatively modifying the inner membrane of dormant spores

(Loshon *et al.*, 2001). Spore resistance to Sterilox is due in large part to the presence of spore coats but, since wet heat is the most commonly used method of spore control, there is still a need to understand how it kills spores and, at least as importantly, what the mechanisms are which protect spores against wet heat as it is used commercially. Factors which contribute to resistance to wet heat include the low water content of the spore core, the amount and type of mineral ions bound to the dipicolinic acid in the core and the protection of DNA by its saturation with α/β- type acid soluble spore proteins (SASP) (Setlow 2006). However, the mechanism of spore killing by wet heat is not understood.

One approach to understanding spore resistance and the mechanisms of spore killing is by producing spores with different resistances. This can be done using mutants which produce spores with reduced resistance (for example SASP- spores) or by altering spore structure by changing sporulation conditions. Melly *et al.*, (2002) have shown that when cells of *Bacillus subtilis* were sporulated at temperatures from 22°C to 48°C those formed at higher temperatures were more resistant to wet heat. Other workers (Sedlák *et al.*, 1993; Heredia *et al.*, 1997) had previously found that heat shock early in sporulation resulted in increased wet heat resistance in the spores formed subsequently. Using proteomics we have examined the effect of heat shock, cold shock, ethanol stress and puromycin stress applied early in sporulation on the proteins formed with a view to determining whether particular stress proteins play a part in increasing heat resistance in the mature spores. For temperature shock sporulating cells were transferred from 30°C to 48°C or 10°C for 30 min. Ethanol stress involved the culture being shocked with 4% (v/v) ethanol for 60 min, and was compared with stress produced by 20μg/ml of puromycin for 60 minutes.

Heat shock 60 minutes into sporulation increased the D_{100} value from 5±0.3 to 11±2, while cold shock reduced the D100 value from 5.2±0.6 to 1.8±0.3 and ethanol and puromycin increased the D_{100} values from 5.8±0.3 to 9.3±0.6 and 7.8±0.5 respectively (Movahedi & Waites 2000; Movahedi & Waites 2002). 2-D gel electrophoresis showed that at least 65 proteins were over expressed or induced as a result of heat shock. Eleven of these were heat specific but these same proteins were all induced after ethanol or puromycin stress when no specific stress proteins were induced. In contrast a total of 13 proteins were either over expressed or induced as a result of cold shock. The identities of eight of these proteins were confirmed and all have previously been shown to be cold shock (but not heat shock) proteins in vegetative cells of *B. subtilis*.

In all cases stress proteins were detected within 15min of the stress being applied. With heat and cold shock the biochemical response was maintained for at least 60min before declining to pre-shock levels after 120min. For both stresses the maximal indication of proteins was apparent 30-60min after the shock. With ethanol or puromycin treatment the eleven heat shock proteins were induced but, unlike heat shock, the levels peaked at 120min after stress and diminished to pre-stress levels at 180min.

The most obvious explanation for these changes in spore heat resistance is that during late germination and outgrowth stress proteins act to repair heat damage in spores. However, as a result of this work, which demonstrated that stress proteins had returned to pre-shock levels well before mature spores have formed, it would seem more likely that heat resistance is altered as a result of changes in spore structure. Melly *et al.*, (2002) found that sporulation at higher temperatures is accompanied by decreases in core water content but that the cortical peptidoglycan in spores formed at various temperatures showed very little difference in structure with only a small, although significant increase, in the percentage of muramic acid with a cross-link in spores formed at higher temperatures.

As discussed by Melly *et al.*, (2002) there may be some large change in the three-dimensional structure of the cortex, some modulation of the pattern of cortical synthesis or modification during sporulation as a function of temperature. The same could apply to stress induced changes. However, it is interesting that when the combined effects of two of these stresses are studied in sequence then the changes in stress proteins follow the changes in spore resistance and cold shock appears to dominate heat and ethanol stress.

It is worth indicating that, apart from the timing of stress during sporulation, no attempts have been made to maximise these effects nor has the effect on any killing agent other than wet heat been studied. From a practical view point in preparing spores for use in monitoring the efficacy of sterilising conditions it is important to use the conditions which produce the most resistance spores. As far as I know stress induced increased resistance is not being used.

I would like to thank Dr Sara Movahedi for setting up the proteomic system and for carrying out this study, the EU for financial support and the Society for awarding a grant from the President's Fund to allow me to attend and speak at the Society's Conference 2005. As it says on the tin, this Fund is open to members of all ages!

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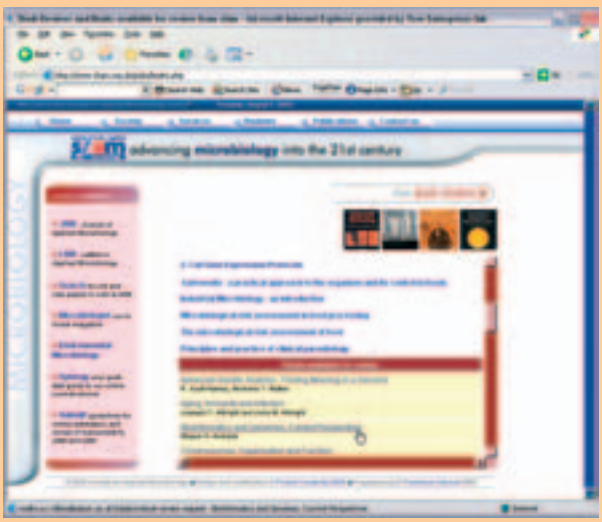

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The Pneumococcus

Edited by EI Tuomanen, T J Mitchell, D A Morrison, B G Spratt
ASM Press 2004
ISBN 1-55581-297-X. $115.99
Reviewed by Sue Lang

Streptococcus pneumoniae, or the pneumococcus as it is perhaps more commonly known, is the leading cause of invasive bacterial infection in children within the UK, associated primarily with bacteraemia and meningitis. It is also responsible for significant levels of morbidity ad mortality in the elderly population and immunocompromised patients. Although a common member of the flora of the nasopharynx it is acquisition of certain serotypes, of which in excess of 90 serotypes based on the complex polysaccharide capsule are currently recognised, and the host response to the bacterium that dictates the disease outcome.

The Pneumococcus, published in 2004, is the most recent of a number of texts in print which focus on aspects of *S. pneumoniae* physiology, pathogenicity, and interaction with the host. This is probably a reflection on the importance of this pathogen and the increase in knowledge within this rapidly changing field rather than a re-evaluation of old information.

This substantial text, edited by leading authors within the field of *S. pneumoniae* research, provides an up-to-date and authoritative account of investigations covering the breadth of pneumococcal microbiology. Following a brief forward by Robert Austrian, a pioneer in the research of pneumococcal disease, a series of 25 concise individual papers are divided between three topics; the bacteria, the host-microbe interactions, and treatment and prevention. Contributions being submitted by a panel of 46 internationally recognised experts. These papers cover cutting edge areas of research including comparative genomics which have followed in the wake of a series of genome sequencing programmes. Other aspects of research covered range from pneumolysin toxin expression and pneumococcal carriage to changes in ecology in response to antibiotics and vaccines.

Beginning with a comprehensive introduction to the item, covering many historical aspects of the subject and concluding with a succinct summary, each topic is accessible to both new and more advanced researchers.

A great strength of this publication is the holistic approach taken to the subject. The authors contributions include not only an in-depth analysis of the pathogen but also address the greater picture of the disease process including host-pathogen interaction, the inflammatory response, carriage and epidemiology.

Although there is variation in the level of detail between each topic, the writing retains clarity throughout. A greater use of colour in the numerous diagrams would have been a welcome addition and greatly contributed to the overall presentation of the volume. I suspect, however, that the inclusion of non-essential colour would have greatly elevated the cost of an already expensive text. At around £80, this book is at the more expensive end of the market, as is generally the situation with highly specialised texts such as this. The inclusion of a comprehensive subject index, not always included in works of this type, is a welcome addition.

Comprehensively referenced and clearly up-to-date, this is a thoroughly readable book and an essential for anyone with an interest in *S. pneumoniae*. This is not the only text published which focuses on the pneumococcus, but it is certainly a volume worthy of a position on the bookshelf of a new researcher, graduate or medical student and potentially a valuable resource within the research lab.

Bioremediation — Applied Microbial Solutions for Real-World Environmental Cleanup

Eds: R M Atlas, J Philp
ASM Press. Washington 2005
ISBN 1-55581-239-2 pp.366
Price: $109.95
Reviewed by J Gwynfryn Jones

This multi-author volume is written (to quote the editors) for both academics and practitioners to provide a detailed knowledge of bioremediation research and real-world applicability of that research. It claims a "truly international perspective" although with five US, 12 British and one Dutch author this perspective might be considered to be somewhat limited.

The case for bioremediation is made in the first chapter and this is followed by guides to the characterization of contaminated land and the legal and regulatory framework for bioremediation, although the latter is confined to the USA and Britain. There are chapters devoted to contaminated soils and groundwater and more specific sections on marine oil spills, metals and radionuclides. Monitoring of the bioremediation process is covered and the book ends with a discussion on preemeptive bioremediation i.e. the application of biotechnology to clean industrial products and processes.

Each chapter is accompanied by a long list of references (373 for the section on soils and aquifers) so the reader certainly has plenty of additional material to hand. The usefulness of such a large number of citations depends on the care with which they have been chosen. What is the reader to make of sentence that refers to an author's ideas on microbial *infallibility* when the reference title relates to the problem of microbial *fallibility*?

The case studies and box figures certainly provide useful examples of bioremediation methodology although this hardly seems to be the sort of book where one would expect brief (and, therefore, unhelpful) descriptions of techniques such as plate counts and MPN methods. The reader will also find that the volume has a strong "soils" bias, although this might be quite reasonable given the distribution of pollution incidents.

My main complaint is that the book is exceptionally badly edited and, as such, required intervention by the publishers. The poor English jars (page 1: "There was a large increase in...compounds that are produced" and "there have been a public demand") but the use of jargon, undefined or remotely defined abbreviations and contorted sentence construction makes reading a labour of love. The editors could have done with the advice given by my professor, to read Ernest Gowers' "The Complete Plain Words" before putting pen to paper. Unfortunately many of the figures and tables do not fare much better as their contents do not stand alone and the reader is left searching the text for interpretation. Indeed, some tables hardly seem necessary. One, entitled "Some effects of pollution and those affected" includes such items as cancer as an effect and humans as the affected, somehow forgetting other animals. This volume required a helpful editorial pen to remove endless repetition, material of little relevance and a plethora of motherhood statements. Readers should also be warned that bioremediation in this volume is confined, almost entirely, to the use of bacteria and fungi. They will search in vain for information on phytoremediation, reed-bed technology etc. The book's title, and far too many of its pages refer to "real-world" cleanup. Unfortunately, a significant proportion of the real world's pollution problems occur in developing countries, usually as the result of the activities of multinational companies. Such countries require low-tech, cost-effective bioremediation solutions. These are not provided here.

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