Microbiologist

The magazine of the Society for Applied Microbiology ■ March 2010 ■ Vol 11 No 1 ISSN 1479-2699

Biofilms
safety in numbers

INSIDE

excellence in microbiology

don whitley scientific

Want instant access to your anaerobes?

Our new portholes provide chamber access in seconds allowing you to manipulate samples as easily as working at the bench.

The rapid cycle airlock transfers 40 Petri dishes in one minute.

So take a little time to contact us today - you'll find we operate an open door policy.

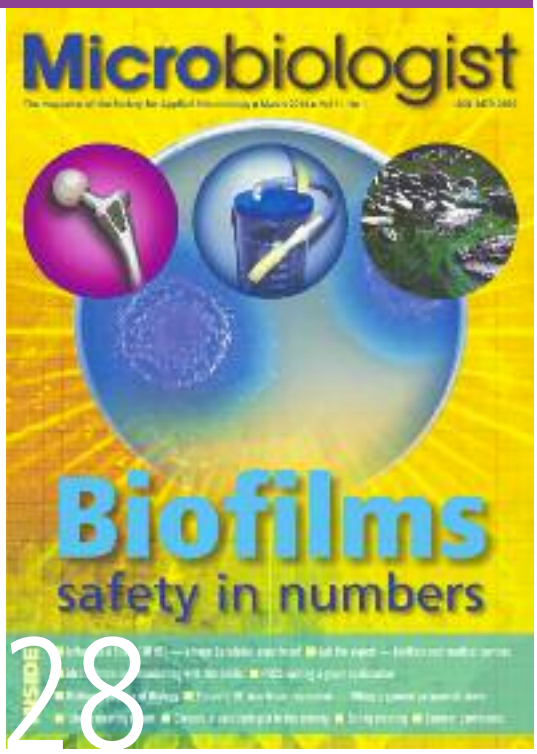
Technical sales: +44 (0)1274 595728

www.dwscientific.co.uk

March 2010 ■ Vol 11 No 1 ■ ISSN 1479-2699

Microbiologist the magazine of the Society for Applied Microbiology

contents



members

publications

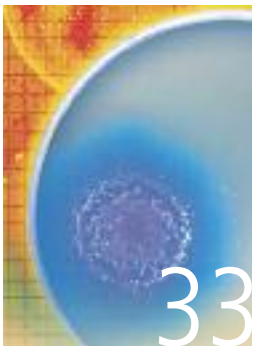
news

features

meetings

commercial

Summer Conference 2010



Secrets of a successful minimalist

information

Microbiologist is published quarterly by the Society for Applied Microbiology. ISSN 1479-2699. Registered in the UK as a charity and Company limited by guarantee. Registered in England and Wales: 6462427. Registered Charity: 1123044

Editor: Lucy Harper. lucy@sfam.org.uk

Contributions: These are always welcome and should be addressed to the Editor at: lucy@sfam.org.uk

Advertising: Lucy Harper. Tel: +44 (0)1234 326709. email: lucy@sfam.org.uk

Design and print: Pollard Creativity. Tel: +44 (0)1933 664700 email: micro@pollardcreativity.co.uk

Cover: 'Biofilms' © Joe Slonik/Pollard Creativity 2010

Society for Applied Microbiology, Bedford Heights, Brickhill Drive, Bedford MK41 7PH, UK Tel: +44 (0)1234 326661. Fax: +44 (0)1234 326678

www.sfam.org.uk

editorial

Lucy Harper talks about biofilms and communities

The "safety in numbers" hypothesis states that by being part of a group, whether human, animal or organization, an individual will come to less harm from predators. This issue of *Microbiologist* draws upon this theme and looks at biofilms, a community of organisms which, with the help of an extracelluar polymeric substance (EPS) matrix, are more tolerant to treatment with antimicrobials than the constituent individual microbes.

In a paper recently published in the *Journal of Applied Microbiology*, S*f*AM member Alex Rickard and his team at Binghampton University, USA have look at biofilms in chronic wounds. Wounds such as diabetic foot ulcers and venous leg ulcers have been known to harbour biofilms and their tolerance to biocide treatments makes such wound infections difficult to treat. Alex and his team have found a weakness in a cell-signalling system which could be blocked to weaken biofilm defenses. To find out more, visit: http://www3.interscience.wiley.com/journal/122603864/abstract

It is the tolerance of biofilms to treatment which forms the basis of our first feature article (page 28). Here, Andrew McBain provides us with a comprehensive introduction to these microbial communities, stating that: "*biofilms exhibit structural complexity indicative of a high degree of (physiological) co-ordination, co-operation and communication*." These assets: co-ordination, co-operation and communication could be applied to many situations in life which require the successful interaction of people or organizations. For example, many of you will know that the biological sciences community has been brought together by the unification of the Institute of Biology with the Biosciences Federation. On page 15 the CEO of the new Society of Biology, Mark Downs, introduces himself and describes the aims of this new organization, whose vision is: "*to represent all who are committed to biology in academia, industry, education and research*."

It is well-known that microbial biofilm communities are ubiquitous and can be found on materials as diverse as rocks in a stream, tooth enamel, and medical devices. They are formed from an enormous variety of microbes, but our second feature article concentrates specifically on *Mycoplasma* biofilms. The formation of biofilms by a large number of mycoplasma species could be "*an important step in disease initiation*" by these intriguing microbes — turn to page 33 to find out more.

The final feature article in this series asks an expert about biofilms which adhere to medical devices. How do these biofilms form and why do they cause a problem? There are a variety of interesting ways in which this problematic issue can be handled, including the use of bacteriophages to prevent biofilm formation and you can find out more on page 37.

The efficacy of biocides against biofilms has been the subject of much debate, and the topic of biocides was the basis of one of the two parallel sessions of this years' Winter Meeting. Held at the Royal Society during its 350th anniversary year, a full report of the meeting can be found on page 20. But don't worry if you missed the S*f*AM Winter Meeting. If all this discussion about microbial communities has sparked your interest and you'd like to find out more about biofilms or bacteriophages, then don't despair — this years' Summer Conference in Brighton has sessions on each of these two topics as well as a session looking at *Listeria*. You can book a place online (www.sfam.org.uk/summer_conference) or by completing the booking form on page 27.

contribute

We are always looking for enthusiastic writers who wish to contribute articles to the magazine on their chosen microbiological subject.

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

Lucy Harper

Microbiologist is published quarterly by the Society for Applied Microbiology, a registered charity. ISSN 1479-2699

Copy Dates:

Vol 11 No. 2 June 2010
Friday 26 March 2010

Vol 11 No.3 Sept 2010
Friday 25 June 2010

Vol 11 No.4 Dec 2010
Friday 24 Sept 2010

Vol 12 No.1 March 2011
Friday 17 Dec 2010

Subscriptions: A subscription to *Microbiologist* is included in the annual S*f*AM membership fee. For further information about the many benefits of membership please see page 6.

Advertising: Information about advertising in *Microbiologist* and how to submit advertisements can be found on the Society website.

Website: our website **(www.sfam.org.uk)** is a timely source of up-to-date information on all Society matters and maintains a comprehensive archive of articles and reports on a variety of microbiological topics.

contact point

Society for Applied Microbiology
Bedford Heights, Brickhill Drive, Bedford MK41 7PH, UK

tel: +44 (0)1234 326661
fax: +44 (0)1234 326678
email: communications@sfam.org.uk
www.sfam.org.uk

society office staff

CHIEF EXECUTIVE OFFICER: Philip Wheat
email: pfwheat@sfam.org.uk
tel: +44 (0)1234 326661

COMMUNICATIONS MANAGER: Lucy Harper
email: lucy@sfam.org.uk
tel: +44 (0)1234 326709

COMMUNICATIONS OFFICER: Clare Doggett
email: clare@sfam.org.uk
tel: +44 (0)1234 327679

MEMBERSHIP & FINANCE CO-ORDINATOR: Julie Wright
email: julie@sfam.org.uk
tel: +44 (0)1234 326846

EVENTS ORGANISER: Sally Cryer
email: sally@sfam.org.uk
tel: +44 (0)1234 761752

OFFICE ADMINISTRATOR: Kate Coggins
email: kate@sfam.org.uk
tel: +44 (0)1234 326661

publications subcommittee

FEATURES EDITOR: Claire Cassar
email: c.cassar@vla.defra.gsi.gov.uk

FEATURES EDITOR: Louise Fielding
email: lfielding@uwic.ac.uk

REGULAR CONTENT EDITOR: Alison Kelly
email: a.kelly@kingston.ac.uk

GRANTS EDITOR: Louise Hill-King
email: louise@hill-king.com

executive **committee**

COMMITTEE MEMBERS

HON PRESIDENT: Professor Geoff Hanlon, School of Pharmacy and Biomolecular Sciences, University of Brighton, Moulsecoomb, Brighton BN2 4GJ
email: g.w.hanlon@brighton.ac.uk

HON GENERAL SECRETARY: Dr Mark Fielder, School of Life Sciences, Kingston University, Penrhyn Road, Kingston upon Thames, Surrey KT1 2EE
email: m.fielder@kingston.ac.uk

HON MEETINGS SECRETARY: Dr Andrew Sails, Health Protection Agency, Newcastle Laboratory, Institute of Pathology, Newcastle General Hospital, Westgate Road, Newcastle NE4 6BE
email: andrew.sails@hpa.org.uk

HON TREASURER: Mr Steve Davies, Microbiology Department, Northern General Hospital, Herries Road, Sheffield S7 5AU
email: steve.davies@sth.nhs.uk

ORDINARY COMMITTEE MEMBERS UNTIL JULY 2010

Dr Louise Fielding, Food Research and Consultancy Unit, Cardiff School of Health Sciences, University of Wales Institute Cardiff, Llandaff Campus, Western Avenue, Cardiff CF5 2YB
email: lfielding@uwic.ac.uk

Professor Andrew Fox, Health Protection Agency North West, PO Box 209, Clinical Sciences Building, Manchester Royal Infirmary, Manchester M13 9WZ
email: andrew.fox@hpa.org.uk

Dr Andrew McBain, School of Pharmacy & Pharmaceutical Sciences, Stopford Building, University of Manchester, Manchester M13 9PT
email: andrew.mcbain@manchester.ac.uk

ORDINARY COMMITTEE MEMBERS UNTIL JULY 2011

Professor Christine Dodd, Division of Food Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD
email: christine.dodd@nottingham.ac.uk

Dr Leon Gorris, Unilever, SEAC Risk Analysis Group, Colworth House, Sharnbrook, Bedfordshire MK44 1LQ
email: leon.gorris@unilever.com

ORDINARY COMMITTEE MEMBERS UNTIL JULY 2012

Mr Mark Reed, Pro-Lab Diagnostics, 7 Westwood Court, Neston Cheshire CH64 3UJ
email: mreed@pro-lab.com

Dr Sally J Cutler, School of Health and Biosciences, University of East London, Stratford Campus, Romford Road, London E15 4LZ
email: s.cutler@uel.ac.uk

Dr Samatha Law, NCIMB, Ferguson Building, Crabstone Estate, Bucksburn, Aberdeen AB21 9YA
email: s.law@ncimb.com

Dr Alison Kelly, School of Life Sciences, Kingston University, Penrhyn Road, Kingston upon Thames, Surrey KT1 2EE
email: a.kelly@kingston.ac.uk

benefits

The Society for Applied Microbiology is the voice of applied microbiology within the UK and was founded in 1931. Society members play a leading role in shaping the future of applied microbiology, and enjoy many benefits, including:

- The opportunity to apply for one of our many grants or funds
- Eligibility to win any of our awards or nominate a candidate for the SfAM Communications Award
- Access to our five peer-reviewed Journals: *Journal of Applied Microbiology*, *Letters in Applied Microbiology*, *Environmental Microbiology*, *Environmental Microbiology Reports* and *Microbial Biotechnology*
- Free access to the entire collection of digitized back files for *JAM* and *LAM* dating back to 1938
- A topical quarterly magazine, *Microbiologist*
- Substantially reduced rates for attendance at SfAM meetings and conferences
- Networking with worldwide professionals in over 80 countries
- Access to private members area of the SfAM website
- Monthly email bulletins with the latest news from SfAM
- Invitation to the annual *Environmental Microbiology* lecture
- Fostering cross disciplinary research
- A 25% discount on the extensive Wiley-Blackwell collection of titles

Detailed information about all these benefits and more can be found on the Society website at: www.sfam.org.uk

GRANTS & AWARDS: Many grants, awards and prizes are available to members including the W H Pierce Memorial Prize and prizes for student oral presentations and posters at the Summer conference. In addition to these substantial awards, the Society has funds to assist members in their careers as microbiologists. These include the President's Fund, Conference Studentships, Sponsored Lecture Grants and the popular Students into Work Scheme.

Full details of all the Society's grants and awards can be found on the website together with PDF downloadable application forms.

JOURNALS: The Society publishes two monthly journals: *Journal of Applied Microbiology* and *Letters in Applied Microbiology*. We also produce this quarterly colour magazine, *Microbiologist*, which contains features, topical news stories and full details of our meetings. The Society is also a partner with Wiley-Blackwell in the monthly journals *Environmental Microbiology*, *Environmental Microbiology Reports* and *Microbial Biotechnology*.

All Full and Student members receive free access to the online versions of the Society's journals, and can also submit papers to our journals via an online submission service.

MEETINGS: We hold three annual meetings; the winter meeting is a one-day meeting with parallel sessions on topical subjects. The spring meeting is a one-day meeting tailored for personnel in clinical microbiology. The summer conference is held every July and comprises a main symposium, a poster session, the AGM and a lively social programme. All members are invited to our prestigious annual lecture held to commemorate the success of our *Environmental Microbiology* journal. We also hold joint ventures with other organizations on topics of mutual interest.

WEBSITE: The website is the best source of detailed information on the Society and its many activities. It has fully interactive membership areas where you can find archive issues of *Microbiologist*, exclusive SfAM documentation and much more.

membership options

■ **Full ordinary membership** gives access to our many grants and awards, online access to the *Journal of Applied Microbiology*, *Letters in Applied Microbiology*, *Environmental Microbiology*, *Environmental Microbiology Reports* and *Microbial Biotechnology*, copies of *Microbiologist*, preferential registration rates at Society meetings and access to the members areas of the website.

■ **Full student membership** confers the same benefits as Full membership at a specially reduced rate for full time students not in receipt of a taxable salary.

■ **Associate membership** is only open to those with an interest in applied microbiology without it being a prime aspect of their job. For example, school teachers and those taking a career break; on maternity leave, or working temporarily in other areas. It does not provide access to any journals or Society grants and awards.

■ **Honorary membership** of the Society is by election only and this honour is conferred on persons of distinction in the field of applied microbiology. Honorary members have access to our online journals.

■ **Retirement membership** is available to Full members once they have retired from their employment. Retired members are entitled to all the benefits of Full membership except grants and access to the Society's journals.

■ **Corporate membership** is open to all companies with an interest in microbiology. Corporate members benefits include:

- Quarter page advertisement in each issue of *Microbiologist* (which can be upgraded to a larger size at discounted rates)
- the opportunity to publish press releases, company news, etc., in each issue of *Microbiologist*
- FREE banner advert on the Society Website with a direct link to your company site.
- Up to three members of company staff attending Society meetings at members' rate (this means a 50% discount on non member registration rate).

JOIN US!

You can apply for membership on, or offline. To apply offline, please contact the Membership Co-ordinator, Julie Wright on **+44 (0)1234 326846**, or email **julie@sfam.org.uk.** Alternatively, write to her at:

The Society for Applied Microbiology, Bedford Heights, Brickhill Drive, Bedford MK41 7PH, UK

www.sfam.org.uk

microbreak

The answers to the microbiology rebuses competition are shown below. Congratulations to **Richard Horsman** whose correct answers were picked from the Editor's in-tray. A £30 Amazon voucher is on its way to you.


Gentamicin


Oxidase


Petridish


Genome


Zoonoses


Halophile


Salmonella

The quiz for this issue is an **anagram competition**. Identify all eight organisms correctly and you could be in with a chance of winning an Amazon voucher. Please send your answers to the Editor before Friday 2 April 2010 to be in with a chance of winning.

anagram	species name
ACHE IS LICORICE	
ANY PROJECTILE CUB JAM	
ONE SUBLIMINAL PEAK EEL	
ILLUSIONARY MAT HELP MUM	
ACCUSATORY HUE CUP LOSS	
SARS — NICER CREAM TEAS	
MUSICIAN SEES OAR	
ROUND APPLE MEAL TIM?	

An Amazon voucher is waiting for the person whose entry is picked first from the Editor's in-tray! The closing date for entries is **Friday 2 April 2010**. The answers will appear in the June 2010 issue of *Microbiologist*.

Name: _____

Address: _____

_____

Photocopy this page and send it to: 'Microbreak', Society for Applied Microbiology, Bedford Heights, Brickhill Drive, Bedford MK41 7PH, UK, or email your answers to the Editor at: lucy@sfam.org.uk.

president's column

Geoff Hanlon outlines some of the important issues currently facing applied microbiologists

I was asked recently to write an article in the Parliamentary and Scientific Committee journal *Science in Parliament* on the challenges faced by applied microbiologists. This was a useful exercise for me as it highlighted the extensive range of problems we face globally where the subject of applied microbiology plays a pivotal role. It also provided an opportunity to bring these issues to the heart of the decision making process. In this column I will outline just a few selected issues facing microbiologists at the present time and try to indicate that these are not merely academic problems but issues which impact all of us in a most profound manner.

Health issues

Healthcare associated infections

When I was chatting to a friend of mine who is Chief Pharmacist at a large, local hospital he voiced the opinion, held by many, that the biggest issue at present in the NHS is the management of healthcare associated infections. Similarly, talking to patients due to enter hospital they frequently say that contracting an infection such as MRSA or *Clostridium difficile* is what they fear most about their impending therapy. Since microbiological issues form such a central focus in this arena it is rather surprising to find that the amount of microbiology taught within the current curricula of both medical and pharmacy undergraduate degrees has decreased to a pitifully small level. Moreover, the teaching of practical aspects of microbiology in general has been severely hampered by financial constraints. This contradiction highlights a lack of recognition for the important work carried out by microbiologists in addressing the most pressing issues facing us today.

Influenza pandemics

Over 40 million people died worldwide in the space of two years during the influenza pandemic of 1918/19. This was an extraordinary number of deaths which overshadows even the ongoing HIV tragedy. Given this historical perspective it is not perhaps surprising that there was such widespread concern over the recent swine flu pandemic as a new strain of H1N1 virus emerged. However, swift responses on the part of politicians and microbiologists put in place systems to limit its impact. We still don't know what awaits us over the coming months but the role of applied microbiologists in the areas of epidemiology, identification, vaccination and treatment will be critical.

Global health concerns

Developing countries continue to face a number of health issues including infections such as TB, HIV, malaria and cholera which are still major causes of mortality and morbidity. While some problems are linked to infrastructural issues such as the supply of clean water and appropriate disposal of sewage waste, health education and training is also important. In many of these countries there is the potential to build up local scientific, particularly microbiological capacity to enable them to address their specific local issues and ultimately to help them develop sustainable economies. Learned societies can have a significant role in this agenda and the part played by the applied microbiologists who are its members will be critical.

Climate change

Bacteria are the most abundant free-living organisms on earth and countless billions of them live in our vast oceans. However, in terms of numbers even these are eclipsed by viruses. A recent special issue of the S*f*AM journal: *Environmental Microbiology*, has focussed on the subject of *Environmental Viruses: Shaping the Biosphere*. This highlighted the fact that there are an estimated 10^{31} viruses (mostly bacteriophages) on the planet and that in our oceans about 100 million metric tons of microbes die every 60 seconds due to viral infection. Thus viral predation accounts for a significant part of the ocean's carbon recycling. The precise role these microbes play in biogeochemical cycles and their part in influencing carbon dioxide levels and hence climate change is not well understood. Even less well understood is what impact rising ocean temperatures and changing acidification will have on the functioning of this gigantic biome. There is a clear need for more work in trying to understand the complex interactions which lie at the heart of this problem so that we may be more able to deal with the issues arising from climate change.

Food safety

Just after WW1 the Ministry of Agriculture appointed Advisory Dairy Bacteriologists to address the parlous state of the dairy industry, the problems of which at that time, impacted on both economic and health issues. These microbiologists solved the problems confronting them and along the way formed the forerunner of our *Society for Applied Microbiology*. Today, the dairy industry is in good shape, at least from a food safety perspective, but that is not to say that problems don't exist elsewhere. The globalisation of the food market, increasing use of minimally processed foods, the desire for organic food production etc., all present microbiological challenges. Recent cases of *E. coli* O157 outbreaks in the UK have focussed our attention on this issue and highlight the fact that we must not become complacent. As in the past we can be sure that members of our society will be at the forefront of efforts to address these issues.

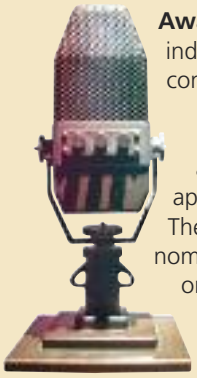
The role of learned societies

Learned societies bring together like-minded specialists to enable them to share their expertise and to act as a voice for their subject discipline, particularly attempting to influence policy makers on matters of importance. This might include putting the case for more microbiology to be introduced into clinically related undergraduate courses or more money to be made available for the effective teaching of microbiology laboratory skills. A further vital function in our case is to effectively communicate the science of applied microbiology to the general public and the media. In this context we work with representative bodies like the *Society of Biology* (recently formed by the amalgamation of the *Institute of Biology* with the *Biosciences Federation*) and organizations such as the *Science Media Centre*.

Our society has members engaged in day-to-day work within microbiology laboratories in academia, the NHS, other government-funded organizations and industry. These people are at the cutting edge in dealing with some of the most important issues facing us at the current time. Learned societies exist to support them in their endeavours and to push these issues further up the political agenda. In particular they are there to highlight the vital role played by this often-overlooked group of specialists. It is essential for the long term prosperity of the UK that sufficient resources are available to support applied microbiology so that the challenges we face can be adequately addressed.

Professor Geoff Hanlon
President of the Society

SfAM Communications Award call for nominations!



The *Sf*AM **Communications Award** aims to recognize individuals who have communicated their work/applied microbiology to the general public. The overall aim of this award is to raise the profile of applied microbiology and *Sf*AM. The award will be for £1000 and nominations must be from Full ordinary or student members with a deadline in April each year. Nominations should be in writing, providing detailed information about all relevant media/communications work of the nominee. Nominations should be made by members of *Sf*AM but nominees do not have to be members of the Society. Nominees could include:

- Professional communicators: broadcasters, authors or science writers/journalists
- Scientists who are recognized science communicators
- Scientists who are not yet recognized science communicators but have significant experience of working with the media
- Teachers/lecturers
- Artists

The nature of the communication can be local, national or international factual or fictional works including: fiction books, factual books, popular science books, newspaper / magazine articles, film, television (series or documentary), lectures or lecture series, classroom demonstrations, works of art / exhibitions or any other format a nominee considers appropriate.

The award will be presented every year at the summer conference dinner and the winner will be asked to give an after dinner speech as a condition of receiving the award. Members who make a nomination are responsible for contacting the nominee to ensure they are available on the date of the summer conference dinner. **The closing date for applications is 9 April 2010.**

To make a nomination, please download and complete a pdf application form from the website: www.sfam.org.uk/grants.php and send five copies of the application together with the nominated newspaper article, magazine, DVD or other medium to the *Sf*AM office.

ceo's column

Philip Wheat reports on the latest developments within the Society

Welcome to my first column of 2010. It is once again pleasing to report that membership numbers are still increasing. Last year over 300 new members joined the Society and I can confidently predict that this year, we will see membership numbers reach over 1700. The Society can also truly call itself an international society. We have members in over 70 countries, with those who do not reside in the UK accounting for over 30% of the membership.

In the last issue of the *Microbiologist* we were able to announce that a full member can now attend the entire summer conference at the Grand Hotel, Brighton, for the highly discounted rate of £250 (see page 25). The decision to reduce the Summer Conference fee was made in part as a result of feedback from the membership questionnaire, where a number of members indicated they felt the cost of the Summer Conference was too high. This year's fee is at least a £200 reduction from previous years and covers three nights accommodation with breakfast in the Grand Hotel. It also covers the provision of dinner for two nights with just an additional £20 cost for attendance at the official conference dinner. In addition, the fee also covers all the social events including a reception on the first evening and access to the trade exhibition where wine will be served. As well as the social programme, this fee includes access to the full scientific programme, where this year we have three themes: Biofilms, *Listeria* and Bacteriophages. There will be 19 presentations in total from national and international speakers covering these three areas. I am writing this article in December 2009 and already we have delegates applying for the Summer Conference next July. The numbers we can accommodate are limited so I strongly recommend that you register as soon as possible to avoid disappointment (visit www.sfam.org.uk/summer_conference.php or see page 25 to book your place).

Another new initiative for 2010 is the creation of Professional Development Groups, the remit of which will be as follows:

- Suggest meeting topics and scientific programmes for their area of interest. These topics may or may not be for the established Winter or Spring Meetings, or Summer Conference.
- Organize local and national practical workshops for their area of interest.
- Act as a source of knowledge and information for any members' enquiries within their area of interest.
- Act as a source of expertise for any relevant consultations on which the Society may be asked to comment.

I am delighted to announce that the first group covering Medical and Veterinary Microbiology will be led by Dr Mark Fielder (m.fielder@kingston.ac.uk). Mark is keen to hear from any member interested in joining the group. We are looking to create at least another two groups by the end of this year, so look out for further announcements concerning this matter. If any member would like more information, please contact me (pfwheat@sfam.org.uk) to discuss in more detail.

I am pleased to announce that the details of 2010 *Environmental Microbiology* lecture have just been finalized (see page 13). The speaker for this event will be **Professor Willy Verstraete** from Ghent University, Belgium. Professor Verstraete is the head of the Laboratory of Microbial Ecology and Technology (LabMet) based at Ghent University. His research and development has a central theme: processes mediated by microbial mixed cultures, which looks at microbial transformations in waters and soils and the gastro-intestinal tract. The title of his presentation will be *"Microbial resource management."* Further details about Professor Verstraete can be found at http://www.labmet. ugent.be/en/?staff:head. The lecture will take place on 11 October 2010 at the Royal Society of Medicine, London and all members of the Society will receive an invitation to this prestigious event.

Philip Wheat
Chief Executive Officer

Call for Nominations to Committee

There will be up to three vacancies on the SfAM Committee in **July 2010.** Nominations are invited from all Full members of the Society for these vacancies.

Nominations must be made in writing and received by the Society Office by **7 May 2010**.

Should nominations exceed vacancies, election will be by a system of postal voting arranged by committee.

membership matters

Membership changes

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

K. Hodgson

Canada

S. Banerjee

Chile

A. Gonzalez

Denmark

V. Kastbjerg

Germany

T. Wassenaar

Greece

K. Kormas

Guatemala

D. Roche Recinos

Ireland

O. Condell

Malaysia

T. Wu

Mexico

F. Tuz-Dzib; Velasco-Bedran

New Zealand

K. Calvert

Nigeria

O. Adejoye; F. Adesina; O. Elutade; C. Kester; O. Kuforiji; O. Obayori; O. Oyetibo; O. Tinubu; A. Uba

Spain

B. Maru

Thailand

S. Lumyong

The Netherlands

P. Chanos

UK

M. Aguas; H. Al Badri; A. Arora; A. Aspin; C. Baker; C. Boinett; F. Bolatito; M. Chattaway; K. Chipasa; A. Concord; D. Costa Bento; M. Cubitt; M. Davies; L. Davies; A. Devani; E. Duddle; N. Elwell; O. Erhabor; E. Evans; R. Fagan; E. Fokou; D. Garcha; M. Gillett; P. Goddard; E. Haas; A. Hall; S. Jayasekara; X. Jian; S. Joseph; L. Joshi; A. Kang; A. Khan; A. Kowalczyk; C. Lam; E. Linley; P. Marsh; L. Moran; J. Nale; M. Nishad; N. Pillai; S. Price; T. Prince; A. Ram; N. Reed; A. Ridley; M. Rizwan; K. Rowe; M. Shahzad; R. Shields; F. Short; K. Shoukat; C. Smith; L. Stewart; S. Thurston; E. Tichy; J. Tyrrell; S. Uthman; J. Villarreal Chiu; S. Wallis; X. Ze

USA

J. Clayton; A. McAuley; R. McDonald

Corporate member

Macaulay Scientific Consulting Ltd

Losses

We were saddened to learn of the deaths of the following members of the Society:

Carol Adair and Ellen Garvie

OBITUARY

Christopher Collins MBE

The death of Chris Collins on Monday 19 October deprived the Society of one of its staunchest supporters, and society in general of a worthy and influential citizen. Chris, as we all knew him, became an internationally acclaimed expert on the mycobacteria, especially those concerned with tuberculosis, and on the prevention of laboratory acquired infections. He had a most successful career though the route he followed had some unusual features.

Christopher Herbert Collins was born in Luton in 1919 and was educated from the age of 11 at a county Grammar School of which, according to his own biographical notes, he had a low opinion. Fortunately he received much valuable instruction in art, music and literature from his aunt. He did well in school examinations but despite obvious academic ability did not attend university; this will seem strange to those who grew up after the Second World War, but not to earlier generations. Few went to university in the 1930s unless they had access to family money or scholarship funding. Chris, lacking both, sought employment and obtained a position as trainee sanitary inspector, and also acted as part-time laboratory assistant to the Medical Officer of Health of the Borough of Luton.

In 1939 he joined the Royal Air Force Volunteer Reserve and had the good fortune to be posted to the RAF Institute of Pathology which enabled him to increase his knowledge of bacteriological practice and so further his career while serving. Indeed, the Service flew him to London to take the intermediate examination of the Institute of Medical Laboratory Technology (IMLT). It was during his service in Iceland that he sustained a crushed foot in an air accident; this caused him considerable trouble in later life.

After the war he was welcomed back to the municipal laboratory at Luton and studied for the qualification of Associate of the IMLT. He became a Fellow in 1951, having provided a dissertation on methods for the cultivation of mycobacteria; this proved to be a turning point in his career. In 1952 he started work in the Public Health Laboratory Service laboratory in County Hall, London, where he was able to pursue his special interest in mycobacteria. In those days, the classification of mycobacteria other than the tubercle and leprosy bacilli was chaotic but Collins was able to introduce some order into the system. As a result of his published work on this topic he was promoted to senior technical officer. In 1965 he qualified as a Member of the Institute of Biology, a qualification equal to a university degree; subsequently he was elected a Fellow of this Institute. For many years he was responsible for diagnostic tuberculosis bacteriology in London and the South East and made many studies on the distribution of the various types of tubercle bacteria in the community. This work revealed that cases of human tuberculosis acquired from cattle still occur in the community decades after the completion of bovine tuberculosis eradication programmes. He made significant contributions to the laboratory study of tuberculosis and, together with his colleagues John Grange and Malcolm Yates, developed tests for distinguishing between the different types of bacteria causing this disease: these tests were eventually adopted by the World Health Organization.

The TB laboratory at County Hall was among

Call for nominations for W H Pierce Prize

Do you know a young microbiologist (under 40 years of age) who has made a substantial contribution to microbiology? If so, why not nominate them for this prestigious and substantial award which is now worth £3,000. The award was instituted in 1984 by Oxoid to commemorate the life and works of the late W H (Bill) Pierce, former chief bacteriologist at Oxoid Ltd and a long time member of the Society. The prize is presented annually at the summer conference. Full members wishing to make a nomination for the 2010 prize should write in confidence to the Hon. General Secretary, Dr Mark Fielder, at the Society Office in Bedford, including a full CV of the nominee and a letter of support. Please note that application is through nomination by Full members of SfAM only and that there are no official forms for this award.

Closing date for nominations is Friday 23 April 2010.

the first to be equipped with microbiological safety cabinets and, with characteristic enthusiasm, Collins embarked on a study of such cabinets and laboratory safety in general. He became known in this field through several publications and this led to collaboration with the safety officer of the Microbiological Research Establishment at Porton Down and appointment to the Special Programme on Safety in Microbiology of the World Health Organization.

On his official retirement in 1985 he was awarded membership, soon followed by Fellowship, of the Royal College of Pathologists, an honour rarely given to those who are not medically qualified. Chris received an MBE in 1972 and was awarded the degree of Doctor of Science in1986. He was also appointed a Research Fellow at King's College Hospital and a Senior Visiting Research Fellow at the National Heart and Lung Institute, now part of Imperial College School of Medicine. He was made an honorary member of the European Biosafety Association (EBSA) in 2002 for his outstanding contribution to biosafety. At the age of 80 he enrolled at the University of Kent at Canterbury to read for an MA which was awarded in 2003 for a thesis entitled '*Cholera and the sanitary revolution of 19th century England*'.

In addition to his practical contribution to medical microbiology, notably in the fields of mycobacteria and laboratory safety, for which he produced some 50 scientific papers, he distinguished himself as an author of many books and monographs. Three of his books have been published in more than one edition. The first of these, *Microbiological Methods* (1964), written in collaboration with his second wife, Patricia M. Lyne, herself a long-serving member of the then Society for Applied Bacteriology, is now in its eighth edition. *Laboratory acquired infections* appeared in 1983 (4th edition 1999) and *Organization and practice in Tuberculosis bacteriology* in 1985; a second edition (1997) of the last mentioned was re-named as *Tuberculosis bacteriology — organization and practice*. At the time of his death he had just finished a new book, provisionally entitled *Filth and fevers*, an account of the revolution in public health practice in the 19th century.

Chris joined the then *Society for Applied Bacteriology* (SAB) in 1963 and served it well by making use of his considerable editorial skills as a co-editor of the *Journal* and as a member of the Editorial Board over many years. He was made an Honorary Member of the SAB in 1994. He was also an organiser of the 25th Society Symposium on *Mycobacterial Disease — Old Problems, New Solutions* in 1996 and co-editor of its Proceedings, published as a Supplement to the *Journal* (Vol. 81).

As a companion, Chris was a delight to be with. He was a great raconteur and a source of numerous anecdotes, many of them highly entertaining. He spoke in glowing terms of the many distinguished microbiologists he had encountered in his long professional career and gratefully acknowledged the help and support that he had received from them. In turn he generously helped and encouraged many fledgling microbiologists. He had a great and abiding respect for genuine leaders, including Sir Graham Wilson and Sir James Howie who were Directors of the Public Health Laboratory Service, but little time for administrators who hide their incompetence behind a veil of self-importance and status. In conversation, Chris revealed himself as a man of wide interests, with a keen but kindly appreciation of the deeds and misdeeds of his fellow humans, and a strong sense of humour which was never far beneath the surface. He was enthralled by the characters in the TV programme '*The Magic Roundabout*' (a preference not unknown among senior academics!) and by the antics of those comic strip adventurers, *Asterix and Obelix*. In a slightly more serious mood he would draw the listener's attention to the subtle differences of character that distinguish one single malt from another. I always enjoyed talking to Chris — somehow he made one feel better. I cannot think of a better epitaph than that.

In 1942 Chris married his cousin Elizabeth and they had two sons. Elizabeth died in 1966 and he married Patricia Lyne two years later: there was one son by this second marriage. Chris died on the day following his 90th birthday.

Fred Skinner

Environmental Microbiology Lecture 2010

The 2010 *Environmental Microbiology* lecture will be presented by **Professor Willy Verstraete** of Ghent University, Belgium. The title of his presentation will be "*Microbial resource management*". Professor Verstraete is the head of the Laboratory of Microbial Ecology and Technology (LabMet) at Ghent University. The lecture will take place on the 11 October 2010 at the Royal Society of Medicine, London and all members of the Society will receive an invitation in due course. Further details about Professor Verstraete can be found at http://www.labmet.ugent. be/en/?staff:head. For members unable to attend, the lecture will be available online immediately after the event.

journalWatch

News about the Society's journals

Journal of Applied Microbiology
The following articles published in 2009 were the most downloaded articles from Journal of Applied Microbiology between January – December 2009:

Applications of cyanobacteria in biotechnology. R.M.M. Abed, S. Dobretsov and K. Sudesh, **Vol. 106**, No. 1, January 2009

The bacteriophages in human- and animal body-associated microbial communities. A. Letarov and E. Kulikov, **Vol. 107**, No. 1, July 2009

Microbial nitrilases: versatile, spiral forming, industrial enzymes. R.N. Thuku, D. Brady, M.J. Benedik and B.T. Sewell, **Vol. 106**, No. 3, March 2009

The effect of climate change on the occurrence and prevalence of livestock diseases in Great Britain: a review. P. Gale, T. Drew, L.P. Phipps, G. David and M. Wooldridge, **Vol. 106**, No. 5, May 2009

Plant growth promotion and biological control of *Pythium aphanidermatum*, a pathogen of cucumber, by endophytic actinomycetes. K.A. El-Tarabily, A.H. Nassar, G.E.St.J. Hard and K. Sivasithamparam, **Vol. 106**, No. 1, January 2009

Letters in Applied Microbiology
The following articles published in 2009 were the most downloaded articles from Letters in Applied Microbiology between January – December 2009:

Comparison of T-RFLP and DGGE techniques to assess denitrifier community composition in soil. K. Enwall and S. Hallin, **Vol. 48**, No. 1, January 2009

Bacterial spoilage of wine and approaches to minimize it. E.J. Bartowsky, **Vol. 48**, No. 2, February 2009

Assessment of the bacterial diversity of breast milk of healthy women by quantitative real-time PCR. M.C. Collado, S. Delgado, A. Maldonad and J.M. Rodríguez, **Vol. 48**, No. 5, May 2009

The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobials. S.F. van Vuuren, S. Suliman and A.M. Viljoen, **Vol. 48**, No. 4, April 2009

Isolation and characterization of alginate-degrading bacteria for disposal of seaweed wastes. J.-C. Tang, H. Taniguchi, H. Chu, Q. Zhou and S. Nagata, **Vol. 48**, No. 1, January 2009

Environmental Microbiology
The following articles published in 2009 were the most downloaded articles from Environmental Microbiology between January – December 2009:

Quorum sensing in *Pseudomonas aeruginosa* biofilms. T. R. de Kievit, **Vol. 11**, No. 2, February 2009

Insights on *Escherichia coli* biofilm formation and inhibition from whole-transcriptome profiling. T. K. Wood, **Vol. 11**, No. 1, January 2009

Comparative day/night metatranscriptomic analysis of microbial communities in the North Pacific subtropical gyre. R. S. Poretsky, I. Hewson, S. Sun, A.E. Allen, J. P. Zehr and M. A. Moran, **Vol. 11**, No. 6, June 2009

Applications of the rep-PCR DNA fingerprinting technique to study microbial diversity, ecology and evolution. S. Ishii and M. J. Sadowsky, **Vol. 11**, No. 4, April 2009

Effect of PCR amplicon size on assessments of clone library microbial diversity and community structure. J. A. Huber, H. G. Morrison, S. M. Huse, P. R. Neal, M. L. Sogin and D. B. Mark Welch, **Vol. 11**, No. 5, May 2009

Environmental Microbiology Reports
The following articles published in 2009 were the most downloaded articles from Environmental Microbiology Reports between January – December 2009:

Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. H. W. Paerl and J. Huisman, **Vol. 1**, No. 1, February 2009

Crystal ball – 2009. **Vol. 1**, No. 1, February 2009

Bacterial diversity associated with freshwater zooplankton. H-P. Grossart, C. Dziallas and K. W. Tang, **Vol. 1**, No. 1, February 2009

Raman tweezers sorting of single microbial cells. W. E. Huang, A. D. Ward and A. S. Whiteley, **Vol. 1**, No. 1, February 2009

Honeybee colony collapse due to *Nosema ceranae* in professional apiaries. M. Higes, R. Martín-Hernández, E. Garrido-Bailón, A. V. González-Porto, P. García-Palencia, A. Meana, M. J. del Nozal, R. Mayo, J. L. Bernal, **Vol. 1**, No. 2, April 2009

Microbial Biotechnology
The following articles published in 2009 were the most downloaded articles from Microbial Biotechnology between January – December 2009:

Bioremediation, a broad perspective. P. van Dillewijn, H. Nojiri, J. Roelof van der Meer and T. K. Wood, **Vol. 2**, No. 2, March 2009

Twenty one important things you should know. C. Michán, J. L. Ramos, C. Daniels, **Vol. 2**, No. 4, July 2009

Microbial degradation of lignin: how a bulky recalcitrant polymer is efficiently recycled in nature and how we can take advantage of this. F. J. Ruiz-Dueñas, Á. T. Martínez, **Vol. 2**, No. 2, March 2009

Uracil influences quorum sensing and biofilm formation in *Pseudomonas aeruginosa* and fluorouracil is an antagonist. A. Ueda, C. Attila, M. Whiteley and T. K. Wood, **Vol. 2**, No. 1, January 2009

A broad range of themes in Microbial Biotechnology. C. Daniels and J-L. Ramos, **Vol. 2**, No. 1, January 2009

Sam Holford
Wiley-Blackwell

bio **focus**

Mark Downs introduces the Society of Biology in his first column for *Microbiologist*

The Society of Biology is a single unified voice for biology:

- advising Government and influencing policy
- advancing education and professional development
- supporting our members
- engaging and encouraging public interest in the life sciences

For further information visit:

www.societyofbiology.org

As most readers will be aware, the Society of Biology formally came into existence on 1 October 2009 following the unification of the Biosciences Federation and the Institute of Biology. Although we plan to build on the heritage of these two important bodies it is important for all of us to see the Society as a new organization with a different outlook and approach to its organizational parents. For the first time we have one body to represent the interests of all biologists in the UK creating a single powerful voice to advise and inform Governments and make a difference. That aspiration can only be realized if we are a nimble organization, quick to respond to opportunities, capable of learning from our mistakes and willing to work in partnership. We also need to be truly proactive to really drive the policy agenda.

At the time of reading this article the Society of Biology will be less than six months old and there will still be plenty of opportunity to shape the way we work. But, to do that we need to understand what matters to our members, how you will judge if we are delivering for you and how best to involve you. Any member of the team here would be delighted to hear your thoughts and, as the newest recruit, I am particularly keen to learn about the way the Society for Applied Microbiology would like to be represented by us.

The Society now has over 70 Organisational Members and nearly 12,000 Individual Members. This represents 80,000 biologists, giving us the legitimacy to speak with authority in all our work.

Our Council have identified four priority areas for 2010:

Firstly, **practical biology**. No matter which biological discipline undergraduates or postgraduates follow, there needs to be the opportunity to practise science at the bench or in the field. It is simply not tenable to expand undergraduate science education without additional resource to facilitate hands-on experience of designing real experiments and interpreting the results. As Keith Gull, Professor of Molecular Biology at Oxford University and Council member of the Society of Biology said in a recent interview to the *Evening Standard* newspaper, responding to Lord Mandelson's announcement on spending cuts, "*A perfect Storm is gathering. Our next generation of scientists will need to look very carefully at the quality of degrees on offer. If we want top scientists—to innovate, to find out fundamental truths and to get us out of recession—this is simply not good enough*" (sic). There has to be the resource to properly fund practical biology both in schools and in the higher education sector. We will be pushing this message at every opportunity, especially in the run up to the election.

Secondly, the **impact of biology**, a central theme in the recent consultation on the Research Excellence Framework. Most biologists accept that the public have a right to know that the money they spend on research is being spent wisely. Its impact on our economy, health care system, environment and society is important to recognize. But it has to be a sophisticated measurement. We plan to build on existing work and present a consistent and clear case around the impact of biology from blue sky research to the most applied. Case studies will be an important part of that and microbiology is surely one of the best sources.

Thirdly, we will continue to work on a pilot **accreditation programme** to report back to the Office for Life Sciences. The Government has asked us to look at ways in which some biological science degrees can be accredited to give greater confidence to students and employers so that they provide the solid grounding needed for employment. There is no doubt that the topic arouses strong views. The academic community does not want to be forced into a corner with no room for innovation in their degree programmes or to become a surrogate for technical training programmes, whilst industry bemoans the lack of hands-on laboratory skills of many graduates. I am convinced there is a route to delivery of a solution that meets the needs of both camps. A lot rests with the terminology used. Any accreditation programme we take forward will benefit from wider consultation and will certainly not be compulsory. It is likely to focus on core requirements for biological science courses to be accredited, such as numerical content, experimental design, opportunity for hands-on experimentation and intellectual rigour. It is certainly not about accrediting individuals or asking for coverage of specific training tasks or a defined list of techniques. For sure, biology is more diverse than chemistry or engineering, but by starting in specific areas real benefit can accrue, along with experience.

Finally, we will of course be talking to all the parties in the run up to the **General Election**, forcing them to focus on their science agenda and representing the interests of biology, raising its profile and using our work on "impact" to argue for investment.

The Society will also be working on many wider education, science policy and public understanding of science issues and, of course, trying to evolve new services to benefit our members. We welcome your suggestions for the Society, if you have data or views to share please email me at markdowns@societyofbiology. org.

Dr Mark Downs, Ph.D, FSB
Chief Executive, Society of Biology

Communicating with the public

The Veterinary Laboratories Agency (VLA) International Conference on Animal Disease 2009 included a session on "**Communicating with the public**" which was co-organised by S*f*AM. The session had a mixed audience of both scientists and the general public and the programme reflected this, covering a variety of perspectives of science communication

The session began with Professor Peter Borriello, VLA Chief Executive, who entertained the non-scientists in the audience with a talk entitled: "*germs for beginners.*" This was an interactive session with Professor Borriello asking the audience questions and comparing aspects of microbiology to real-life situations. He went through the basics of organism classification, general categories of sites of infection and defence mechanisms. During this section of the talk, he removed one of the barriers to effective science communication, scientific jargon, by comparing the human body to a fort, where pathogenic microbes are the enemy. He asked the audience how the fort (body) can defend itself against attack from the enemy (microbes). One answer was 'the walls' which, in human body terms translated as 'the skin'. This entertaining and informative talk continued with descriptions and explanations of some bacterial virulence factors, as well as electron micrographs of some pathogenic bacteria accompanied by very clear descriptions of their clinical effects. Finally, Peter demonstrated the importance of effective communication of microbiology to the public with some poignant images of people showing symptoms of, among other diseases, meningitis. This reminded the audience that, although we constantly live safely with microbes, awareness is important as some of them can cause serious problems.

Next Chris Thorns, Science Director of the VLA, provided a fascinating overview of the work done by the VLA as well as taking us through the history of this important organization. The aims of the VLA are: "*to safeguard public and animal health, protect the economy and enhance food security through world-class veterinary research and surveillance.*" Of the 1300 staff, the majority (850) are based at Weybridge—-a specialist facility where the VLA (previously the Central Veterinary Laboratory) has been based since 1917. Chris described the testing, surveillance, diagnostic and reference aspects of the work of the VLA and listed the main disease areas of research, including bovine tuberculosis and influenza. He illustrated his talk with pictures of the Weybridge site developing over time. This included the construction of the M25 which, for those who've not visited the site, is deceptively close. Chris explained that the VLA is world-renowned and recognized by the World organisation for animal health (OIE), Food and Agriculture Organization (FAO) or World Health Organization (WHO) for more than 15 animal and zoonotic diseases, including avian influenza, rabies and BSE.

Following a coffee break, the audience was presented with the agency perspective of science communication from two high profile organizations, the Food Standards Agency (FSA) and the Health Protection Agency (HPA). The first of these came from Terrence Collis of the Food Standards Agency (FSA) which has received both positive and negative publicity. A recent example was the publicity associated with a study on the nutritional benefits of organic foods. One headline described the study as "*a cancerous conspiracy to poison your faith in organic food*". Terrence explained the FSA's policy for openness and transparency. This means it can be difficult to spin an FSA story. He also discussed ways in which the FSA increase their profile, through a twitter presence and a blog written by their Chief Scientist, Andrew Wadge. This has proved very effective as a means of commenting on relevant news stories using evidence to back up or counter a particular story. Last year one paper covered a story on eating bacon, claiming in their headline "*bacon butties will kill you*". The blog post that followed this article argued that

media**watch**

microbiology and the media

If you have any views on science in the media which you think should feature in this column, please send them to the Editor at: **lucy@sfam.org.uk**

our 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.

eating bacon was fine in moderation, using scientific evidence to reassure readers of the facts about eating bacon. The combination of all these communications strategies has led to the FSA being trusted by the public and treated as an authority by journalists.

Viv Brealy from the Health Protection Agency (HPA) followed by discussing the changes in the way the HPA are communicating science. They began their communications strategy on a reactive basis, responding to health issues as they arose in the media, but this is changing. Viv explained that the communications strategy of the HPA is in line with the vision of the organization to: "*reduce key infections, minimise the health impact of hazards and to reduce harm in incidents and emergencies*". To ensure the general public are receiving the correct information about health, the HPA carried out a survey to find out where people go for information about health, who they trust to give them this information and the health issues about which they are concerned. This enabled the HPA to target their communications effectively. The importance of up-to-date information was illustrated by the repetition of this survey with the aim of changing current practice to ensure the public are receiving the information they need.

The journalist's perspective followed, with Vivienne Parry, a broadcaster and journalist, providing an entertaining take on her top 10 tips for scientists speaking to the media. Without spoiling the fun for any members who have yet to see Vivienne speak, some of her top tips include:

1. Read, watch, listen—you need to be aware of the nature of the programme/publication for which you're being interviewed.
2. React quickly—keep in mind that journalists have tight deadlines, so if you can't help, it's more useful to a journalist if you let them know this straight away. But make it clear if you would like to help in the future.
3. Mind your language—is the vocabulary you're using easy enough for your auntie Maureen, who knows nothing about science, to understand?
4. Practice—learn to keep it simple. Local radio and TV are good ways of getting practice. Finally watch or listen to everything you do, learn your nervous twitches and try to stop yourself from repeating potentially distracting behaviour.
5. Be committed—passion and enthusiasm for your subject will come across in an interview.

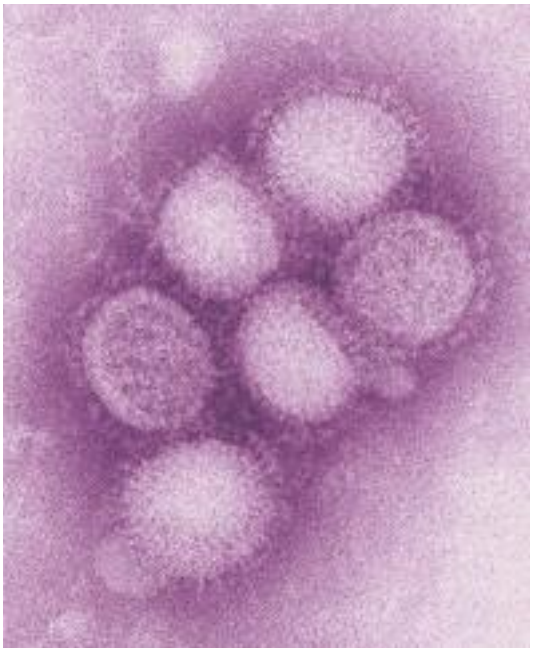
Finally, Vivienne offered some general advice from her own experiences: if a print journalist is quoting you, ask them to repeat the quote back. Establish how much the journalist knows already. Be human: when an interviewer asks is it safe, they want to know if you would drink it, or even let your children swim in it.

The penultimate speaker was S*f*AM Hon. General Secretary, Mark Fielder who provided a scientist's perspective of talking to the media. Mark firstly posed the question "*why should scientists talk to the media*?" Mark answered this question with reference to a number of sensational headlines, emphasising the point that if scientists who are experts in a certain area don't talk to the media, somebody else will and this may not lead to accurate reporting. Mark then went on to discuss his own mixed experiences of talking to the media. One piece of advice he offered was to make sure you know who you are talking to, illustrating the point by describing a negative experience he had previously when talking to someone with a specific agenda. However, Mark has not let this deter him from speaking to the media, and he countered this with a description of a very positive experience he's had talking to a reputable journalist and the extensive coverage generated as a result. Mark encouraged all scientists to talk to the media as the public are often funding scientist's research, so it's right that they find out how their money is being spent. He provided tips on who to ask for help—seeking assistance from your Press Officer, or taking up media training can help. Also, it's important to know your boundaries and only talk about that which is relevant and not protected by legislation. Finally he urged the scientists in the audience not to complain about the 'old fart' on the telly unless they are prepared to give it a go themselves.

The final presentation was given by Anthony Hilton of Aston University whose talk was entitled '*does our message reach the young*?' He began by showing the audience video footage of young people of varying ages who were asked what they knew about microbiology. The video demonstrated that many young people had little, if any knowledge of microbiology. Anthony explained that whilst microbiology is on the school syllabus, schools rarely have the resources or infrastructure to provide laboratories which are sufficiently equipped for microbiology practical classes. As many school students are kinaesthetic learners (they learn by doing) practical lessons are one of the best ways of teaching children about microbiology. Anthony then went on to talk about his own work with young people, including a Wellcome Trust funded outreach project called: "*The world of microbiology*". This project takes a lorry fully kitted out as a microbiology lab to different schools. The scientists involved then present lectures and practical classes to the school children to teach them about hygiene, foodborne infection, sexually transmitted infections (STIs) and other aspects of microbiology. Through a pre- and post-course questionnaire it has become apparent how much the students learn through these classes. Anthony concluded his talk by suggesting that yes our message can reach the young… "*but it needs help*".

Following the talks, Professor Borriello facilitated a lively discussion session which involved input from members of the public as well as government scientists and science communicators. Many thought-provoking questions were raised such as: should all scientists communicate? And: why are younger bench scientists not often encouraged to comment on media stories? Many universities are now involving their younger researchers in communication skills training to try and redress the balance and to remove the perception of science and scientists as somehow mysterious and remote from the general public. It may take some time, but with the help of organizations such as the Science Media Centre and Sense About Science, as well as Press Officers of Universities and Learned Societies, the accurate reporting and communication of science is no longer just an aspiration. The session finished with a thought "*If you can't explain Nuclear Physics to a barmaid, there's nothing wrong with her, there's something wrong with you.*"

Lucy Harper and **Clare Doggett**
Society for Applied Microbiology



Influenza A Swine (H1N1) is conducting a huge Darwinian experiment

Professor **John S Oxford** presents us with an interesting perspective on the current influenza pandemic

This year we are all part, willing or not, of a great experiment as influenza A California/4/09 H1N1, the first Swine influenza isolate in Orange County California, sweeps the planet. The virus is the fittest of the fittest, displacing epidemic influenza A (H3N2) and H1N1 viruses alike. The H3N2 virus had been with us causing epidemics and death each year since 1968 when it emerged suddenly in South East Asia (Stuart-Harris, Schild & Oxford, 1988) becoming dominant quite quickly and displacing its predecessor influenza A (H2N2). The seasonal influenza (H1N1) has a more curious origin having escaped from a laboratory in South East Asia in 1977.

But I want to push back even further, to 1918. During the years of the First World War a novel influenza A (H1N1) virus emerged in Europe (Oxford, *et al.*, 2002) and spread worldwide, aided by the dominant British Navy as millions of young soldiers returned home to Australia, New Zealand, Canada, South Africa and the USA. This current influenza A/Swine virus still has some genes from the Spanish influenza of 1918, albeit like a cousin six times removed. The 1918 virus, has quite rightly I believe, been called the Mother Of All Influenza Viruses. It forced out the preceding Russian pandemic of 1889, probably H3, and dominated the world until 1957. But even the new Asian influenza A H2N2 virus of 1957 retained some H1N1 genes, as did the next pandemic influenza A H3N2 virus of 1968 and now as does influenza A/Swine (H1N1). The A/Swine virus is a triple reassortant.

But there is yet another way to look at this great Darwinian experiment. Incidentally, I am sure Darwin is chuckling in his grave at all this, seeing the crux of his theory in action in front of his eyes over a period of months rather than millions of years. A/Swine (H1N1) has caused fewer deaths than in an 'ordinary' seasonal influenza year. How has this conundrum arisen? The crux of the matter is fitness and survival. The new A/Swine (H1N1) virus fits so beautifully, so smugly, into its ecological niche in the human airway, despite a modest reproduction number (Ro) of 3.3 and generation time of two-to-seven days, that it has pushed 99% of seasonal influenza A virus aside. There is another piece to the Darwinian jigsaw puzzle: the A/Swine (H1N1) virus finds itself up against an almost impenetrable immune barrier in the over-60's. Those who lived in the 1950's, the last years of world dominance by the 'Mother, Of All Influenza Viruses' have a deep immune imprint of influenza A (H1N1). Influenza virologists bandy around a favourite phrase to confound all other infectious disease scientists, that of 'Original Antigenic Sin'. In essence the first influenza A virus we encounter in life leaves an indelible immune memory as a footprint. Whenever our immune memory is re-stimulated, even 50 years later, we tend to respond by reliving our first experience as previous virgins. And why not! This means that the over-60's will respond well to A/Swine (H1N1) vaccines. Most importantly the vast majority of over-60's have not suffered from A/Swine virus, at least not yet.

This year of the new pandemic, in which seasonal influenza A (H3N2) would normally kill large numbers of the over-60's, it has been displaced by our super fit, super spreader A/Swine (H1N1). The same over-60's group cannot be infected with A/Swine (H1N1) because of their Original Antigenic Sin, and hey presto, no deaths! But my story of biology, immune reactions and memory of the past is not finished. If only it was! If this was the entire story then we could thank the Almighty for allowing the emergence of what could be viewed as a novel live flu vaccine with spreadability, mild infection, no mortality in the elderly and ability to displace influenza A H3N2 which would otherwise kill. What a novel vaccine, able to displace an otherwise killer seasonal influenza A H3N2! Not one of us have ever thought of this scenario as

we planned our reverse genetic experiments to create new live attenuated vaccine viruses. But the A/Swine (H1N1) virus has a sting in its tail. In essence how do you tell parents grieving after the death of their four year old or the husband of a deceased pregnant woman that this is a 'mild virus'? Professor Sir Liam Donaldson (Chief Medical Officer for England and the UK's Chief Medical Adviser) put it succinctly "*perception of mildness should not be an excuse for inaction*."

The influenza A/Swine H1N1 virus pandemic, at least so far, has indeed been moderately mild with 10% of the population infected, case fatality rate around 0.02 and an Ro of 1.8 (Fraser, *et al.*, 2009, Neuman, Noda & Kawaoka, 2009). But viewed another way, years of active life lost, another tale emerges because the death of a four-year-old deprives 'us' of 80 years of life whereas the death of an 80-year-old deprives us of, say 10 years. You may ask why I use the phrase 'deprives us'? Well, John Donne gives the answer in his Elizabethan poem '*No man is an island, for whom the bell tolls*'. Even 400 years ago he realised that we all live here on planet earth together and the bell tolls for us all when a single person dies, wherever and whenever this happens.

The new influenza A/Swine (H1N1) virus has a complex genetic structure and could just as easily have arisen in South East Asia like most pandemic and indeed epidemic genetic variants to be transported to Mexico around Christmas 2008. The overt clinical problem became apparent there by the Spring, causing consternation worldwide (Belshe, 2009) and epidemic anxiety (Ofri, 2009). One of the main objectives of global pandemic planning, kicked into action after the emergence and threat of avian influenza (H5N1) in 2003, is to reassure the Home Front that every arrangement is calmly in place with stocks of antivirals and vaccines. Last minute rushes to the airport by politicians to collect Tamiflu and images of masked people in the street, as in the Ukraine recently, and in Mexico a year ago, dissipate confidence and lead to chaos.

The current World Health Organization (WHO) level 6 alert is unlikely to be alleviated until 2011, when we will have more measure of the virus. Animal modelling has shown that the new virus descended more deeply into the air sacs than epidemic and seasonal influenza (Itoh, *et al.*, 2009). Pathology of human cases in the USA also shows a deep seated infection akin to previous pandemics in 1918, 1957 and 1968.

The new vaccines produced rapidly because of prior investment for an expected H5N1 pandemic, induce protective antibody rapidly after a single dose in the over four-year-olds. Rapid use of the neuraminidase inhibitor Tamiflu reduces the time of virus excretion and is a powerful aid in seriously ill persons. Darwin would not have wished a virus upon us, even one so illustrative of his survival of the fittest which can kill four-year-olds, pregnant women, the obese and diabetics. But he would have pondered that at least we have applied modern science to contain the virus.

We are not bereft of ammunition. Scientific societies, like in Darwin's own country, are still prone to anxiety moods. Virologists have squeezed out global smallpox to two holding laboratories in Novosibirsk and Atlanta and polio is very close to teetering into the abyss. All the accumulated knowledge about influenza as the 'new acquaintance' at the time of John Donne, helped by collaboration between the UN, WHO governments and the large pharma groups of the world, has enabled us to come out fighting. We have the tools, the vaccines (Davies, 2009) and neuraminidase inhibitors and we are using them. The fight back will continue. As Darwin could predict, to become the biggest virus in the world block, A/Swine H1N1 must now mutate one step further: it has now to break through the Original Antigen Sin immune barrier of the over-60's. So next year (2011) we could see many more deaths than this year. But we will not sit quietly by and view with detachment the deaths of 20,000 persons, as we did in 1990/2000. To my mind influenza will never be the same again to doctors, virologists and citizens. This could be a truly lasting legacy of the 2009 H1N1 pandemic of this most Darwinian of viruses.

references

- Belshe, R.B. (2009). Implications of the emergence of a novel H1 influenza virus. N Engl J Med, **Vol. 360**: pp2667-8.
- Davies J. (2009). Swine Flu Vaccines: Reaching the Finish Line. *Cell*, **Vol. 139**: pp449-451
- Fraser, C. *et al*. (2009). Pandemic Potential of a Strain of Influenza A (H1N1): early findings. *Science*, **Vol. 324**: pp1557-1561
- Itoh, Y., Shinya, K., Kison, M. *et al* (2009). *In vitro* and *in vivo* characterization of new swine-origin H1N1 influenza viruses. *Nature*, **Vol. 460:** pp1021-1025
- Neumann, G., Noda, T., Kawaoka, Y. (2009). Emergence and pandemic potential of swine-origin H1N1 influenza virus. *Nature*, **Vol. 459**: pp931-939
- Ofri, D. (2009). The Emotional Epidemiology of H1N1 Influenza. *N Engl J Med*, **Vol. 361**: pp2594-2595
- Oxford, J.S., Sefton, A., Jackson, R., Innes, W., Daniels, R.S and Johnson, N.P.A.S. (2002). World War I may have allowed the emergence of "Spanish" influenza. *The Lancet*, **Vol. 2**: pp111-114
- Stuart-Harris, C.H., Schild, G.C. and Oxford, J.S. (1985). Influenza, the Viruses and the Disease. Edward Arnold London, pp264

Professor John S Oxford
Professor and Scientific Director
Retroscreen Virology Ltd

Winter Meeting 2010 report

Royal Society, London, UK, Monday 11 January 2010

Advances in biocide development

Tuberculosis

Difficult weather conditions didn't prevent delegates getting to the hallowed halls of the Royal Society in its 350th anniversary year for the S*f*AM Winter Meeting 2010. The meeting covered *Mycobacterium tuberculosis* and biocides.

The morning session was chaired by S*f*AM President, Geoff Hanlon. The proceedings started with the fifth Denver Russell Memorial Lecture, followed by two taster presentations which gave a flavour of the parallel sessions to follow.

Gerald McDonnell of STERIS Ltd, UK presented the fifth Denver Russell Memorial Lecture: a review of biocide research, describing how our understanding in this field has developed in recent years. Having explained that biocides were historically less well studied than anti-infectives, such as antibiotics, he gave examples of work which has challenged our established methods of testing biocides.

There is a traditional hierarchy of microorganisms ranging from those thought to be highly resistant to biocides (sporulated bacteria) to those thought to be easily inactivated by biocides (enveloped lipophilic viruses). It is now becoming apparent, however, that different hierarchies exist for different biocides. Furthermore, organisms which are used as models have been shown to be inappropriate, for example, polioviruses are used to represent non-enveloped viruses yet parvoviruses, which are increasingly associated with clinical disease in humans, are less readily inactivated.

Gerald discussed the various organism groups which should be considered when testing the efficacy of biocides. Viruses, bacteria, mycobacteria, protozoa and prions are all relevant yet many of these are often overlooked. The final challenge that was presented to us was the transmissibility of protein precipitation diseases such as Alzheimer's disease. Our biocidal practices do not currently tackle this emerging issue.

Douglas Young of Imperial College London, UK summarised the aims of the Global Plan to Stop TB and highlighted the need to tackle both active and latent TB. In order to achieve the Global Plan's elimination target by 2050 the number of TB infected individuals should be reduced by 6% per annum. Currently we are only achieving a reduction rate of 1% per annum. Douglas explained the need to target preventive therapy against 'at risk' subgroups of latently infected individuals and also the need to use drugs which attack both growing and non-growing bacterial subpopulations. Novel anti-TB drugs are being developed, including nitroimidazole

information

For more information about the Society's meetings please visit the website at: www.sfam.org.uk

You can also find details of this year's meetings on pages 23 to 27 of this issue of *Microbiologist*

derivatives. These are used in addition to conventional TB drugs and are active against mycobacteria in anaerobic conditions, as encountered in caseous necrotic lung lesions.

Douglas concluded by describing CT/PET imaging techniques which facilitate the monitoring of specific lesions, enabling us to investigate differences between responding and non-responding lesions during therapy.

The morning lecture on biocides was delivered by Ian Hosein of North Middlesex NHS Trust, UK. He focussed on the use of biocides in a hospital setting, using light-hearted yet thought-provoking anecdotes.

The complexities of a healthcare environment comprising staff, sick patients and visitors were cited as reasons why the NHS cannot operate in the same streamlined fashion as the food and car industries. Ian explained that healthcare associated infections (HAIs) are generally not a result of biocide failure *per se* but rather a failure in their implementation. The Organizational-Behavioural-Technical model was presented as one way to successfully manage staff such that they understand appropriate usage of biocides and are motivated to use them correctly. This was illustrated by the introduction of alcohol gel to replace existing handwashing measures in a Welsh hospital. In his summary, Ian advocated the need for realistic expectations of biocides and a balanced view regarding the technical and organizational aspects of their application.

Louise Hill-King

The afternoon session continued with the earlier theme of latent tuberculosis, this time focussing upon its detection. Ajit Lavani (Imperial College London) delivered an excellent presentation on the development of the ELISpot and its ELISA equivalent. These assays quantify the production of interferon gamma from specific T cells giving both sensitive and specific detection of those infected. Indeed, subsequent analysis has confirmed the utility of ELISpot for detection of contacts following exposure. Interestingly, use of this assay has demonstrated that infected individuals can clear acute tuberculosis. This assay has enormous diagnostic value both for prediction of those that might develop acute infection and for those exposed that are subsequently able to clear infection.

Our next speaker took us back in time to the impact of tuberculosis in historic times. This fascinating lecture by Helen Donoghue described the co-evolution of *Mycobacteria* with their human hosts. Even humans from Neolithic times showed evidence of infection; however obtaining quality data from such ancient samples can be a challenge. Similar molecular techniques (Single Nucleotide Polymorphisms Analysis, Variable Number Tandem Repeat and spoligotyping) have been applied to remains from Hungary revealing earlier evidence of European lineages of *M. tuberculosis*. Other less expected challenges of such research interests are explaining the presence of mummified human remains in ones luggage to customs officials!

Grace Smith followed with an excellent presentation of contact tracing of tuberculosis patients in the Midlands. She described the local high incidence of infection and the value of molecular diagnostics such as VNTR for analysing whether cases were associated or not. Indeed, this laboratory analysis can be used to justify subsequent epidemiological analysis. On an individual level, this can determine whether infection is exogenous re-infection or reactivation. The typing scheme itself has recently been modified, changing from a 15 to 24 locus scheme. Assessment of the additional benefits of these additional markers is eagerly awaited.

Mike Hutchings gave the final presentation of the day on bovine tuberculosis. This presentation gave refreshing insights into this problem such as the grazing preferences of cattle and

their lack of aversion to grazing urine contaminated pasture, yet their lack of grazing faecal-contaminated grass. Effective transmission does apparently require close proximity between susceptible host and the contaminated source of infection. Understanding of the social interactions of possible reservoir wildlife species and the habits of livestock is pivotal to understanding the dynamics of infection and revealing potential weak links that might be utilized for future intervention campaigns.

Sally Cutler

The four speakers in the biocides session were from diverse backgrounds, each bringing a different perspective to the debate concerning biocides and how we can and should be using them. Ludger Grunwald from Ecolab, Germany, brought us details of European legislation which seemed to catch some members of the audience by surprise. European legislation in the form of the Biocidal Product Directive (BPD) is expected to be introduced across the European Union in the next decade with the aim of harmonizing the use of active ingredients and the product formulations they are contained within.

Gerald McDonnell of STERIS Ltd (right), receives the Denver Russell Memorial lecture award from Professor Geoff Hanlon and Jean-Yves Maillard of SfAM

Whilst well intentioned it seems that a number of unforeseen consequences have arisen during the conception and construction of the BPD. Trying to identify all areas of biocide use has lead to issues surrounding how to define a biocide as such and not as a medicinal product or cosmetic. Also, the cost can be an issue (a single active agent in a single formulation could cost a minimum of €2.2 million). Finally, there has been a precipitous reduction in the number of active ingredients in circulation since the start of the process. This legislation, once accepted, will have an impact on the likely introduction of any new and/or novel ingredients in the future.

John Holah from Campden BRI presented on biocide testing from a regulatory perspective. This speaker introduced the discrepancies between biocide stakeholders (manufacturers, regulators, test houses and academics) and their specific requirements, which seldom coincide. Currently, European disinfectant testing methods are generated by two bodies, European Committee for Standardization (CEN) and Organization for Economic Development and Co-operation (OECD) each with different testing methods. This was an interesting talk which emphasised that whilst there is movement on legislative requirements concerning active agents, it seems this is lacking in the design of standard methods for testing as a result of conflicting points of view. It seems we are still labouring under criteria established in the nineteenth century which need to be updated in terms of technology and relevance. A very good final point was raised, namely that "harmonization is compromise".

The final two speakers were academics discussing the area of biocide resistance and current research being undertaken in the action of biocides against biofilms. Jean-Yves Maillard from Cardiff University discussed bacterial resistance mechanisms to biocides and highlighted the need for further research in this area as a result of high level and constant use. This is particularly relevant in healthcare settings; areas where highly resistant bacteria persist. Research is necessary to determine whether biocide resistance is selected for by constant use, as well as to determine whether sub-lethal dosing confers other characteristics such as cross-resistance.

When discussing any form of bacterial testing invariably planktonic or adhered cells are alluded to, however, Rodney

Donlan of the CDC made a strong case for including biofilms in biocide evaluation. Biofilm involvement in many infections as a result of adhering to indwelling devices e.g. heart valves and replacement joints, is well known. Their formation is thought to impart tolerance to biocides, therapeutics and environmental stresses; therefore there is a case to include a biofilm model in the standard biocide testing regimes. Rodney Donlan also brought evidence from his current research into the association of bacteria with protozoa in biofilm formation, an exciting development.

Overall, this was a well rounded session with speakers representing all areas involved in biocide research.

Alison Kelly

The Trade Show attendees enjoyed a busy time during the coffee and lunch breaks and with no additional snowfall – for one day at least – delegates were all set for a smooth journey home.

Friday 16 April 2010

Spring Meeting

Latest developments in gastrointestinal infections

4th broadening microbiology horizons in biomedical science

■ Including the Procter & Gamble Lecture

The Stratford Q Hotel, Stratford upon Avon, UK

BOOK NOW USING THE FORM ON THE NEXT PAGE!

CPD ACCREDITATION 6 CREDITS

Programme

09:15 - 10:15	**Coffee, tea, trade exhibition and registration**
10:15 - 10:20	**Chairman's welcome**
10:20 - 11:00	**Procter and Gamble lecture** Peter Borriello,Veterinary Laboratories Agency, UK
11:05 - 11:35	**Recent developments in *Salmonella*** John Threlfall, HPA Colindale, London, UK
11:35 - 12:05	***E coli.* O157** Geraldine Smith, HPA Colindale, London, UK
12:05 - 12:35	**Cryptosporidia** Rachel Chalmers, NPHS, Swansea, UK
12:35 - 14:00	**Lunch and trade exhibition**
14:00 - 14:30	***Campylobacter* — a food microbiologist's perspective** Frieda Jorgensen, HPA, Bristol, UK
14:30 - 15:00	**Developments in culture media** Steve Dimmer, Oxoid, UK
15:00 - 15:30	***Clostridium difficile*** Jim Gray, Birmingham Children Hospital, UK
15:30 - 16:00	**The gut health of animals and foodborne zoonoses** Martin Woodward, VLA, UK
16:00	**Finish, tea and coffee**

The programme for this meeting was correct at the time of going to press

BOOKING FORM and INVOICE

SfAM SPRING MEETING FRIDAY 16 APRIL 2010

Only ONE person per form please. CLOSING DATE FOR REGISTRATIONS: Friday 9 April 2010
EARLY BIRD DISCOUNT of £30.00 is applied to all bookings made before **Friday 19 March 2010**

Cancellation policy: Up to 30 days prior to the event all cancellations will be subject to a 10% cancellation fee, up to 14 days prior to the event there will be a 50% cancellation fee, and no refunds will be given on cancellations made within 7 days of the event.

***Non members please note:** You can add 1 year's membership to your event booking using this form, then register at the member rate and spend the same amount of money or less!

FEES	Before 19/03/2010	Between 20/03/2010 and 9/04/2010
Full member	£50 ☐	£80 ☐
Student member	£30 ☐	£60 ☐
Honorary member	£30 ☐	£60 ☐
Associate member	£30 ☐	£60 ☐
Retired member	£30 ☐	£60 ☐
Student non member and IBMS member	£75 ☐	£105 ☐
Non member	£100 ☐	£130 ☐

*ADD MEMBERSHIP TO YOUR BOOKING

Add Student membership (£25.00): ☐ **Add Full membership (£50.00):** ☐

YOUR DETAILS

Title:_____ **First Name:**_____ **Family Name:**_____

Address:_____

_____ **Postcode:**_____

Tel No:_____ **Fax No:**_____ **Email:**_____

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

_____

YOUR NAME BADGE

Please enter the information below in **BLOCK CAPITALS** as you would like it to appear on your name badge

First Name:_____ **Family name:**_____

Organization/Affiliation:_____

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.** We accept payment **ONLY** by the following credit and debit cards: VISA, Mastercard, Eurocard, Delta, Electron, JCB, Maestro and Solo.

☐ **Cheque enclosed** ☐ **Please charge my *Mastercard/Visa card /Debit card*** *(please delete inapplicable items)*

TOTAL Amount enclosed/ to be charged: £_____

Card number: ☐☐☐☐ ☐☐☐☐ ☐☐☐☐ ☐☐☐☐ Solo Cards only: ☐☐☐

Issue No. ☐☐☐ Expiry Date: ☐☐☐☐ Start Date: (*Debit cards only*) ☐☐☐☐

Security Code (last 3 digits on reverse of card): ☐☐☐ Cardholder's address to which credit card statement is sent:

_____

Signature:_____ Date:_____

Please return the completed form by fax (post if you are enclosing a cheque) to: **The Society for Applied Microbiology, Bedford Heights, Brickhill Drive, Bedford MK41 7PH, UK. Tel: 01234 761752 Fax: 01234 328330. Email: meetings@sfam.org.uk**

Monday 5 - Thursday 8 July 2010

Summer Conference

Applied microbiology with sessions on:

- **Biofilms:** buzzwords in biofilms
- ***Listeria*:** new perspectives on an old pathogen
- **Bacteriophages:** applied bacteriophage technology

■ Including the Lewis B Perry Memorial Lecture

CPD ACCREDITATION APPLIED FOR

The Grand Hotel, Brighton, UK

STUDENTSHIP GRANTS

■ Don't forget we offer studentship grants to enable student members to attend Society meetings. The grant covers registration, accommodation, meals (where appropriate) and modest travel expenses.

■ To be considered for a studentship grant please complete the application form at **www.sfam.org.uk/grants.php**

For more information about SfAM grants visit: www.sfam.org.uk/grants.php

CALL FOR ABSTRACTS!

■ We are now accepting abstracts for posters and the Student session at the 2010 Summer Conference in Brighton. These can be on any topic in applied microbiology.

■ There are prizes of **£150**, **£100** and **£50** available to winners of first, second and third prize for posters. For the best student oral presentation there is a prize of **£300!**

For more information or to submit your abstract visit: www.sfam.org.uk/summer_conference.php

REDUCED RATES!

Summer Conference delegate fees REDUCED for 2010!	Full member		Student, Associate Honorary and Retired member	
	Early bird before 7 June	From 8 June	Early bird before 7 June	From 8 June
Full conference with accommodation	£250	£300	£200	£250
Full conference no accommodation	£100	£150	£50	£100
Day rate	£50	£100	£25	£75

■ We are also delighted to announce that the Summer Conference 2010 fee includes dinner in the hotel restaurant every evening.

■ Please note that there is a small supplement of **£20** payable to attend the drinks reception and conference dinner on the evening of Wednesday 7th July 2010.

INFORMATION

■ To download an abstract form or to register online visit: **www.sfam.org.uk/summer_conference.php**

For further information on the Summer Conference please vist the website or contact Sally Cryer. Email: sally@sfam.org.uk. Telephone: 01234 761752

Programme

Monday 5 July

14.00 onwards	**Arrive and register**
	Workshop session: Science and the media
18.00-18.50	**Lewis B Perry Memorial Lecture:**
	Contagious bovine pleuropneumonia: in search of the origins and virulence of lung sickness Robin Nicholas, Veterinary Laboratories Agency, UK
19.00-20.00	**Drinks reception**
20.00	**Evening at leisure**
21.30	**Quiz night**

Tuesday 6 July

Session 1: Applied bacteriophage technology

Chair:	**Andy Sails**
09.00-09.35	**Phage therapy in human infections** Alexander Sulakvelidze, Intralytix, USA
09.35-10.10	**Uses of phage in the animal food production chain** Ian Connerton, University of Nottingham, UK
10.10-10.45	**Bacteriophages for the treatment of *Pseudomonas aeruginosa* infections** David Harper, Biocontrol, UK
10.45-11.15	**Coffee and Tea**
11.15-11.50	**Using bacteriophages to develop DNA vaccines** John March, Big DNA, Edinburgh, UK
11.50-12.25	**Bacteriophages for the prevention of *Listeria monocytogenes* in food** Steven Hagens, EBI Food Safety, The Netherlands
12.25-13.25	**Lunch**
13.25-14.00	**Optimized by evolution: phage and its enzmyes for detection and control of pathogenic bacteria** Martin J. Loessner, Institute of Food Science and Nutrition, ETH, Switzerland
14.00-14.35	**The challenge of using phage in food and veterinary diagnostics** George Botsaris, Cyprus

Session 2: *Listeria*: new perspectives on an old pathogen

Chair:	**Christine Dodd**
14.35-15.10	***Listeria* and listeriosis: emergence and re-emergence** Jim McLauchlin, Health Protection Agency, UK
15.00-15.45	**The challenge of quantifying *Listeria* from foods and environmental samples** Martin Wagner, Austria
15.30-16.00	**Coffee and tea**
16.00-16.35	**Molecular sleuthing and listeriosis** Kathie Grant, Health Protection Agency, Centre for Infections, UK
16.35-17.10	**Ecology of *Listeria* in the environment and in animals** Kendra Nightingale, Colorado State University, USA
17.10-18.10	**Student session**
17.15-19.30	**Trade show**

Wednesday 7 July

Session 2: *Listeria*: New perspectives on an old pathogen (continued)

09.00-09.35	**What does not destroy *Listeria*, makes it strong—adapting to stress *in vitro* and *in vivo*** Colin Hill, University College Cork, Ireland
09.35-10.10	**Renaissance Bacteria — *Listeria* persistence in the food environment** Cath Rees, University of Nottingham, UK
10.10-10.40	**Coffee and Tea**
10.40-12.00	**Attended poster viewing**
12.00-13.00	**Lunch**

Session 3: Buzzwords in Biofilms

Chair:	**Jo Verran**
13.00-13.35	**Gene transfer in oral biofilms** Adam Roberts, Eastman Dental Institute, UK
13.35-14.10	**Biofilm development and dispersion** Karen Sauer, Binghamton University, USA
14.15-14.45	**Coffee and tea**
14.45-15.45	**Student presentations**
Chair:	**Geoff Hanlon**
15.45-16.15	**W.H. Pierce Prize Lecture**
16.15-16.45	**AGM**
19.00	**Drinks reception and dinner**

Thursday 8 July

Session 3: Buzzwords in Biofilms (continued)

Chair:	**Geoff Hanlon**
09.00-09.05	**Introduction to the New Lecturer Research Grant**
09.05-09.40	**Co-aggregation and signalling** Alex Rickard, (recipient of a SfAM New Lecturers Grant), Binghamton University, USA
09.40-10.15	**Evolution** Daniel Rozen, Manchester University, UK
10.15-10.45	**Coffee and tea**
10.45-11.20	**Subpopulation interactions during *Pseudomonas aeruginosa* biofilm formation** Tim Tolker-Nielsen, University of Copenhagen, Denmark
11.20-11.55	**Programmed cell death** Jeremy Webb, Southampton University, UK
12.00-13.00	**Lunch and close**

This programme was correct at the time of going to press. For the latest programme please visit us online at www.sfam.org.uk

BOOKING FORM and INVOICE

SfAM SUMMER CONFERENCE 5 — 8 July 2010

CLOSING DATE FOR REGISTRATIONS: Monday 21 June 2010
EARLY BIRD DISCOUNT of £50.00 is applied to all bookings made before **Monday 7 June 2010**

Cancellation policy: Up to 30 days prior to the event all cancellations will be subject to a 10% cancellation fee, up to 14 days prior to the event there will be a 50% cancellation fee, and no refunds will be given on cancellations made within 7 days of the event.

***Non members please note:** You can add 1 year's membership to your event booking using this form, then register at the member rate and spend the same amount of money or less!

FEES BEFORE 7 JUNE 2010	Full Member	Student, Honorary, Associate & Retired Member	Student Non -Member	Non - Member
Full Conference Rate inc. accommodation	£250.00 ☐	£200.00 ☐	£400.00 ☐	£600.00 ☐
Conference Rate exc. accommodation	£100.00 ☐	£50.00 ☐	£100.00 ☐	£200.00 ☐
Conference Day Rate:	£50.00 ☐	£25.00 ☐	£50.00 ☐	£100.00 ☐
FEES BETWEEN 8 JUNE and 21 JUNE 2010	**Full Member**	**Student, Honorary, Associate & Retired Member**	**Student Non -Member**	**Non - Member**
Full Conference Rate: (inc accommodation)	£300.00 ☐	£250.00 ☐	£450.00 ☐	£650.00 ☐
Conference Rate: (no accommodation)	£150.00 ☐	£100.00 ☐	£150.00 ☐	£250.00 ☐
Conference Day Rate:	£100.00 ☐	£75.00 ☐	£100.00 ☐	£150.00 ☐

Conference Day Rate delegates please tick the day you wish to attend: Mon 5th ☐ Tue 6th ☐ Wed 7th ☐ Thur 8th ☐

CONFERENCE DINNER: full conference fees include dinner in the hotel every night; there is a small supplement of £20 payable to attend the conference dinner on Wednesday evening. If you wish to attend this please tick the box: £20.00 ☐

*ADD MEMBERSHIP TO YOUR BOOKING

Add Student membership (£25.00): ☐ Add Full membership (£50.00): ☐

YOUR DETAILS

Title:_____ First Name:_____ Family Name:_____

Address:_____

Postcode:_____ Tel No:_____ Email:_____

Special dietary or other requirements:_____

YOUR NAME BADGE

Please enter the information below in **BLOCK CAPITALS** as you would like it to appear on your name badge

First Name:_____ Family name:_____

Organization/Affiliation:_____

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.** We accept payment **ONLY** by the following credit and debit cards: VISA, Mastercard, Eurocard, Delta, Electron, JCB, Maestro and Solo.

☐ **Cheque enclosed** ☐ **Please charge my *Mastercard/Visa card /Debit card*** *(please delete inapplicable items)*

TOTAL Amount enclosed/ to be charged: £_____

Card number: ☐☐☐☐ ☐☐☐☐ ☐☐☐☐ ☐☐☐☐ Solo Cards only: ☐☐☐

Issue No. ☐☐☐ Expiry Date: ☐☐☐☐ Start Date: (*Debit cards only*) ☐☐☐☐

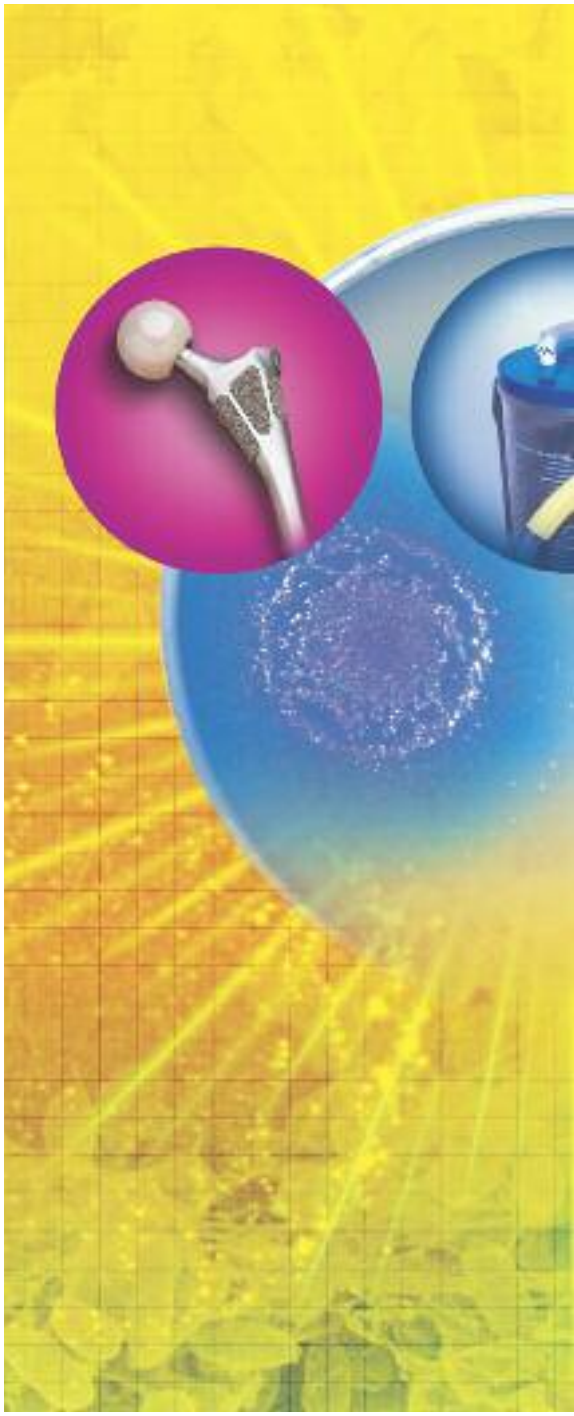
Security Code (last 3 digits on reverse of card): ☐☐☐ Cardholder's address to which credit card statement is sent:

Signature:_____ Date:_____

Please return the completed form by fax (post if you are enclosing a cheque) to: **The Society for Applied Microbiology, Bedford Heights, Brickhill Drive, Bedford MK41 7PH, UK. Tel: 01234 761752 Fax: 01234 328330. Email: meetings@sfam.org.uk**

Biofilms:

an introduction to their significance and recalcitrance

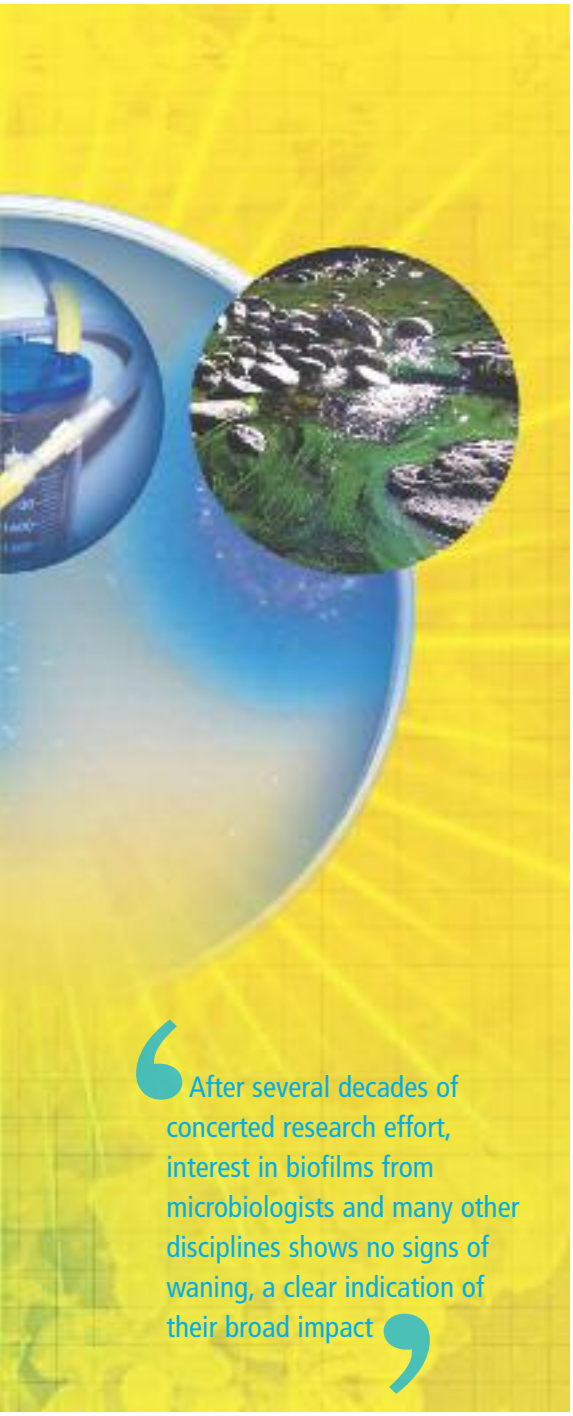


The term "biofilm' refers to sessile (i.e. attached) modes of microbial growth that occur in close association with surfaces (substrata). The definition can be extended to include growth in dense communities where a surface may be absent, such as in floccules and to other forms of interfacial microbial growth. Whilst microbiologists have studied sessile microbial growth for many years, in dental plaque or as "microbial films", there has been a major expansion in interest and systematic biofilm-related research over the past 30 years or so since the term "biofilm" was coined. Biofilms, which represent the predominant mode of microbial growth in many non-sterile environments, differ fundamentally from the planktonic forms upon which much of our understanding of microbial behaviour has been based. Their importance to humankind is beyond doubt, and is manifested through interactions with built/industrial environments (through biofouling, biocorrosion, biodegradation and contamination), the natural environment (nutrient cycling), their involvement in disease (at surgical sites, implant-associated, otitis media, dental caries) and in the maintenance of health (e.g. through microbiome colonization-resistance and gut nutrient sequestration). One of the most troublesome aspects of biofilms is their remarkable ability to survive antimicrobial treatments. Whilst our understanding of the mechanisms responsible for biofilm recalcitrance has improved considerably in recent years, strategies for their control (where physical cleaning or removal is not possible) remain elusive and the adage that prevention is better than cure remains true.

Introduction

When a bacterium encounters a surface, which will probably already have been "conditioned" by the adsorption of molecules from the bulk phase, a series of events may follow which result in the formation of a biofilm. Simplistically, bacteria adhere to surfaces (Kolenbrander & London 1993) and extracellular polymeric materials are deposited which ultimately confer three-dimensional structure as the biofilm "matrix" (Flemming *et al.*, 2007). Microcolonies form through bacterial division and individual cells and (co)aggregates (Aspiras *et al.*, 2000) may be recruited from the bulk phase through coadhesion. Biofilm maturation occurs over a variable timescale, and biofilms exhibit structural complexity indicative of a high degree of physiological co-ordination, co-operation and communication; evidence for the involvement of various forms of quorum sensing is compelling (Camilli & Bassler 2006; Williams & Camara 2009). Bacteria can "escape" extant biofilms and colonize elsewhere via a process termed bacterial dispersion, which can also occur by physiologically coordinated mechanisms (Davies & Marques 2009). Figure 1 illustrates a simplified scheme for biofilm development and dispersion. Whilst there is still much to learn, molecular microbiologists have identified biofilm formation pathways involving the differential regulation of many operons, during all stages of biofilm development (Petrova & Sauer 2009).

Most definitions essentially describe biofilms as microbial cells that grow in association with surfaces and which are embedded within self-produced matrices of extracellular polymeric material (Costerton *et al.*, 1994). The key to understanding the physiological significance of the biofilm mode of growth is to view it as a fundamental and ancient form of microbial adaptation that is probably the dominant mode of microbial growth in nature. Figure 2 depicts a nascent *Pseudomonas aeruginosa* biofilm imaged using environmental scanning electron microscopy, stained to show matrix material. Many important biofilm characteristics can be attributed to the matrix which confers immobilization,



> After several decades of concerted research effort, interest in biofilms from microbiologists and many other disciplines shows no signs of waning, a clear indication of their broad impact

binds the biofilm to the substratum and is also responsible for high cell densities and three-dimensional structure. These properties, which viewed superficially may seem unremarkable, result in discontinuity of nutrient availability and metabolic product accumulation. This generates phenotypic heterogeneity which is manifested in distinct patterns of gene expression and immediate functional or phenotypic changes that include rapid decreases in antimicrobial susceptibility.

Practically any non-sterile aqueous environment will support the growth of biofilms and their most common manifestation is as biofilm communities, composed of multiple eubacterial phyla and, depending on the environment, they may also incorporate archea and fungi, together with protozoan and metazoan grazers. Single-species biofilms are less common, but can be found in most biofilm laboratories, in many types of infection (Hall-Stoodley *et al.*, 2004) and they are also associated with contamination problems in manufacturing processes. This is particularly apparent where the prevailing conditions are sufficiently selective to discourage multi-species growth or the plant sufficiently closed to prevent microbial ingress.

The problems caused by biofilms are a significant driver of biofilm research and, from the early days it was apparent that both industrial and clinical microbiologists had much to gain by better understanding their roles in fouling, contamination and general wastage in many industries. It was also apparent that a better understanding of infections associated with medical implants was required as they are at best challenging and in many cases, impossible to treat without removal of the implant. Biofilms have also been implicated in public health problems such as contamination of water supplies, air-quality issues and in foodborne infections. Dental microbiologists, who pioneered biofilm research through their interest in dental plaque (an archetypal model biofilm) have benefited from the increased critical mass through increased research attention on parallel issues in non-dental biofilms, since the fundamental principles hold true.

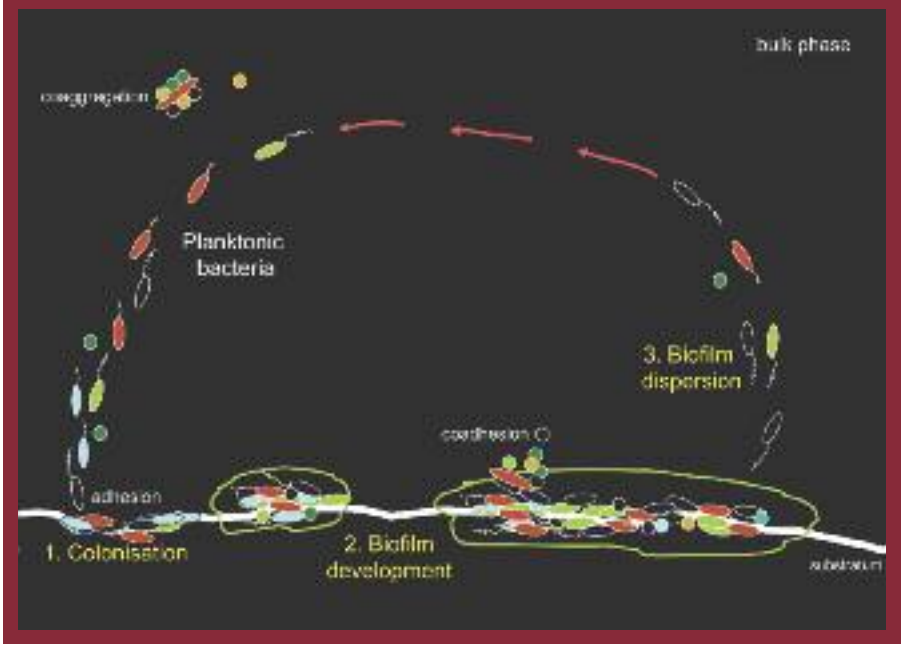
The beneficial aspects of biofilms, whilst considerable, have received less attention. Industrial solid-state fermentations utilize biofilms, as do the trickling filters used in sewage treatment. Biofilm-associated microorganisms play crucial roles in terrestrial and benthic nutrient cycling and microorganisms that comprise the human microbiome mostly grow in association with surfaces, whether on the skin, teeth, mucosal surfaces or on food particles within the lumen of the intestine. Whilst these microbial communities may be implicated in the aetiologies of various diseases; most of the time they contribute to host health through participation in digestive processes in the intestine or through the prevention of infection though immuno-modulation and the process of colonization resistance. The mechanisms responsible for colonization resistance include the production of inhibitory substances such as bacteriocins, competition for binding sites and competition for nutrients.

Arguably, the most notable feature of biofilms is their chemical recalcitrance; biofilms are almost always difficult to inactivate using chemical treatment regimes that would be effective against planktonic organisms (Gilbert *et al.*, 2002; O'Toole & Stewart 2005). Reports in the literature suggest that biofilms can be up to 1000 times less susceptible to antimicrobials than are planktonic cells. Whist this could be viewed as worst-case figure, experience in the clinic and elsewhere has proven beyond doubt that biofilms present a persistent and recurrent problem. For this reason, this short overview article will pay special attention to current understanding of the reasons for biofilm recalcitrance. Some references for further reading have been listed in Table 1.

Biofilm recalcitrance: mechanisms and theories

Biofilm-related contamination or infection problems are commonplace. Such problems are often characterized by chemical recalcitrance and recurrent episodes, where fouling, contamination or infection proves difficult to eradicate or treat with regimes that should be effective, based on planktonic susceptibility tests. Understanding the reasons for biofilm recalcitrance is an important step in devising effective treatment regimes or adapting existing strategies for maximum effectiveness. Whilst it seems that our understanding of this phenomenon is still incomplete, we do know that no single mechanism is responsible and that the reasons for biofilm recalcitrance differ from those responsible for resistance in clinical isolates that are detectable in planktonic Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) tests. Biofilm resistance is mostly a phenotypic phenomenon which reflects multi-cellular defence mechanisms and dispersed biofilm cells generally have comparable susceptibility to planktonic cells (Gilbert *et al.*, 2002). The

Figure 1. *A simplified model of biofilm development showing that planktonic bacteria adhere to substrata and develop into a biofilm with the associated deposition of enveloping matrix material. Dispersion from mature biofilm enables cells to colonize elsewhere. The diagram also shows coaggregation and coadhesion. By this model, planktonic cells may be viewed as a stage in the biofilm lifecycle. For a variety of informative biofilm material, please see the website of the Center for Biofilm Engineering (Montana, USA)*



following section will outline the mechanisms that are believed to be involved.

i) Penetration failure

Poor penetration of chemical treatment agents into the biofilm matrix was probably the originally proposed biofilm resistance mechanism, much like other mechanisms however it provides only a partial explanation. Since an antimicrobial must penetrate the full depth of a biofilm in order to inactivate the cells present, such a mechanism will certainly delay eradication (figure. 3). The problem however is that the diffusivity of biofilms is normally sufficient to allow significant penetration and biofilms additionally contain water channels that have been likened to primitive circulatory systems that increase fluid transport. Depending on the chemical composition of the antimicrobial however, it may react with matrix material: cationic antimicrobials, can for example bind to anionic sites within the biofilm matrix, markedly reducing their penetration and bioavailability. This mechanism has been termed reaction diffusion limitation. Additionally, biofilm matrices can bind extracellular products of bacterial growth, including hydrolytic enzymes such as β-lactamases and other drug-inactivation enzymes and this accounts for a process termed enzyme-mediated reaction-diffusion limitation where the biofilm matrix acts as an immobilized enzyme system for inactivating drugs such as the penicillins. Whilst these forms of diffusion-limitation can exacerbate penetration-failure and account for extended survival times in biofilms, they do not alone explain biofilm recalcitrance. This is because, given time, and providing there is sufficient antimicrobial in the bulk phase, the gradual process of penetration will quench the binding and/or reaction sites and additionally, progressive cell death will occur.

ii) Phenotypic heterogeneity

Growth-rate is an important mediator of bacterial susceptibility to a range of antimicrobials: slow-growing or dormant cells generally exhibit considerably lower susceptibility than their faster growing counterparts and this has been termed drug-indifference. High cell densities and the resultant considerable variation in phenotypes and growth-rate within biofilms therefore provide another partial explanation of recalcitrance. However, it is important to consider why such physiological variation occurs. Biofilms are inherently physiologically heterogeneous, with localised variations in nutrient availability, cell density, oxygen concentrations, bacterial end product concentrations, and physicochemical conditions. Since nutrient and oxygen penetration into biofilms may be limited, concentration gradients become established. The resultant phenotypic variation is reflected in differential susceptibility of individual cells or phenotypically-differentiated cell clusters. A wide range of susceptibilities towards a given antimicrobial may therefore be expressed within a biofilm. As with diffusion-limitation, this provides a compelling but incomplete explanation for biofilm recalcitrance.

iii) Biofilm-specific phenotypes

An important contributory mechanism of biofilm recalcitrance relates to the fact that moderate reductions in susceptibility to bacterial antibiotics and biocides can occur very soon after initial adherence to a surface, before a structure recognizable as a biofilm has formed. This will obviously contribute to overall reductions in susceptibility and could alone result in therapeutic failure if dealing with a marginally effective antibiotic, but alone it does not account for high-level

Figure 2. *Early stages of biofilm formation of Pseudomonas aeruginosa. The biofilm was imaged using environmental scanning electron microscopy. Ruthenium red stain was used to improve the visualization of the biofilm matrix. The outline of individual cells can be seen (e.g. a), as can the enveloping matrix (b). A 20mm size bar has been given included at the foot of the Figure. Image courtesy of G. Humphreys, University of Manchester*

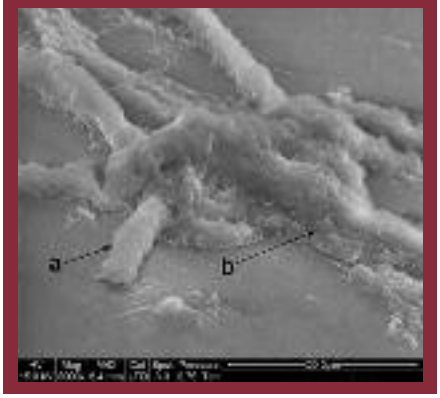


Table 1. Some further reading

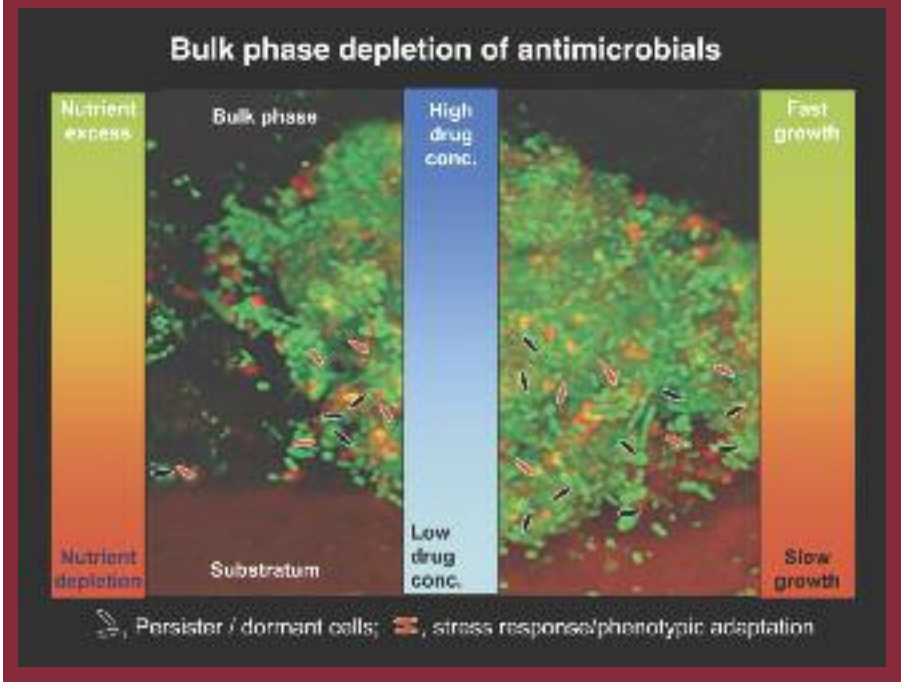
Areas covered	Explanation/significance	References
Adhesion	An initial stage of biofilm formation	Kolenbrander and London (1993)
Biofilm development pathways	Coordinated gene expression during biofilm formation	Petrova and Sauer (2009)
Matrix	Matrix deposition starts early in the biofilm formation process	Flemming *et al.*, (2007)
Coaggregation	Aggregation between phylogentically distinct bacteria. Believed to be important in the assembly of multi-species biofilms, particularly dental plaque	Kolenbrander and Andersen (1986)
Coadhesion	Adhesion onto extant biofilms	Aspiras *et al.*, (2000)
Quorum sensing	Involved in the co-ordination of biofilms	Camilli and Bassler (2006) Williams and Camara (2009)
Differential mutation rates in biofilms	Mutation rates may be enhanced in biofilm and/or mutants may accumulate through protection	Conibear *et al.*, (2009)
Biofilms and infection	"Foreign body" infections have always been challenging. We now know that these are associated with biofilms	Hall-Stoodley *et al.*, (2004)
In vitro modelling	A variety of systems have been developed to grow biofilms in the lab to enable holistic and reductionist investigations	Hope and Wilson (2006) Dalwai *et al.*, (2007)
Biofilm recalcitrance	A key biofilm characteristic	Gilbert *et al.*, (2002), O'Toole and Stewart (2005) Levin and Rozen (2006)
Bacterial persistence	An important biofilm survival mechanism	Spoering and Lewis (2001)
Programmed cell death in biofilms	Evidence of physiological co-ordination of viability	Webb *et al.*, (2003)
Biofilm dispersion	Mechanisms by which bacteria leave biofilms	Davies and Marques (2009)
A general review	Providing a very useful overview	Costerton *et al.*, (1994)

recalcitrance. Another hypothesis proposes that reduced rates of antimicrobial penetration and the resultant exposure to sub-lethal levels of an antimicrobial will provide sufficient time for physiological adaptations that could confer reduced susceptibilities through perhaps, the up-regulation of efflux pumps, the adoption of dormant phenotypes and the general stress response. These phenotypic adaptations do occur in biofilms but it has been additionally proposed that diffusible signals termed "alarmones" could act as an early warning system, perhaps released altruistically by killed (lysed) cells in order to "warn" other bacteria of the imminent arrival of chemical stress (Gilbert *et al.*, 2002). Whilst some studies indicate that such mechanisms may be active and can decrease susceptibility in exposed cells, their chemical composition, distribution and significance in biofilms has yet to be confirmed.

iv) Persisters

As correct as the above partial explanations are, they do not singly or in combination provide a satisfactory explanation of the extent of biofilm recalcitrance often observed. The simple and elegant persister hypothesis however fills many of the gaps and is based on observations that were published over 60 years ago by Joseph Bigger in the now defunct *Irish Journal of Medical Science* and in the *Lancet* (Bigger 1944). Bigger demonstrated that although penicillin was bactericidal for *Staph. pyogenes* it consistently failed to sterilise broth or serum containing *Staph. pyogenes* due to the presence of "*cocci which are called persisters at a frequency of approximately one per million of the staphylococci originally present and for which penicillin is bacteriostatic*

Figure 3. *A schematic diagram to illustrate the major mechanisms of biofilm recalcitrance as currently understood. These are i) poor antimicrobial penetration; ii) gradients of nutrients and oxygen leading to variations in growth rate and other phenotypic variability (including stress-specific phenotypes and dormancy) and iii) persisters*



and only very slowly, if at all, bactericidal".

These persisters account for the tailing seen in many bacterial kill curves that has often wrongly been attributed to the antibiotic being expended. Bigger suggested that persisters are dormant forms (and thus indifferent to penicillin) and that they account for the fact that bacteria can be isolated from the pus of penicillin-treated patients, after treatment with penicillinase. Importantly, he suggested that failure to cure staphylococcal infections in man with penicillin could be due "to the presence in the body of persisters…" Another fascinating paper, published in the *Journal of General Microbiology* (Gunnison *et al.*, 1964) made similar observations for *Staph. aureus*, again with penicillin and discounted the possibility that persister cells were the result of stable genetic alteration by showing: i) that the offspring of surviving cocci (persisters) exhibited the same sensitivity as the original population and ii) that successive exposure of the offspring of persisters did not increase the proportion of survivors nor their resistance. The recalcitrance of persisters was clearly demonstrated in this report where the proportion of survivors was not changed even by a 1000-fold increase in the dose of penicillin, or addition of streptomycin. The Gunnison paper stated that, "Indirect evidence indicates that persisters survived because at the time of first contact with the penicillin they were in a state unfavourable to initiation of division or cell wall synthesis" (i.e. they were drug indifferent). Sporadic reports have appeared in the literature relating to bacterial persistence over the years and around nine years ago a link between persistence and biofilm resistance was proposed. A paper by Spoering & Lewis (2001) presented susceptibility data for four different antimicrobial agents and *Pseudomonas aeruginosa* (a very common test bacterium in biofilm studies) and suggested that biofilm recalcitrance is a function of slow growth rate and persister cells. This turned out to be an important hypothesis and persisters now form an important part of most explanations of biofilm recalcitrance. The theory is that biofilm recalcitrance often represents a pyrrhic victory, since the majority of cells are inactivated, but the persister fraction remains to renew the biofilm once the inimical stress has been removed. It is proposed that the persisters represent a particularly effective survival mechanism when expressed within biofilms, since biofilms protect the persisters from phagocytes (*in vivo*) or from predation and other physical stresses in other environments (Figure 3).

Conclusions

After several decades of concerted research effort, interest in biofilms from microbiologists and many other disciplines shows no signs of waning, a clear indication of their broad impact and significance. Biofilm recalcitrance is clearly associated with several different mechanisms, most of which are attributable to, or exacerbated by the growth of cells within exopolymer matrices, a key biofilm characteristic. Biofilm matrices confer considerable physiological heterogeneity, may reduce the rate of antimicrobial penetration and are associated with bacterial dormancy and the physical protection of persister cells

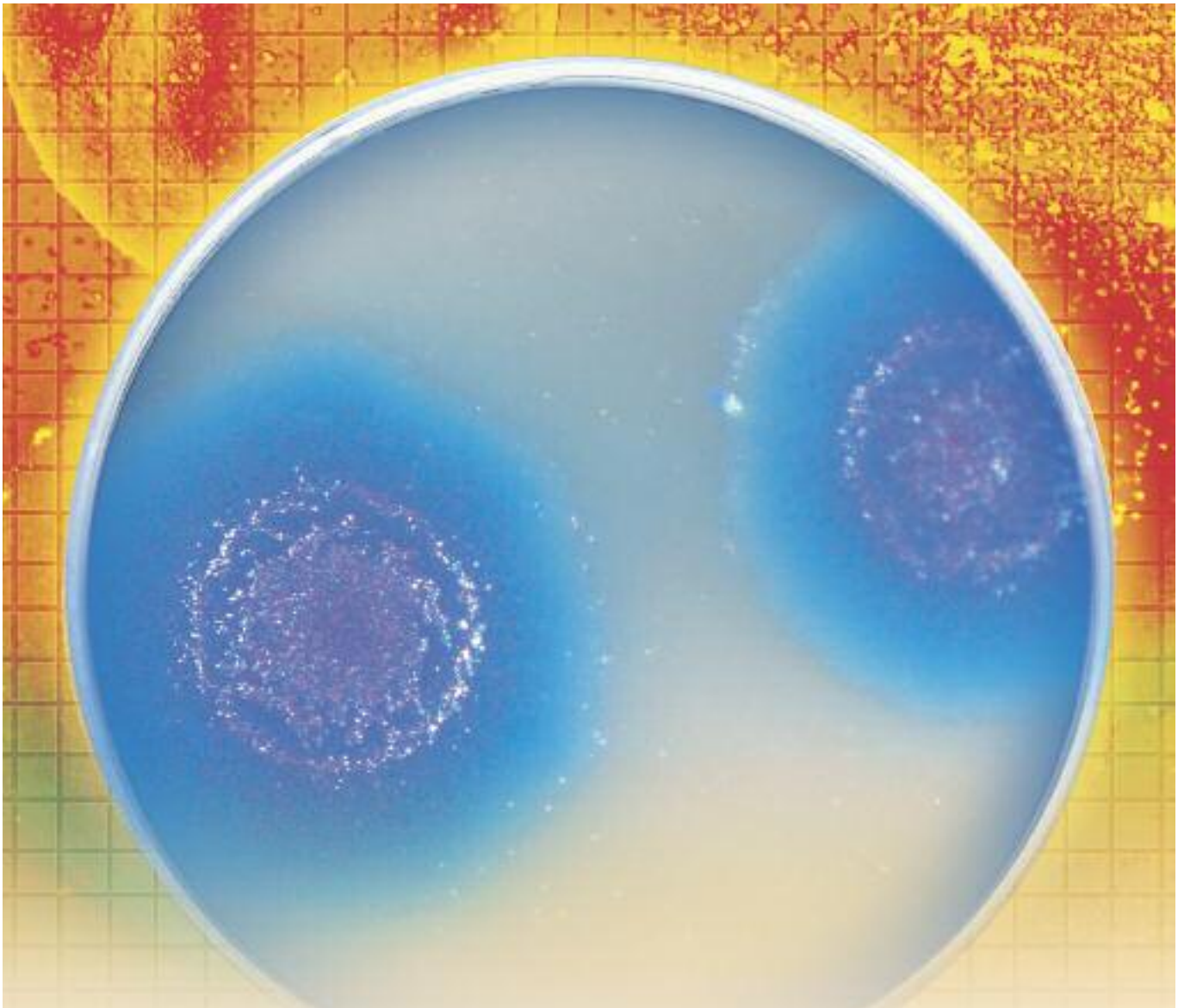
references

- Aspiras, M.B., Kazmerzak, K.M., Kolenbrander, P.E., McNab, R., Hardegen, N. and Jenkinson, H.F. (2000) Expression of green fluorescent protein in *Streptococcus gordonii* DL1 and its use as a species-specific marker in coadhesion with *Streptococcus oralis* 34 in saliva-conditioned biofilms *in vitro*. *Appl Environ Microbiol* **Vol. 66**, pp4074-4083.
- Bigger, J.W. (1944) Treatment of staphylococcal infections with penicillin. *Lancet* **Vol. 2**, pp497-500.
- Camilli, A. and Bassler, B.L. (2006) Bacterial small-molecule signalling pathways. *Science* **Vol. 311**, pp1113-1116.
- Conibear, T.C., Collins, S.L. and Webb, J.S. (2009) Role of mutation in *Pseudomonas aeruginosa* biofilm development. *PLoS One* **Vol. 4**, ppe6289.
- Costerton, J.W., Lewandowski, Z., DeBeer, D., Caldwell, D., Korber, D. and James, G. (1994) Biofilms, the customized microniche. *J Bacteriol* **Vol. 176**, pp2137-2142.
- Dalwai, F., Spratt, D.A. and Pratten, J. (2007) Use of quantitative PCR and culture methods to characterize ecological flux in bacterial biofilms. *J Clin Microbiol* **Vol. 45**, pp3072-3076.
- Davies, D.G. and Marques, C.N. (2009) A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. *J Bacteriol* **Vol. 191**, pp1393-1403.
- Flemming, H.C., Neu, T.R. and Wozniak, D.J. (2007) The EPS matrix: the "house of biofilm cells". *J Bacteriol* **Vol. 189**, pp7945-7947.
- Gilbert, P., Maira-Litran, T., McBain, A.J., Rickard, A.H. and Whyte, F.W. (2002) The physiology and collective recalcitrance of microbial biofilm communities. *Adv Microb Physiol* **Vol. 46**, pp202-256.
- Gunnison, J.B., Fraher, M. A. and Jawetz, E. (1964) Persistence of *Staphylococcus aureus* in Penicillin in vitro. *Journal of General Microbiology* **Vol. 35**, pp335-349.
- Hall-Stoodley, L., Costerton, J.W. and Stoodley, P. (2004) Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol* **Vol. 2**, pp95-108.
- Hope, C.K. and Wilson, M. (2006) Biofilm structure and cell vitality in a laboratory model of subgingival plaque. *J Microbiol Methods* **Vol. 66**, pp390-398.
- Kolenbrander, P.E. and Andersen, R.N. (1986) Multigeneric aggregations among oral bacteria: a network of independent cell-to-cell interactions. *J Bacteriol* **Vol. 168**, pp851-859.
- Kolenbrander, P.E. and London, J. (1993) Adhere today, here tomorrow: oral bacterial adherence. *J Bacteriol* **Vol. 175**, pp3247-3252.
- Levin, B.R. and Rozen, D.E. (2006) Non-inherited antibiotic resistance. *Nat Rev Microbiol* **Vol. 4**, pp556-562.
- O'Toole, G.A. and Stewart, P.S. (2005) Biofilms strike back. *Nat Biotechnol* **Vol. 23**, pp1378-1379.
- Petrova, O.E. and Sauer, K. (2009) A novel signaling network essential for regulating *Pseudomonas aeruginosa* biofilm development. *PLoS Pathog* **Vol. 5**, ppe1000668.
- Spoering, A.L. and Lewis, K. (2001) Biofilms and planktonic cells of *Pseudomonas aeruginosa* have similar resistance to killing by antimicrobials. *J Bacteriol* **Vol. 183**, pp6746-6751.
- Webb, J.S., Thompson, L.S., James, S., Charlton, T., Tolker-Nielsen, T., Koch, B., Givskov, M. and Kjelleberg, S. (2003) Cell death in *Pseudomonas aeruginosa* biofilm development. *J Bacteriol* **Vol. 185**, pp4585-4592.
- Williams, P. and Camara, M. (2009) Quorum sensing and environmental adaptation in *Pseudomonas aeruginosa*: a tale of regulatory networks and multifunctional signal molecules. *Curr Opin Microbiol* **Vol. 12**, pp182-191.

acknowledgements

- The author acknowledges Peter Gilbert (1951–2008) for many insightful discussions and for his significant contributions to the study of biofilm recalcitrance.

Andrew J McBain
School of Pharmacy and Pharmaceutical Sciences, University of Manchester, UK



Secrets of a successful minimalist — safety in numbers?

Mycoplasmas are small Gram-positive bacteria that have minimal genomes having undergone degenerative evolution. During their evolution from their *Lactobacillus* ancestors they selectively lost a large portion of their genome and retained only the genes necessary for survival and replication in their natural environment, the living host. They lack many genes including those for cell wall synthesis, for the production of all 20 amino acids, as well as genes encoding enzymes of the citric acid cycle and the majority of all other biosynthetic genes (reviewed in Razin, Yogev & Naot, 1998). Despite often being thought of as the primitive 'poor cousins' of other bacteria as they lack so many genes they are actually highly evolved and have discarded genes they don't require in favour of a tiny, streamlined genome. They have survived successfully with a reduced genome effortlessly acquiring their essential requirements from their host *in vivo*. Mycoplasmas have become masters of exploiting their hosts and have evolved to fill almost every conceivable niche with practically every animal species known to man acting as host to at least one mycoplasma species. As mycoplasmas have a highly successful but minimal genetic complement they are considered to be model organisms for the study of essential functions in living cells.

Despite their small size and tiny genome mycoplasmas cause a wide range of diseases in humans and animals. In humans *M. pneumoniae* is responsible for atypical pneumonia and infection with other mycoplasmas is implicated in a wide range of disease symptoms including infertility, arthritis and possibly even chronic fatigue

Figure 1. *Cow showing signs of respiratory distress due to CBPP*

syndrome. Undoubtedly, the most important animal mycoplasma disease is contagious bovine pleuropneumonia (CBPP—see *Microbiologist*, Vol 10 No.4, pp41), which is caused by *Mycoplasma mycoides* subspecies *mycoides* small colony (MmmSC). CBPP is a severe respiratory disease of cattle that has a significant economic impact (Figure 1). CBPP is currently absent in Europe but widespread in Africa where it causes high mortality and morbidity. Even today very little is known about how mycoplasmas cause such severe disease as CBPP and how they persist in the host or the environment. However, even in 1896 when the cause of the disease was unknown, there were indications that the organism may persist in the environment and it was written that "*Many stables have been found in which the disease would appear and reappear after the slaughter of affected herds, the destruction of the stable, the burning of the lumber, the removal of the accumulations beneath the floors, and the thorough disinfection prevented the recurrence of the plague in new stables built on the same premises. It is conclusively shown that under some conditions stables may retain the infection for a considerable time and that when restocked disease may break out again.*" (Salmon, 1896). Similarly, contagious agalactia is a disease of sheep and goats typified by mastitis, arthritis and keratoconjunctivitis. It is caused by *Mycoplasma agalactiae* and is known as 'mal di sito' ('disease of the place') in Italy because of its ability to contaminate the environment and infect successive flocks on a farm. It has never been explained how mycoplasmas that are seemingly so fragile and lacking a cell wall could survive in the environment.

Mycoplasmas are intriguing as they have very few known virulence factors such as toxins, cytolysins and invasins seen in other bacteria yet cause severe disease. Despite extensive research, virulence factors have only been found in a few mycoplasma species and these include the production of hydrogen peroxide (Miles, Taylor & Varsami, 1991) which causes oxidative damage, the carbohydrate capsule (Almeida *et al.*, 1992) which is inflammatory, the ability to scavenge arginine from host cells (Sasaki *et al.*, 1984) which effectively starves them and T-cell mitogens (Tu *et al.*, 2005) which disrupt the immune system and can cause toxic shock. Many highly pathogenic mycoplasma species still have no known virulence factors. Previously it has been difficult to explain how mycoplasmas manage to cause such severe and chronic infection given their paucity of virulence factors.

In many other bacterial species, adherence to a solid surface and biofilm formation are important steps in the initiation of disease. The possibility of environmental persistence and virulence in the host could also be explained by biofilm formation in mycoplasmas.

Biofilms are sessile bacterial communities that live attached to each other and/or surfaces enclosed in a sugary exopolysaccharide matrix. Biofilm structure is highly variable and dependent on a number of factors, including the organism, the surface, the surrounding nutrient environment and the rate of flow of any aqueous interface. Biofilms may vary in configuration, from sparse amorphous masses to highly complex, organized structures with mushroom-like cell stacks interspersed with fluid-filled channels. These channels have been compared with circulatory systems, leading to the view that biofilms can be considered analogous to primitive multicellular organisms .

The most important and widely studied property of biofilms is their vastly increased recalcitrance to antimicrobials and host defences (see page 28). Compared with planktonic cells, biofilms are commonly ten to one thousand times more resistant (Mah & O' Toole, 2001). Within the host, unattached bacteria can be cleared by antibodies and phagocytes and are susceptible to antibiotics. However, adherent biofilm cells are resistant to antibiotics, phagocytes and antibodies. In addition, biofilms can cause host damage as phagocytes are attracted but phagocytosis is frustrated leading to the release of phagocytic enzymes which damage surrounding tissue and exacerbate infection. As well as enabling chronic infection of hosts, biofilms may cause bouts of acute infection when planktonic cells are periodically released from the biofilm.

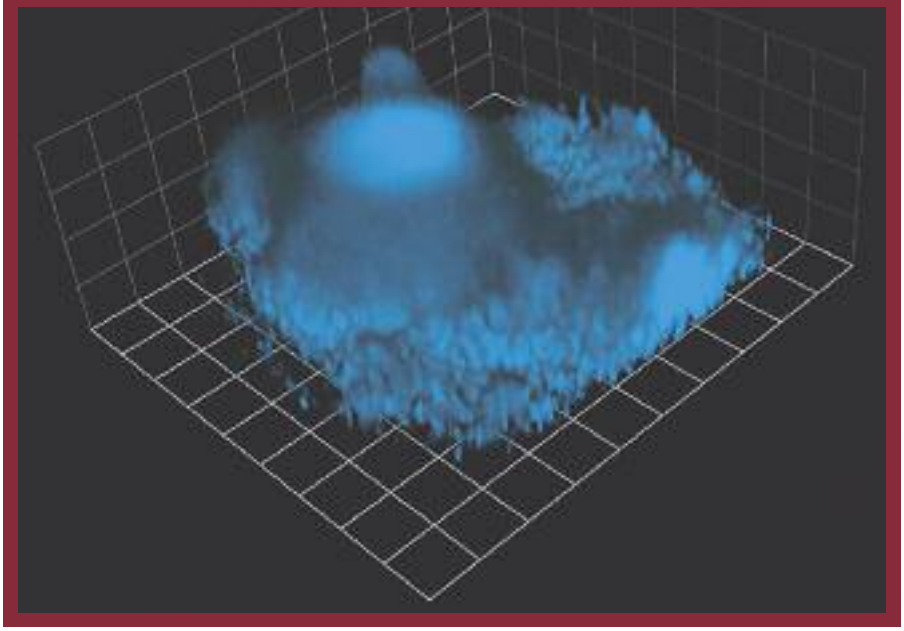
Studies of biofilm formation by mycoplasmas carried out by the Mycoplasma Group at the Veterinary Laboratories Agency have shown that biofilms are formed by the vast majority of mycoplasma species (McAuliffe *et al.*, 2006). Present in at least 20 mycoplasma species, the ability to form biofilms is found in diverse species from all groups of mycoplasmas. No true link between the ability to form a biofilm and virulence has as yet been determined, with some non-pathogenic species such as *M. cottewii* and *M. yeatsii* forming prolific biofilms. Intriguingly some highly virulent species, which are known

to have a polysaccharide capsule, did not form prolific biofilms in some biofilm systems, leading to the suggestion that the capsule may be hydrophobic and prevent adherence. Biofilm formation is not simply a laboratory phenomenon and is likely to be an important step in disease initiation in the host as it has recently been shown to occur *in vivo* in the mouse pathogen *M. pulmonis* (Simmons *et al.*, 2009).

Perhaps one of the most important findings of our studies relates to the persistence of mycoplasmas when grown as a biofilm. This is seen most convincingly in MmmSC. Certain strains of MmmSC were found to survive for over 20 weeks when grown as an adherent biofilm on a bare surface exposed to the atmosphere (McAuliffe *et al.*, 2008). Obviously this finding may have implications for disease control as it has previously been assumed that MmmSC, and other *Mycoplasma* species, would not survive in the environment. In fact, they may have the potential of surviving outside of their host for considerable amounts of time particularly in the temperate climate of Europe. We also found mycoplasma biofilms to be considerably more resistant to detergents, oxidative stress, heat and desiccation. In many instances biofilms were ten to one thousand fold more resistant than their free-living counterparts (McAuliffe *et al.*, 2006, 2008). As mycoplasmas lack the many global and specific stress responses that other bacteria use it may well be that biofilm formation has evolved to become their best method of surviving in a harsh and ever changing environment, be it the host or the outside world.

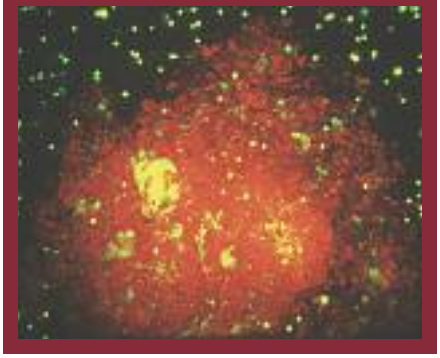
Despite years of research, the reason why biofilms are so much more resistant to stress than their planktonic counterparts is still not well understood, but a number of mechanisms are thought to act synergistically (reviewed in Mah & O' Toole, 2001). It has been proposed that multiple mechanisms of resistance exist which vary in importance depending on the species of bacteria present. These resistance mechanisms include the failure of antimicrobials/host cell defenses to penetrate the biofilm, decreased growth rate, induction of stress responses, the presence of persister cells, high cell density and induction of a specific 'biofilm phenotype' (Stewart, 2002). To date there is no *in vitro* evidence to show that mycoplasma biofilms inhibit the action of antibiotics but the patent lack of success of chemotherapy to treat mycoplasma infections, particularly in mastitis, strongly suggests biofilm involvement.

Figure 2. *Confocal image of Mycoplasma biofilm with Live/Dead staining, showing persistence of live cells within microcolonies*



Studies of the morphology of mycoplasma biofilms using confocal scanning laser microscopy showed that they have a highly differentiated structure like that seen in biofilms formed by 'higher' bacteria. Stacks were seen several cells high with channels in-between. All biofilms were covered with a thick polysaccharide layer and DNA containing glyocalycx was found in some instances (Figure 2). Microscopy has provided further evidence of the contribution of biofilm growth to the persistence of some mycoplasma species. When mature biofilms that were starved of nutrients were observed using confocal microscopy and Live/Dead staining it was found that live cells persisted in the centre of biofilm microcolonies even after all other cells around the edge of the biofilm had died (Figure 3). It seems likely that mycoplasma cells in the centre of the biofilm are protected from environmental stress compared with free-living counterparts or those at the edge of the biofilm.

Figure 3. *Confocal image of calcoflor white stained Mycoplasma biofilm. A thick layer of polysaccharide (stained blue) can be seen covering stacks of cells within the biofilm*



It is often thought that in 'normal' bacteria programmed changes in gene expression result in a specific biofilm phenotype that is more resistant to stress and exhibit differential gene expression compared with planktonic cells. Most bacteria have enormous adaptive capability and can modulate and reprogramme gene expression in response to the changing environment. In other bacteria, master regulators such as *rpo*S, *sin*R, *sig*B and *sar*A that can switch on a different subset of genes, are crucial in biofilm formation. Bacterial communication via quorum sensing is essential for gene regulation during biofilm formation in many other bacterial species. Intriguingly, mycoplasmas lack all of the known regulatory systems that are involved in biofilm formation in other bacterial species; they only have one sigma factor

for RNA polymerase and they lack quorum sensing systems and any of the specific genes linked to biofilm growth in other bacterial species. This raises the question: what is the genetic basis of biofilm formation in a minimal genome?

As mycoplasmas are rather lacking in regulatory mechanisms it seems unlikely that biofilm formation could be regulated by a complex regulatory system similar to those seen in higher bacterial species. Recently it has been shown in *M. pulmonis* that biofilm formation occurs completely due to chance events that cause rearrangements in variable surface antigens and is not due to complex changes in programmed gene expression (Simmons *et al.*, 2007). Our studies of mycoplasma biofilms have revealed that several genes are linked to biofilm formation. These include elongation factor Tu, the phosphotransferase system (PTS) glucose-specific transporter IIB component, phosphoenoylpyruvate protein phosphotransferase, fructuose-bisphosphate aldolase class II, pyruvate dehydrogenase and a number of membrane-linked and hypothetical proteins.

Interestingly pyruvate dehydrogenase and elongation factor Tu are thought to play a role in the binding of the human pathogen *M. pneumoniae* to the extracellular matrix component fibronectin and are linked to cell adhesion (Layh-Schmitt *et al.*, 2000). The PTS system proteins and Tu have also been linked to stress response in *M. pulmonis* (Fehri *et al.*, 2005).

Many of the proteins we have found linked to biofilm formation are involved in carbohydrate catabolism. Previously it has been shown that some catabolic enzymes are not simply limited to substrate turnover and other functions have been ascribed in other bacterial species (Welin *et al.*, 2004). It seems a common theme in mycoplasmas, that in order to cope with a minimal genome, a single gene often has multiple and varied functions. As has been seen in other bacterial species, we suspect that the carbohydrate capsule and exopolysaccharide may also play an important role in biofilm formation and function. As research continues to increase our understanding of how mycoplasmas regulate gene expression in the face of changing conditions, we also continue to search for an elusive 'biofilm master regulator'.

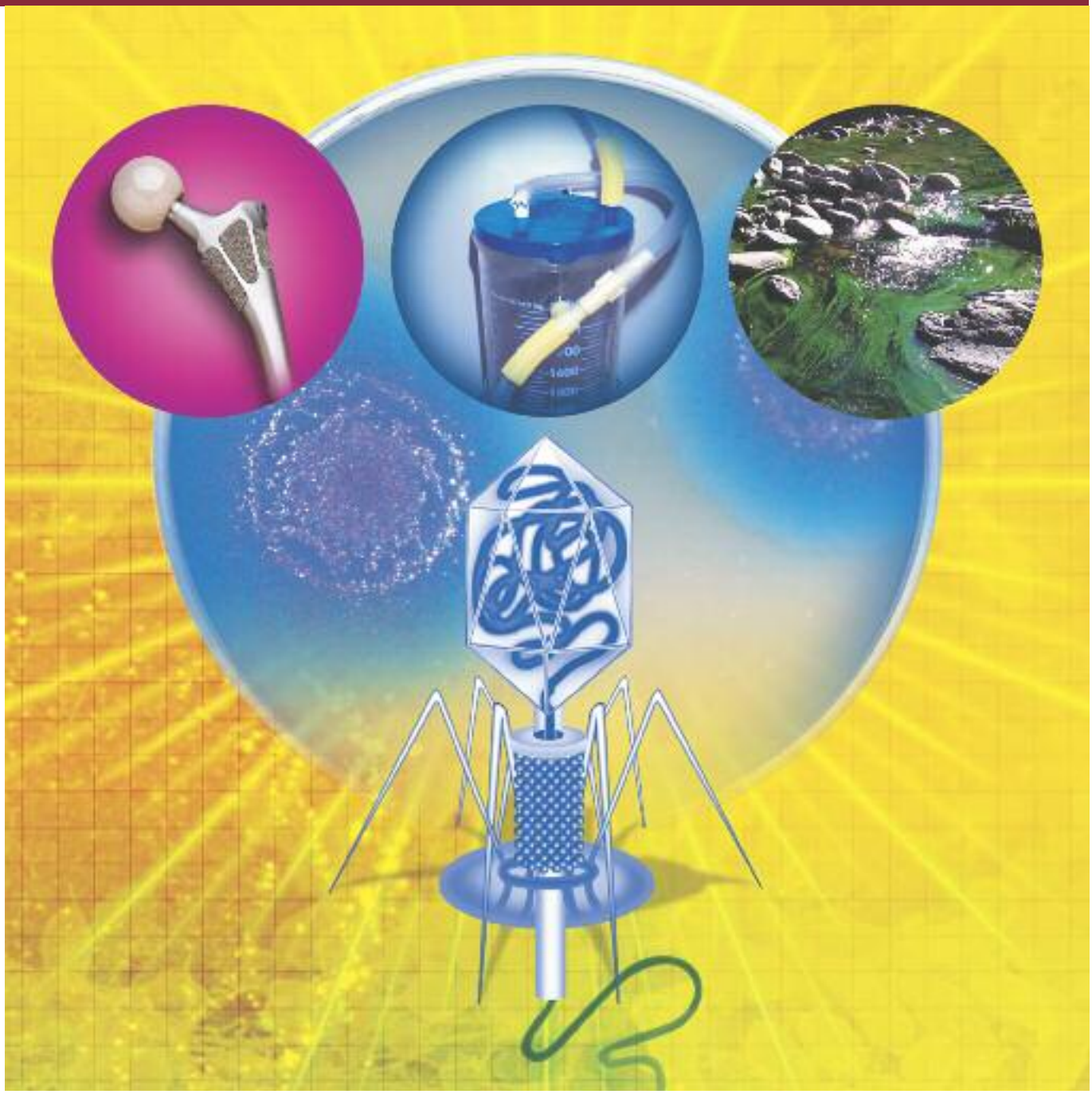
It has previously been suggested that as the inclination for bacteria to become surface bound is so ubiquitous in diverse ecosystems, there is a strong survival and/or selective advantage for surface dwellers over their free-ranging counterparts (Dunne, 2002).

We propose that the biofilm mode of growth offers a selective advantage as it contributes to the persistence of many mycoplasma species in both the environment and the living host. As mycoplasmas possess only a minimal genome it seems likely that biofilm growth is beneficial, if not essential, for many mycoplasmas as otherwise it is likely that the genes necessary for this mode of growth would have been lost during degenerative evolution.

Laura McAuliffe
Veterinary Laboratories Agency

references

- Almeida, R.A. and Rosenbusch, R.F. (1991). Capsule like surface material of *Mycoplasma dispar* induced by *in vitro* growth in culture with bovine cells is antigenically related to similar structures expressed *in vivo*. *Infect Immun* **Vol. 59**, pp3119-3125.
- Costerton, J., Stewart, W.P.S. and Greenberg, E.P. (1999). Bacterial biofilms: a common cause of persistent infections. *Science* **Vol. 284**, pp1318-1322.
- Dunne,W.M. Jr. (2002). Bacterial adhesion: seen any good biofilms lately? *Clin Microbiol Rev* **Vol. 15**, pp155-66.
- Fehri, L.F., Sirand-Pugnet, P., Gourgues, G., Jan, G., Wroblewski, H. and Blanchard, A. (2005). Resistance to antimicrobial peptides and stress response in *Mycoplasma pulmonis*. *Antimicrob Agents Chemother* **Vol. 49**, pp4154-4165.
- Layh-Schmitt, G., Podtelejnikov, A. and Mann, M. (2000). Proteins complexed to the P1 adhesin of *Mycoplasma pneumoniae*. *Microbiology* **Vol. 146**, pp741-747.
- Mah, T.F. and O'Toole, G. A. (2001). Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol* **Vol. 9**, pp34-39.
- McAuliffe, L., Ayling, R.D., Ellis, R.J. and Nicholas, R.A. (2008). Biofilm-grown *Mycoplasma mycoides* subsp. *mycoides* SC exhibit both phenotypic and genotypic variation compared with planktonic cells. *Vet Microbiol* **Vol. 129**, pp315-24.
- McAuliffe, L., Ellis, R.J., Miles, K., Ayling, R.D. and Nicholas, R.A. (2006). Biofilm formation by mycoplasma species and its role in environmental persistence and survival. *Microbiology*. **Vol. 152**, pp913-922.
- Miles, R. J., Taylor, R.R. and Varsani, H. (1991). Oxygen uptake and H_2O_2 production by fermentative *Mycoplasma* spp. *Med Microbiol* **Vol. 34**, pp219-223.
- Razin, S., Yogev, D. and Naot, Y. (1998). Molecular biology and pathogenicity of mycoplasmas. *Microbiol Mol Biol Rev* **Vol. 62**, pp1094-1156.
- Salmon, D.E. (1896). Special report on diseases of cattle and cattle feeding. Pub.Washington government printing office.
- Sasaki, T., Shintani, M. and Kihara, K. (1984). Inhibition of growth of mammalian cell cultures by extracts of arginine-utilizing mycoplasmas. *In Vitro* **Vol. 20**, pp369-375.
- Simmons, W.L. and Dybvig K. (2009). Mycoplasma biofilms *ex vivo* and *in vivo*. *FEMS Microbiol Lett* **Vol. 295**, pp77-81.
- Simmons, W.L., Bolland, J.R., Daubenspeck, J.M. and Dybvig, K. (2007). A Stochastic Mechanism for Biofilm Formation by *Mycoplasma pulmonis*. *J Bacteriol* **Vol. 189**, pp1905-1913.
- Stewart, P.S. (2002). Mechanisms of antibiotic resistance in bacterial biofilms. *Int J Med Microbiol*. **Vol. 292**, pp107-13.
- Tu, A.H., Clapper, B., Schoeb, T.R., Elgavish, A., Zhang, J., Liu, L., Yu, H. and Dybvig, K. (2005). Association of a major protein antigen of *Mycoplasma arthritidis* with virulence. *Infect Immun* **Vol. 73**, pp245-249.
- Welin, J., Wilkins, J.C., Beighton, D. and Svensater, G. (2004). Protein expression by *Streptococcus mutans* during initial stage of biofilm formation. *Appl Environ Microbiol* **Vol. 70**, pp3736-3741.



Ask the expert — biofilms and medical devices — the basics

After the S*f*AM Winter Meeting, I was lucky enough to have the opportunity to interview one of the speakers, **Dr Rodney Donlan** of the Centres for Disease Control and Prevention (CDC), USA. I asked him a little more about biofilms and using bacteriophage to control biofilm formation on medical devices

Q In this issue of *Microbiologist* we've learnt a lot about biofilm recalcitrance as well as *Mycoplasma* biofilms, but can you explain the basics to us — what exactly are biofilms?

A When we think of biofilms we think of organisms that are associated with a surface or interface. They are relevant for a number of different situations: organisms can grow as biofilms in aquatic systems, for example or soil systems, on particles or on rock surfaces. Have you ever slipped while crossing a mountain stream? You can blame the native algae and bacteria for covering the rock surfaces with a slimy biofilm. Biofilms can also be relevant in a number of industrial processes or in drinking water systems and are important in human tissues, for example, the oral flora will form biofilms on tooth surfaces, leading to dental caries or periodontal disease.

Biofilms are also very important in public health and may play a role in infections associated with medical devices.

Q How can biofilms result in hospital acquired infections and other complications?

A Biofilm organisms can colonize indwelling medical devices, e.g. indwelling catheters, prosthetic joints — hips or knees, or prosthetic heart valves. When these devices are put into the patient, if they're contaminated with certain species of bacteria, these bacteria may grow and form a biofilm: a community of organisms embedded in an extracellular polymeric substance (EPS) matrix. When the biofilms then go through the normal growth process, some of the organisms detach from the biofilm and can enter the bloodstream where they can colonize other parts of the body and cause an infection in the patient. They can also cause complications such as blockage of urinary catheters, malfunction of artificial voice prostheses, or the faulty operation of medical devices. These biofilm bacteria may also produce endotoxins and other pyogenic substances which may be associated with patient complications such as fever.

In terms of formation we tend to look first of all at contamination of the device as it's being put into the patient. For example an intravascular (IV) catheter is inserted through the skin into a vein and when that happens, there are times when the bacteria on the skin surface or the hands of the physician could contaminate that device. These organisms could colonize the device and form a biofilm, so in that case you might expect skin flora or environmental flora to comprise this microbial community. Biofilms can also occur through what's called hemotogenous seeding, where organisms from an infection at another location in the body colonize the medical device surface. For example, with a prosthetic joint we might see biofilms occurring months or more than a year after the device was put into the patient.

Q Which organisms can cause or create biofilms on medical devices?

A A number of different kinds of bacteria are associated with medical devices: we see Gram-positive organisms from the skin flora, for example *Staphylococcus* species. The coagulase negative staphylococci are probably the most common bacteria we find on medical devices causing infections, followed by *S. aureus*. We can also find Gram-negative bacteria including *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and we can even see fungi such as the *Candida* species. We probably don't know the full spectrum of organisms that can be found. Also, the communities of organisms will depend on the type of device. If we look at prosthetic joints for example, we might find some of the anaerobes like *Propionibacterium* in the biofilms. If we look on urinary catheters we'll find in predominance the Gram-negatives, for example *Proteus mirabilis*, *E. coli*, *Kl. pneumoniae*.

Once a bacterium has adhered to a device surface it will express different genes controlling production of bacterial components necessary for biofilm formation.

Q How do biofilms create a niche for the growth of antibiotic resistant organisms?

A At the CDC we examined a biofilm which had formed on a medical device taken from a patient from whom a drug-resistant organism was isolated. It happened to be a nephrostomy tube and we found very complex mixed species biofilms growing on the device. We also isolated from that device vancomycin-resistant *Staphylococcus aureus*. We know from some published studies that biofilm organisms have the ability to transfer resistance by means of plasmids from resistant to susceptible organisms. We also know that the biofilm provides conditions which are optimal for that process to occur, either by conjugation or transformation. So once the organisms are in the biofilm they can be potentially protected from antimicrobial agents in the biofilm structure and seed the environment or other parts of the body as organisms grow and disperse from the biofilm. We also know that biofilm organisms are very tolerant to many different types of antimicrobial agents, such as biocides, disinfectants, and antimicrobial drugs.

Q What makes biofilms tolerant to antimicrobial agents?

A We know that this is true from a number of different studies and different mechanisms have been proposed for what we call tolerance or recalcitrance. One possible mechanism is that the EPS will retard the penetration of the antimicrobial agent into the biofilm. Biofilm cells may also alter the immediate chemical and physical microenvironment and alter the activity of the agent. This might also result in reduced growth rates of the organisms which could affect the ability of agents which rely upon active cellular growth to inhibit or kill the cells. Biofilm bacteria may exhibit specific stress responses. There is also a possibility that a small percentage of cells in the biofilm, called persisters are dormant and are recalcitrant to antimicrobial treatments. The reality is almost certainly a combination of different mechanisms.

Q What measures have been taken to combat problematic or dangerous biofilms and can you tell us a bit about each method please?

A There are many different measures which have been investigated in the prevention of biofilm formation. Studies have shown that ultrasound, when combined with antimicrobial drugs such as gentamicin, will reduce biofilms in animal and *in vitro* models. In these studies, the ultrasound was applied in the animals using a transducer and the combination treatment was significantly more effective than treatment with only the antimicrobial drug.

Microbially-produced signalling molecules of various kinds can be used to disperse cells from a biofilm. Studies have shown that a fatty acid messenger, *cis*-2-decenoic acid that is normally produced by *Ps. aeruginosa*, will disperse biofilms of this and a number of other clinically relevant microorganisms, including biofilms of the yeast *Candida albicans*. This idea takes a natural process and uses that process to destabilize the biofilm structure.

It is also possible to use enzymes which target the EPS matrix and I think this is an important approach. The problem is, we need to know which extracellular polymers are in the biofilm and therefore which ones we should be looking to target. Previous studies have used depolymerase enzymes which were

purified against various types of polymers and used to destabilize the biofilm structure. Certain bacteriophages naturally produce depolymerase enzymes and a recent paper reported that a gene for the enzyme dispersin B, could be cloned into a bacteriophage. When this gene was expressed the phage could degrade the extracellular polymers of the phage's host *E. coli* strain. This breaking down or hydrolysis of polymers in the biofilm could actually eradicate the biofilm from the surface and render the cells more susceptible to treatment with antimicrobial agents. At the CDC we have taken bacteriophages which are lytic towards specific bacteria, and we've placed them on to surfaces to try to prevent the formation of bacterial biofilms on that surface. The same can be done with phage in reverse where we grow a biofilm and then add the phage to penetrate the biofilm and destabilize its structure.

The advantages of using bacteriophage are that in a biofilm we're dealing both with the bacterial cells and with the extracellular structure we've already talked about. The ideal situation in the control of biofilm formation would involve the use of a treatment which is not only highly effective against the bacterial cells, but is also effective against the structure of the biofilm. So having a phage that can kill the bacteria and can break down the extracellular part of the biofilm is advantageous. In addition, phages are fairly easily isolated from the environment and we can combine phage together to make the method more effective. Furthermore, we know from the literature that progeny phage will actually penetrate into a biofilm as opposed to being washed out from the biofilm so we know there's an advantage there.

Q You've mentioned the advantages of using phage, but are there any disadvantages to using this method of biofilm control?

A As with any control method there are disadvantages. One such disadvantage is bacterial-phage resistance, which we do see, though the rates are fairly low: usually around one in ten million. This means that if we add phage to a biofilm, not all of the biofilm organisms will be killed — most of them will, but some will survive. One way of attempting to remove the biofilm completely is to develop cocktails of combinations of phages to target the bacteria. Unfortunately we're always going to have to deal with this issue of resistance which is why I would suggest the use of multiple methods for controlling biofilms in addition to phage.

Q Have you found any specific mechanisms that can be used to control the development of biofilms on medical devices?

A We've shown that phage can be placed onto a surface and remain viable and lytic. So we know that when we challenge that surface with host bacteria, we can significantly reduce the number of bacteria that colonize the surface. I would see that as advantageous for a number of different treatments, not only for medical devices but possibly all surfaces in the healthcare environment. If we designed a treatment effectively with the right combinations of phage, we might be able to protect surfaces from that initial bacterial challenge.

Also, there are probably ways in which we can stick phage to materials more effectively. Our approach has been to incorporate the phage into a hydrogel coating, but I think by using better methods, we could control the number and density of phage on a surface.

Another approach might be what we call a phage lock, where we add the phage to a contaminated colonized surface or device and see if this treatment can then reduce the biofilm and eradicate it from that surface.

Some of these concepts could be applied to other sorts of device or surfaces relevant in public health.

Q What do you see as the future for biofilm control?

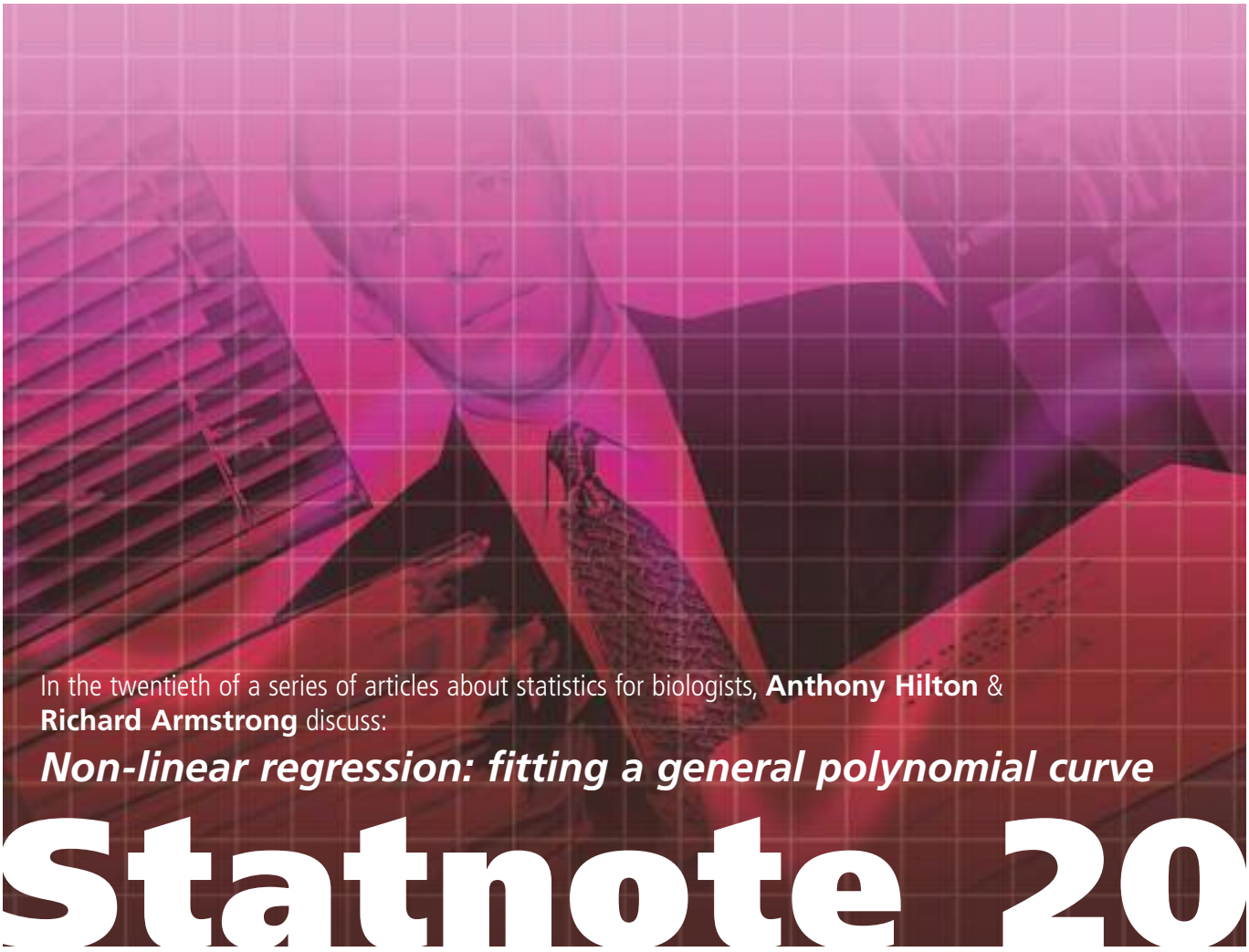
A There are a number of important directions for biofilm control research. Something that was a focus during today's meeting (*Sf*AM Winter Meeting 2010 — see page 20 for full report) is the need for standardized protocols for testing. If we understand that biofilms are ubiquitous then we need standardized protocols for their testing and further evaluation. We need methods that can be undertaken by laboratories and practiced in a healthcare environment: for example how the data from one sort of device or test can be reliably compared with data from another sort of device or test.

The second aspect would be to consider the role of biofilms in disinfection. At the CDC we have looked at the role of free living protozoa and biofilms in the survival of pathogens in, for example, water systems. We need to understand the role protozoa play in a biofilm in protecting organisms from disinfectants or biocides.

Also, from a medical devices perspective, it's important to look at different methods that would not only prevent biofilm formation with combinations of treatment that can be tolerated by the patient, but also treatments that might be used to eradicate or remove the biofilm. A lot of the clinical studies that have been published examine the effect of novel treatments on, say, device- associated infections, but they often don't really look for biofilms. So the treatment might be effective in reducing infection rates but it's not clear whether the biofilm is being eradicated or just treated and reducing in number. In this case, when the treatment is removed, the biofilm returns.

Another interesting direction would be to look for novel methods of detecting initial adhesion of organisms onto biomaterials, for example in the bloodstream. If we had a way to detect when a catheter has become colonized by organisms within hours, we might be able to treat that device effectively, whereas if we don't find out for days or weeks that the device has been colonized, the biofilm becomes untreatable. So I think novel technologies for detecting early biofilm formation would solve many of the problems in a healthcare setting associated with biofilm formation. There is a lot we still don't fully understand about biofilm formation and treatment — it's an exciting area and a superb example of microbiology in a truly applied sense.

Lucy Harper
Communications Manager



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

Non-linear regression: fitting a general polynomial curve

Statnote 20

An investigator may have no knowledge of the theoretical relationship connecting two variables (X,Y) but it may still be necessary to fit a curve to the data, e.g., to be able to predict Y for new value of X. If after plotting the data, simple curvature appears to be present, an immediate question may be whether a curved line would fit the data rather than a straight line. Without prior knowledge of the shape of the curve, fitting a second-order (quadratic) polynomial is often the best approach in the first instance. Essentially, the goodness of fit of a second-order polynomial is compared with that of a straight line; analysis of variance (ANOVA) being used to test the difference between the two fits (Hilton & Armstrong, 2009a). With more complex curves, polynomials of higher order may be necessary to provide the best fit to the data. Hence, polynomials of order 1, 2, 3…n, can be fitted successively to the data and with the addition of each extra term, a further 'bend' is added to in the curve. Hence, third-order 'cubic' curves are 'S' shaped and fourth-order 'quartic' curves have four 'bends' and may appear to be 'double peaked'. An investigator may then have to decide which of the curves provides the 'best' fit to the data. This Statnote describes two statistical methods:

1) to determine whether a simple curved line fits better than a straight line and
2) the fitting of a more complex polynomial-type curve.

Scenario A: does a curved line fit better than a straight line?

We return to the scenario first described in Statnote 19 (Hilton & Armstrong, 2009b). Soil has various horizons and forms a complex series of microhabitats. If soil has a uniform structure, the abundance of soilborne organisms declines markedly within a few centimeters of the surface and continues to decrease with depth. To examine the relationship between the population density of soil fungi and soil depth, the number of fungal colonies at different depths was measured in a sandy soil at a site in the West Midlands. The number of fungal colonies was estimated by the dilution plate

Table 1. Analysis of variance (ANOVA) to test the departure of a set of data from a linear regression (Data from Statnote 19, Table 1)

Source	*SS*	*DF*	*MS*	*F*	*P*
Linear regression	26396.509	1			
Second-order regression	32832.989	1			
Reduction	6436.48	1	6436.48		
Deviations from Second-order	8685.738	8	1085.717	5.93	<0.05

SS=Sums of squares, *DF*=Degrees of freedom, MS = Mean square, F = Variance ratio, P = probability

Figure 1. *Testing whether there is a significant departure from a linear regression. Both a straight line and a second-order (quadratic) polynomial are fitted to the data and analysis of variance (ANOVA) is used to determine whether a significant proportion of the remaining variance after fitting the linear relationship is accounted for by the quadratic polynomial*

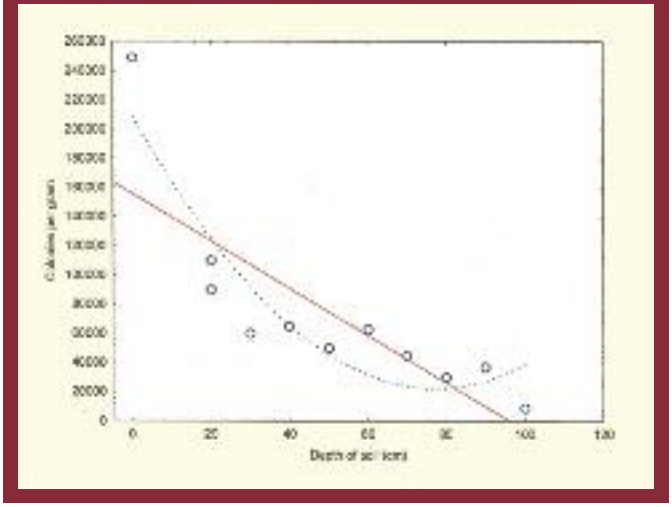
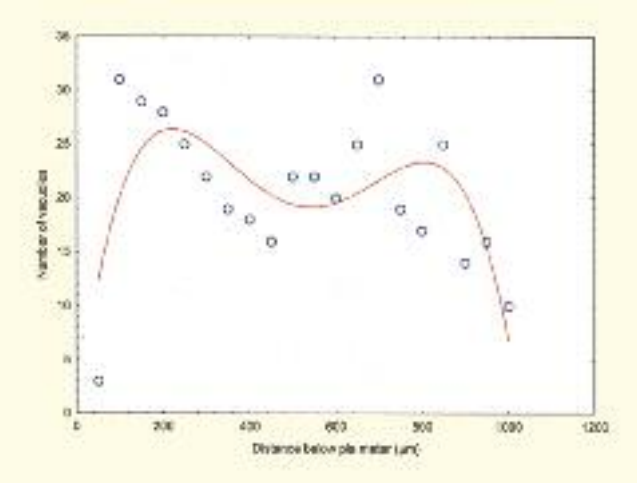


Figure 2. *Fitting a fourth-order polynomial curve to the distribution of vacuoles in an area of cerebral cortex in a case of Creutzfeldt-Jakob disease (CJD). The distribution is bimodal with a peak of vacuole density in the upper cortex close to the surface of the pia mater and a second peak in the lower cortex*



method from a profile dug into the soil at the sample site. Samples of soil of one gram were taken at varying depths down to a maximum of one metre. In Statnote 19, it was assumed that the shape of the curve describing the decline in fungal colonies with increasing soil depth was negative exponential. In the present analysis, no prior knowledge of the possible shape of the decline in numbers with soil depth is assumed. The data are presented in Statnote 19 (Table 1) and comprise measurements of the number of fungal colonies (Y) in relation to soil depth (X).

How is the analysis carried out?

First, a straight line is fitted to the data and an ANOVA carried out to obtain the sums of squares (SS) of the deviations from a linear regression (Hilton & Armstrong, 2009a). A second-order (quadratic) polynomial curve is then fitted to the data, i.e., an equation of the form:

$Y = a + bx + cX^2$.....(20.1)

Hence, $Y = a + bx$ is the equation of a straight line and the addition of the term cX^2 describes the degree of curvature of the data. A second ANOVA is then carried out to obtain the SS of deviations from the curved regression. The difference between the linear and curvilinear SS measures the reduction in SS of the Y values achieved by fitting the curvilinear rather than the linear regression. This difference is then tested against the deviation from the curved regression using an 'F' test. If the 'F' ratio of the mean square reduction in SS to the mean square of the deviation from a curvilinear regression is significant, then the curved relationship is a better fit to the data than the linear. Second-order regressions will often work well for estimation and interpolation within the range of the data even if the actual relationship between Y and X is not strictly quadratic. Extrapolation beyond the range of the measured X data for estimation of Y, however, is extremely risky. If several values of Y are available at each X, then the goodness of fit of the line can be tested more rigorously (Snedecor & Cochran, 1980).

Interpretation

The analysis of variance of the data is shown in Table 1. The difference in the SS as a result of fitting a second-order polynomial curve compared with a linear regression line is calculated and its mean square tested against the mean square deviation from a second-order polynomial. A value of $F = 5.93$ was obtained which is significant at the 5% level of probability ($P < 0.05$). Hence, the second-order polynomial curve is a significantly better fit to the data than the linear regression line.

Scenario B: fitting a general polynomial-type curve

The human disease Creutzfeldt-Jakob disease (CJD) is caused by unusual proteinaceous infectious agents called *prions* (Armstrong, 2003). Characteristic of the brain pathology caused by prions is the development of vacuolation ('spongiform change') within the cerebral cortex resulting from the death of neurons. An investigator wished to determine if the extent of the vacuolation varied across an area of cerebral cortex from pia mater to white matter. The specific objectives were to determine which cortical laminae were significantly affected and, therefore, which aspects of brain processing were likely to be impaired (Armstrong *et al.*, 2002). To obtain the data, five traverses from the pia mater to the edge of the white matter were located at random within an area of cortex. The vacuoles were counted in 50 x 250μm sample fields; the larger dimension of the field being located parallel with the surface of the pia mater. An eye-piece micrometer was used as the sample field and was moved down each traverse one step at a time from the pia mater to the white matter. Histological features of the section were used to correctly position the field. Counts from the five traverses were added together to study the vertical distribution of lesions across the cerebral cortex. Hence, the data comprise estimates of the density of vacuoles (Y) at different distances below the pia mater of the brain (X) and are presented in Table 2.

How was the analysis carried out?

Polynomials of order 1, 2, 3…n, were fitted successively to the data. With each fitted polynomial, the regression coefficients, standard errors (SE), values of 't', and the residual mean square were obtained. From these statistics, a judgment can be made as to whether a polynomial of sufficiently high degree has been fitted to the data. Hence, at each stage, the reduction in the SS is tested for significance as each term is added. The analysis is continued by fitting successively higher-order polynomials until a non-significant value of 'F' is obtained. As a precaution, it is usually good practice to check the next order polynomial after a non-significant term has been fitted. This analysis may be part of the regression option within statistical software (e.g. STATVIEW software) but in some packages can be found within the general linear modeling option (e.g. STATISTICA software).

Table 2. Fitting a general polynomial type curve. Data are the density of vacuoles across an area of brain from pia mater to white matter in a case of Creutzfeldt-Jakob disease (CJD)

Distance below pia mater (µm) (X)	*Number of vacuoles (Y)*	*Distance below pia mater (µm) (X)*	*Number of vacuoles (Y)*
50	3	550	22
100	31	600	20
150	29	650	25
200	28	700	21
250	25	750	19
300	22	800	17
350	19	850	25
400	18	900	14
450	16	950	16
500	22	1000	10

Analysis of variance:

Source	*DF*	*SS*	*MS*	*F*	*R²*
Total variation	19	825.8			
Reduction to linear	1	72.782	72.782		0.09
Deviations from linear	18	753.018	41.834	1.74 ns	
Reduction to quadratic	1	80.391	80.391		0.16
Deviation from quadratic	17	672.627	39.566	2.03 ns	
Reduction to cubic	1	77.004	77.004		0.16
Deviation from cubic	16	595.62	37.23	2.06 ns	
Reduction to quartic	1	327.7	327.7		0.49
Deviation from quartic	15	267.92	17.86	18.35***	

DF=Degrees of freedom, *SS*=Sums of squares, *MS*=Mean square, *F*=Variance ratio, R^2=Multiple correlation coefficient, *ns*=Not significant, *** = P < 0.001

Interpretation

The analysis (Table 2) suggests that the linear, quadratic, and cubic polynomials did not significantly fit the data. However, the quartic polynomial was significant (F = 11.67, P < 0.01) suggesting a complex curved relationship between the distribution of the vacuoles and distance below the pia mater consistent with the vacuoles affecting specific cortical laminae. Incidentally, the fit to the fifth-order polynomial (not shown) was not significant. The fit of the fourth-order polynomial to the data is shown in Figure 2. The bimodal distribution of the vacuoles is clearly evident with peaks of density in the upper and lower cortical laminae. It may be possible to predict which aspects of cortical processing may be affected in patients with CJD from such a distribution of the vacuoles.

There are various strategies that can be employed to decide which degree of polynomial actually fits the data best and these depend on the actual objectives of the investigation. First, as each polynomial is fitted, the reduction in the SS is tested for significance. The analysis is then continued by fitting successively higher-order polynomials until a non-significant value of 'F' is obtained. The final polynomial giving a significant 'F' is then chosen as the 'best' fit to the data. Second, it may be obvious that a simple relationship such as a linear or quadratic polynomial would not fit the data and that a more complex curve is required. In this case, examination of the value of the 'multiple correlation coefficient' (R2) may give an indication of the correct polynomial to fit. Subsequently, 'F' tests can be used to choose the most parsimonious model. This is the approach we adopted in the present scenario. Third, the objective may be to obtain the best possible predictions of *Y* from *X*. Hence, polynomial curves varying up to the 10th order can be fitted to the data and curve of best fit selected on the basis of visual inspection and the highest possible regression coefficient obtained.

Conclusion

In some circumstances, there may be no scientific model of the relationship between *X* and *Y* that can be specified in advance and indeed the objective of the investigation may be to provide a 'curve of best fit' for predictive purposes. In such an example, the fitting of successive polynomials may be the best approach. There are various strategies to decide on the polynomial of best fit depending on the objectives of the investigation.

references

- Armstrong R.A. (2003) Getting your proteins in a twist: disease caused by prions. *Microbiologist,* **Vol 4**. No.3 September 2003, pp32-35.
- Armstrong R.A., Cairns N.J., Ironside J.W and Lantos P.L. (2002) Laminar distribution of the pathological changes in the cerebral cortex in variant Creutzfeldt-Jakob disease (vCJD). *Folia Neuropathol,* **Vol. 40**: pp165-171.
- Hilton A and Armstrong R.A. (2009a) Statnote 16: Fitting a regression line to data. *Microbiologist,* **Vol 10**. No.1 March 2009,pp40-42.
- Hilton A and Armstrong R.A. (2009b) Statnote 19: Non-linear regression: Fitting an exponential curve to data. *Microbiologist,* **Vol 10**. No.4 December 2009, pp44-45.
- Snedecor G.W. and Cochran W.G. (1980) *Statistical methods*, 7th Ed. Iowa State University Press, Ames Iowa. Chapter 9.


Anthony Hilton


Richard Armstrong

Dr Anthony Hilton[1] and Dr Richard Armstrong[2]

[1]Biology & Biomedical Sciences and [2]Vision Sciences, Aston University, Birmingham, UK

Writing a grant application

In the loop pecs

News from the S*f*AM Postgraduate and Early Career Scientist Committee

Congratulations!

Congratulations go to PECS member **Manita Mehmi** of Aston University who has been awarded her PhD in Pharmaceutical Microbiology.

If you know a S*f*AM student or early career scientist who has been awarded a PhD/ prize/award then get in touch, email Sumeet Kaur at: **s.kaur@londonmet.ac.uk**

Researchers are dependent on external funding to support their work. As a result, competition for funding is fierce and writing a successful grant application is critical to a productive research career.

Preparation and planning are vital for a grant application to be successful. When planning your application, first identify the funding body/grant for which you wish to apply. Next, consider performing literature reviews, determining availability of equipment and areas of expertise within your organization and identifying ethical requirements for the project.

A successful proposal should have the potential to make a significant contribution in the field and be economically feasible. Therefore, the application is like a sales-pitch: after reading it the reviewers need to be convinced that your project is worth funding. So as well as being clear, the proposal should demonstrate an emerging supposition backed up by findings published in current literature. It should also demonstrate a certain amount of novelty in approach, as well as explaining how it will enhance understanding in the field. A rationale for the proposal should be provided, along with reasons for the choice(s) of methodology and expenses involved.

Grant funding bodies will provide you with a set of guidelines which include everything from font size to word limit and it is important that you adhere to these instructions. The application should be clear and concise and the aim and objectives should be directly associated with the expected outcomes. Some organizations also require further information such as location, resources, a curriculum vitæ (CV) and budgetary details from the applicant. An application may be supported by research that has already been conducted by the applicant, applicant's group or other groups. This can include published or unpublished data and personal communications. If required, graphs or other illustrations can be included in the appendix rather than the main body of the application. The proposal should also specify the strategies that would be employed for data analysis.

In general, most grant applications require justification at two levels. Firstly, the applicant must be able to demonstrate that the proposal is innovative and will add to understanding in the field. Secondly, expected expenditure needs to be justified: is the potential output of the project worth the financial investment?

Upon completion of the application, it can be helpful to take a break before looking through it again. This will enable you to identify any areas that need improvement. Also, don't be afraid to seek advice from experienced grant recipients whose help can be invaluable. Applications must be submitted before the deadline, along with a covering letter and a copy of the latest CV of the principal investigator.

If your application is rejected, feedback can be requested and your application resubmitted following incorporation of the suggested changes. Finally, there could be conditions attached to the funding, for example, to give a poster or oral presentation at a particular conference, or publish work in a particular journal. Some organizations may provide extra funding for this. Writing a grant application is only the first step in the process; once funding is attained the journey begins.

Sumeet Kaur
PECS Communications Officer

careers

A parasitologist in the making

Telling people about my profession tends to be a conversation stopper. Either they don't want to hear about it because it sounds so disgusting or they want me to describe in minute detail exactly what my job entails. I have come to the conclusion that people in general enjoy distasteful stories especially whilst at the dinner table.

So how did I become the Scheme Manager of the United Kingdom National External Quality Assessment Service (UKNEQAS) Parasitology. This occupation not only involves the preparation and distribution of parasitological specimens, but also travelling around the world to collect the specimens. When I left school I never envisaged that in 30 years hence I would be processing feces, blood and other repulsive specimens that constitute UKNEQAS Parasitology. Although my mother did say that as a toddler I had an unnatural fascination for dog feces on the pavement and as a school child, David Livingston in the African jungle, was my hero. So I suppose from that early age, the writing was on the wall.

At school I studied science subjects and then did a BSc in Microbiology at the University of Glasgow. My favourite modules were parasitology and mycology mainly because the professors who taught these subjects were interesting characters and had tales and pictures of parasites and fungi that any normal person would find revolting. The head of department was a fearsome lady who taught us microbiology using a non-electrical monocular light microscope. Little did I know that this would pave the way for my future career.

By this time I knew that I wanted to work in Africa but was told in no uncertain terms I was being too ambitious. The advice given to me was to obtain some experience before conquering the parasites of the Tropics so my first place of employment was in the Microbiology Department, Bellshill Maternity Hospital, a post which I loved. Unlike University, I examined my Gram stains using an electric binocular microscope. During these four years I learnt the foundations of Clinical Bacteriology and became State Registered with the Council for Professions Supplementary to Medicine (CPSM, now the Health Professions Council—HPC). I also became an Associate of the Institute of Biomedical Science (IBMS) and studied for the Special Examination during which I enjoyed yet more parasite stories from a very enthusiastic parasitologist from the Scottish Parasitology Diagnostic Laboratory. I passed the examination and was awarded a Fellowship of the IBMS. However, Africa was still beckoning and I decided to join the Volunteer Missionary Movement with the intention of fulfilling my dream.

During a bleak, cold November in 1981, two volunteers left Glasgow and arrived in hot humid Lagos, Nigeria. I still remember the anticipation, bewilderment and excitement of arriving in a place so different from Glasgow. It seemed chaotic and I didn't understand what was being said although everyone was speaking English. Fortunately my friend had worked there previously and knew the routine. We were met by a priest from the diocese in which we would be working. After two days of travelling on very bad roads and experiencing landscapes from tropical rainforest to savannah, we arrived at our destination, St Brendan's Missionary Hospital in Bacita, Kwara State run by the order of Our Lady of Apostles. This was to be my home for the next two years.

Whilst in Africa, I set up a laboratory and trained some local people in laboratory procedures. I encountered parasites galore, both fecal and blood and was constantly learning. I experienced the vagaries of living in an African village, water shortages, no electricity for days at a time, deaths due to malaria, measles and pneumonia, poverty and malnutrition. Despite all these hardships, the people remained colourful and happy, always ready to give a gift of oranges, mangos or eggs. Work continued during water and electricity shortages. After all, we could draw water from the village well and I had my monocular light microscope which did not require electricity to continue work in the laboratory. We had a gas cooker in the house so cooking wasn't a problem and we didn't have hot water or television anyway. I loved my experience in Nigeria. I loved the exuberance of the people, the Nigerian food, the missionary nuns and priests and being in Africa. All too soon my two years came to an end and with heavy heart, I went back to the cold of Glasgow.

Soon after arriving back in Glasgow, I rekindled some old laboratory acquaintances and found myself working in the Microbiology Department in Stobhill Hospital in which the Scottish Parasite Diagnostic Laboratory was based. I was being exposed to parasites again but unfortunately for me, there are not many parasites in Scotland. However, many of the positive specimens came from a new parasitology scheme which had just been set up by UKNEQAS for microbiology. Like most microbiology laboratories, I was rotated round the different sections but my favourite section was the parasitology and fecal section as it was headed by eccentric people like those who taught me whilst training. I remember being involved in diverse projects like squashing ticks to look for *Borrelia burgdorferi* or *Babesia* parasites. Exciting stuff!

Although I enjoyed my experience in Stobhill Hospital, I got the five year itch and went to work first in a private laboratory in Moers, Germany and then the British Military Hospital (BMH), Rinteln. This was a great experience, since

soldiers were always being sent to exotic places and returning with "friends" i.e. blood and fecal parasites. Working in Rinteln was fun and also educational. I discovered there that most of the parasitologists I admired so much had all been in the Royal Army Medical College and were Scottish to boot. However, it was in Rinteln that my life would change forever in a monumental way.

It was in Rinteln I met the man who was to be my husband. He was a Warrant Officer and although my boss, he shared my love for parasites and also fungi, the edible type. One of our pastimes was to go into the forest and gather mushrooms. Sometimes we wondered if we would still be alive the following day! It was when he was posted back to the Royal Army Medical College in Millbank, London that I started scouring the IMBS Gazette for a job to be near him. It was there in December, 1991 that I saw the job of my dreams. A post in the recently established UKNEQAS Parasitology based in the esteemed Hospital for Tropical Diseases, London. I telephoned the Laboratory Manager and told him I wanted the job and asked him to send me an application form. I made many assumptions on that day, one of them was that I would be selected for an interview for the job, so I handed in my notice to the BMH, Rinteln and came to London. Fortunately I did get an interview and was appointed to the post.

On 6 January, 1992, I started my dream career in UKNEQAS Parasitology in the Department of Clinical Parasitology, Hospital for Tropical Disease. This is a subscheme of UKNEQAS for microbiology which operates from HPA, Colindale. I was in my element working with fecal specimens full of parasitic eggs and larvae and blood specimens with all types of blood parasites. The icing on the cake was working with likeminded enthusiastic people. My tasks back in 1992 included selection, preparation and quality control of the specimens for UKNEQAS Parasitology distributions, dispatch of specimens to participants, analyses and presentation of distribution results and developmental work for the schemes. I was the only member of staff.

But where did the specimens come from? The blood specimens came from the diagnostic laboratory who alerted me when one contained a nice malaria or microfilaria species. When this happened, it was down tools and about 1000 blood slides were made and stained. Acquiring fecal specimens was a more interesting quest. Where would I obtain the variety of specimens to send 1mL to 500 laboratories? The answer lay in my Missionary connections. I wrote to the matron of St Brendan's Hospital, Nigeria and told her my problem. She was now in St Joseph's Clinic, Nkwanta, Volta Region, Ghana. She informed me that there were an abundance of fecal parasites in Nkwanta and would be willing to help me collect them. So ten years after returning from Nigeria, there I was again in a mission hospital in Ghana screening the local population for parasites.

Over the next six years I returned to Nkwanta and tested schoolchildren and selected villagers for fecal parasites. I relied on the help of translators and primary health workers to explain my requirements. So off I went with the team, armed with my monocular light microscope. I would set up my microscopy laboratory under a tree facing the sun and start screening anyone with abdominal pain or diarrhoea. I always acquired specimens with a wide range of parasites and because all the villages were centred around Lake Volta, there was no shortage of both *Schistosoma haematobium* and *Schistosoma mansoni*. The volunteers were very willing to be screened and supply large amounts of specimen.

My missionary friend was then posted to a mission hospital in Bugisi, Tanzania where I went to for several years to collect specimens. The advantage was that she knew my requirements and I arranged my visits around the rainy season and when there were gatherings of villagers in the diocese. Like Nkwanta, Ghana my visits were always very fruitful.

Other specimen collection trips have been to Iquitos, Peru; Gilgit, Pakistan; Medical Research Council, Gambia; Mumbai, India and Menoof, Egypt. In these locations I had the privilege of being part of a team from the Hospital for Tropical Diseases involved with teaching Parasitology. Teaching is an essential part of the work wherever we collect specimens.

In the early days, there were never any problems bringing the specimens back to the Hospital for Tropical Diseases. The specimens were packed into our hand luggage with the duty free strategically placed on top and questions were never asked at Customs. Nowadays, all the specimens have to be transported back by airfreight which can be more problematic, particularly if the specimens are held up in customs.

Seventeen years later, I am still enjoying my work as much as I did when I started in 1992. I now have two additional members of staff helping me with the day-to-day running of the schemes. The number of schemes has increased from two to six with the addition of Toxoplasma *IgG* and *IgM* serology, parasite serology and blood lysates for Rapid Diagnostic tests for Malaria. The number of participants continues to increase for all the schemes. In these seventeen years I have completed an M.Sc in Clinical Parasitology awarded by University College London; a Certificate in Management, awarded by the Open University; courses in Adult Education and Quality Management. I am continually being invited to give presentations to our European participants. To date I have been to Italy, Romania, Portugal, Netherlands and Sweden not to mention journal clubs in Britain and the IBMS congress. I am one of the tutors involved in the Parasitology Update courses held in the University of Westminster and also tutor MSc. and Clinical Scientist students.

My day-to-day work remains very challenging with a steady stream of new initiatives and a great deal of variety to keep me motivated. I feel privileged to belong to the organization UKNEQAS which provides an external quality assessment service for clinical laboratories and helps ensure that the results of investigations are reliable. I also feel honored to be employed in the Hospital for Tropical Diseases where the ghosts of the early pioneers of tropical medicine still influence the employees. More importantly, I feel extremely fortunate to be able to visit my beloved Africa annually to collect specimens for the scheme and be involved in training programmes there. In conclusion, I forever feel fortunate at being in a profession that inspires and stimulates me.

Monika Manser
UKNEQAS Parasitology

Overseas Development Award
Report on a training visit to the UK

I was born in 1976 in Cankuzo, Burundi where I received my primary school education. My secondary school was in Ruyigi and then I attended Muyuga College where I gained my State Diploma in Science and Humanities. The Rwandan Government sent me to Algeria, to study first at the University of Algiers and subsequently at the University of Oran where I graduated in 2006 with a Diplôme Ingénieur in Microbial Biotechnology Engineering.

For the past two years I have been employed as Supervisor of the Microbiology Laboratory in the Rwanda Bureau of Standards (RBS). The mission of RBS is 'to improve the quality of life of Rwandan people through the effective application of Standardization, Quality Assurance, Metrology and Testing'. In my laboratory we examine a wide range of food, water, cosmetics, toiletries and other products for total microbial numbers and for pathogenic organisms such as the *Enterobacteriaceae*, staphylococci and vibrios. The RBS requires my laboratory to become accredited because if analyses are requested of an accredited laboratory we have to send the samples to Uganda and this is much more expensive and time consuming. But we did not know what we needed to do to become accredited and we also needed to learn how to improve our laboratory quality assurance, determine measurement uncertainty and other critical aspects.

I had read an article by Professor Basil Jarvis on measurement uncertainty and wrote to ask whether it would be possible to obtain training in this and other matters. He discussed my request with Dr Janet Corry of the University of Bristol Veterinary School and other colleagues, and he told me that they might be able to obtain a grant to enable me to visit the UK to study. I was so pleased when I was told that *Sf*AM had awarded me an **Overseas Development Award** to pay my fares and living expenses in the UK. Professor Jarvis and Dr Corry arranged my training programme and Dr Corry booked my flights and accommodation. I feel very privileged to have been able to come to the UK.

The author in the Microbiology laboratory at Campden BRI with Tina Shilham and Julie Archer

I arrived on Sunday 31 August, flying from Kigali to London via Nairobi and then on to Aberdeen, where I attended the Food Micro 2008 conference. This provided me with the opportunity to learn many things about aspects of modern food microbiology; in particular, I learned much about examination of foods for *Listeria* spp. and *Campylobacter* spp. and also about mycotoxins in foods. I met many interesting people and was pleased to visit the *Sf*AM stand. After the conference, I traveled with Dr Corry to Langford, staying overnight near York, and visiting Stirling Castle, York Minster and many other places on the way.

My first week at the Bristol University Veterinary School at Langford was spent in the microbiology laboratories where I learned how they carry out various microbiological examinations and saw the media preparation area and other departments, including the molecular microbiology laboratory and real-time PCR assays. In the second week I received instruction on laboratory accreditation from Dr Susan Passmore, a UKAS accreditor for food microbiology laboratories, who also went with me to visit the Langford Laboratory of the Veterinary Laboratory Agencies to see how they organized their work as an accredited laboratory. Then Dr Alan Hedges and Professor Jarvis introduced me to many aspects of quantitative microbiology, statistics, estimation of measurement uncertainty, Laboratory Quality Assurance and related matters.

My final week was spent at Campden BRI (the newly-formed organization resulting from a merger of Campden and Chorleywood Food Research Association and Brewing Research International), with Dr Mike Stringer, Dr Roy Betts, Julie Archer and other microbiologists. On the first day I attended the annual Oxoid Industrial Seminar that was focused this year on Accreditation and Method Validation, EU and Codex activities on Microbiological Criteria and *Listeria* control. For the following three days I was based in the Microbiological Analytical Services Laboratory, observing and participating in the work of the routine laboratory and also attended selected sessions of a scheduled "Basic Microbiology Practical Course". My last day was spent in the Laboratory Accreditation Group of Campden BRI, which is a recognized provider of microbiology and chemistry laboratory accreditation in the UK and overseas.

Of particular importance to me was the 'hands on' instruction on operation of a laboratory quality system including sample reception and handling, evaluation and internal auditing of laboratory records, evaluation of staff performance and practical aspects of laboratory accreditation.

My four-week visit to the UK has been of great benefit to me in understanding what needs to be done to improve the Quality Systems at the RBS in order to obtain laboratory accreditation. This will also benefit my organization and my country.

I would like to thank Dr Corry, Professor Jarvis and *Sf*AM for making my visit possible and the RBS for granting me leave of absence to undertake this training.

Alphonse Mbabazi
Rwanda Bureau of Standards, Kigali, Rwanda

Laboratory Fellowship

DNase I footprinting assay: same technique, different strategies

Unveiling DNA-protein interactions is of significant importance when studying regulation of gene expression. Indeed, most known regulation is carried out through binding of proteins to particular DNA regions.

Finding proteins that might have DNA regulatory functions *in silico* is nowadays a fairly quick approach. Predictions can be made based on canonical features such as DNA binding motifs, cofactor binding sites and protein-protein recognition elements. *In silico* approaches can also be helpful in finding DNA sequences that might bind to a protein. Different lab experiments can be designed to confirm these predictions. High throughput approaches are available and are helpful in finding such sequences (De Silva *et al.*, 2008).

DNA mobility shift assays (electrophoretic mobility shift assays — EMSAs) are a good starting point to confirm interesting prediction data. The principle of this technique is based on a change in the electrophoretic mobility of a DNA molecule when it is bound to a protein. Interpretation of EMSA results must consider critical aspects of DNA-protein interactions such as pH, salinity and protein affinity for DNA, among others. It is also important to place the result in the right genetic context: localization of transcriptional start and promoter regions in agreement with EMSA results are a strong indication that a particular operon is regulated by a particular protein.

A complementary approach to EMSA is DNA footprinting. This technique allows mapping of the DNA region where a regulatory protein binds. The protein is first bound to labeled DNA and then the DNA is cleaved either by specific exonucleases or chemical agents at a low frequency per molecule. The most common enzyme used for this purpose is DNase I. When using the conventional procedure, the reaction is analyzed by denaturing gel electrophoresis and the detection normally involves autoradiography of the nucleic acid. The "single hit" DNA enzymatic treatment will generate a series of oligonucleotides of characteristic length displaying a ladder pattern. This pattern is interrupted in those regions where DNase I did not have access, most likely due to steric hindrance imposed by the bound regulator protein.

Identification of the protected regions is carried out by running, in parallel, a sequencing reaction of non-treated labeled DNA. An additional possible finding in the autoradiogram is the presence of intense bands in samples incubated with a DNA binding protein and DNase I. These bands reflect enhanced cleavage of the DNA by DNase I and are probably the result of DNA conformational changes induced by the bound protein or due to protein-protein interactions. Even though the nature of these changes remains elusive, these so called "hypersensitive sites" typically flank a DNA region where the regulator binds.

For a laboratory with no experience in isotopic manual DNA sequencing, setting up a footprinting experiment can be time consuming and expensive. Currently, there are alternatives that have none of these drawbacks (Sandaltzopoulos & Becker, 1994; Yindeeyoungyeon & Schell, 2000; Zianni *et al.*, 2006). They involve using fluorescently labeled primers and capillary-based DNA sequencer/genetic analyzers, now available in most core facilities.

DNase I treated samples analyzed by capillary electrophoresis give a fragment pattern that is very similar to the densitometry trace of an autoradiogram of the same sample analyzed by isotopic manual DNA sequencing (Zianni *et al.*, 2006). The multiplex capability of the automated capillary sequencers together with the available genotyping software allows a thorough comparison of the fragment pattern generated after DNase I treatment of naked DNA and DNA-protein samples. The DNase I footprinting assay can be set up separately for both fluorescently labeled DNA strands. The resulting electropherograms can be superimposed using appropriate size standards as reference. The protected as well as the hypersensitive regions from both DNA strands can be determined and compared to the electropherogram of naked DNA. The nucleotide sequence of the protected region can be obtained by running a DNA sequencing reaction using fluorescently labeled primers instead of fluorescently labeled ddNTPs. By these means, differences in migration rates of DNA fragments containing fluorescently labeled ddNTPs as compared to DNA fragments with non-fluorescently labeled ddNTPs are avoided.

In conclusion, DNase I footprinting procedures can be set up in automated capillary DNA sequencer analysis instruments, resulting in several advantages as compared to the traditional footprinting procedure. Among the greatest advantages are the use of non isotopic and more stable reagents, the time saving nature of the technique and the fact that the sample can be reanalyzed the same day if needed.

I am grateful to Dr Teresa Frisán for the opportunity to visit her lab and Dr María Massucci s group for sharing their academic experiences. My visit to Karolinska Institute in Sweden was supported by an SfAM Laboratory Fellowship.

References

- De Silva, E.K., Gehrke, A.R., Olszewski, K., León, I., Chahal, J.S., Bulyk, M.L. and Llinás, M. (2008). Specific DNA-binding by apicomplexan AP2 transcription factors. *Proc Natl Acad Sci USA*, **Vol 105**, pp8393-8398
- Sandaltzopoulos, R. and Becker, P.B. (1994). Solid phase DNase I footprinting: quick and versatile. *Nucleic Acids Res.* **Vol 22**, pp1511-1512
- Yindeeyoungyeon, W and Schell, M.A. (2000). Footprinting with an automated capillary DNA sequencer. *Biotechniques* **Vol 29**, pp1034-1041
- Zianni, M., Tessanne, K., Merighi, M., Laguna, R. and Tabita, F.R. (2006). Identification of the DNA bases of a DNase I footprint by the use of dye primer sequencing on an automated capillary DNA analysis instrument. *J Biomol Tech*. **Vol 17**, pp103-113

Caterina Guzmán-Verri
Universidad Nacional, Costa Rica

Students into Work Grant report

information

am I eligible - can I apply?

Yes — if you are FULL member who can offer an undergraduate microbiology student the chance to obtain work experience. If you would like to read about the experiences of students who have benefited from this grant, you can do so in each issue of *Microbiologist*.

For further information visit:
www.sfam.org.uk/grants.php

Towards understanding the bactericidal mechanisms of action of oxidizing agents

I have been working for ten weeks in the Pharmaceutical Microbiology Laboratory at the Welsh School of Pharmacy at Cardiff University, under the supervision of Dr Jean-Yves Maillard. This ten week project was part of an ongoing research programme which aimed to understand the interactions of oxidizing agents with macromolecules and microbial cell components. Oxidizing agents such as hydrogen peroxide, peracetic acid (PAA) and chlorine dioxide are used for high-level disinfection in the healthcare environment. Oxidizing biocides have many advantages. They are highly reactive and fast active biocides and their degradation results in non toxic species. Their rapid efficacy is thought to be linked to their rapid penetration into the bacterial cell. Their hydrophilic nature and low molecular weight allow them to pass through the many water filled porins of the bacterial membrane (Denyer & Maillard, 2002). Although, as their name suggests, their antimicrobial activity is due to the oxidation of many microbial components, the interactions between oxidizing agents and key bacterial components at the molecular level has not been well described.

This short project aimed to provide background information on the activity of hydrogen peroxide against *Escherichia coli* K12 (ATCC 25257) used as a bacterial model. This organism was chosen because of the amount of information available on its structure and genome in the literature.

Propagation and enumeration of this bacterium was performed according to standard procedures. Working *E. coli* suspensions were prepared from washing overnight bacterial growth from LB slopes at 37°C with tryptone sodium chloride (TSC) solution and resuspension of the pellet in TSC following centrifugation at 11,000 g. The enumeration protocol was based on the drop counting method (DCM; Miles & Misra, 1938). Optical density (OD_{500}) standardization of washed cultures was performed using diluted suspensions of the washed suspension. Results were validated using statistical analysis (Minitab®) to compare differences between OD_{500} measurements and viable counts. Washed bacterial suspensions were adjusted to approximately 10^9 cfu/ml, following the standard curve obtained from the standardization experiment. Counting techniques (viable count and OD_{500} measurements) were validated to ensure consistency in the results and operator skills.

One of the main objectives of this project was to determine the susceptibility of *E. coli* K12 to hydrogen peroxide and to find concentrations of the oxidizing agent that would damage the cell without completely destroying its structure. A range of concentrations of hydrogen peroxide (0.01% to 7.5%) was investigated. To ensure that biocide activity was quenched after a set exposure time, sodium thiosulfate was used as the neutralizer and its use validated (Martin *et al.*, 2008). The efficacy test protocol used here was based on a carrier test (EN 13697, 2001): a washed bacterial inoculum (approximately 10^9 cfu/ml) was dried on the surface of stainless steel disks (grade 2B finish) and exposed to 0.1 ml hydrogen peroxide at different concentrations. After a 30 second exposure, disks were placed face down in a bottle containing 5g of glass beads and 10ml of neutralizer. The survival of the bacterial inoculum to drying was investigated as a control. Surviving bacteria following exposure to hydrogen peroxide were enumerated using the drop count method, and a Bioscreen C microbial analyser. The use of the Bioscreen jointly with viable count (DCM) can provide an indication of the amount of damage sustained by a bacterial population following exposure to a detrimental agent (Lambert *et al.*, 1998) and as such provides useful information on the effect of a biocide at a low (sub-cidal) concentration. Following recovery of the disks in the neutralizer, a Bioscreen plate was inoculated with a serial dilution of the neutralized suspension together with a dilution of unexposed washed suspension and appropriate controls. Readings ($OD_{420/580}$) were taken every 15 minutes for 14 hours. All the experiments were performed in

triplicate. Results showed that hydrogen peroxide at a concentration of 0.025% was the most appropriate to use in future experiments, since a lower concentration (0.01%) has little effect against the bacterial cells while higher concentrations were bactericidal.

Having identified a sub-cidal concentration of hydrogen peroxide that affected the bacterial cell, the next step in this project was to investigate the effect of hydrogen peroxide on outer membrane proteins (OMPs). OMPs can be used as markers to test the oxidizing effect of the biocide. Following exposure to hydrogen peroxide, OMPs were extracted and purified using a modified sarkosyl method. Protein concentration was determined by the modified Lowry procedure (Markwell *et al.*, 1978). Two exposure protocols were used: a) the direct exposure of a dried bacterial suspension (approximately 10^{11} cfu/ml) on stainless steel disks for 30 sec and, b) the overnight exposure of a bacterial suspension inoculated on a tryptone soya agar plate containing 0.025% hydrogen peroxide. The change in exposure protocol resulted from the drying step producing a 2 log reduction in bacterial number in the carrier test and the recovery of a too low concentration of OMPs. The overnight exposure to hydrogen peroxide also failed to recover enough OMPs to be analysed on an SDS gel. This was a setback at the end of this project and time constraints unfortunately did not allow further progress in this work.

I am really pleased to have contributed to the initial part of this research programme, hopefully generating some useful results. I would like to thank S*f*AM for giving me the opportunity to work in this laboratory, allowing me not only to improve my technical skills, but also to experience a new working environment. Through this project I had the opportunity to gain additional practical microbiological skills increasing my biological knowledge and perspective and also to gain an insight into research, its success and setbacks

Martha Mei Ling Chen
Cardiff University

References

- Denyer, S.P. and Maillard, J.-Y. (2002). Cellular impermeability and uptake of biocides and antibiotics in Gram-negative bacteria. *Journal of Applied Microbiology*, **Vol 92**, 35S-45S
- European standard (pr)EN 12054. Chemical disinfectants and antiseptics. Products for Hygienic and Surgical Handrub and Handwash. Bactericidal activity. Test method and Requirements (phase 2, step 1). European Committee for Standardization, Brussels. EN 12054; 1995
- Lambert, R.J., Johnston, M.D. and Simons, E.A. (1998). Disinfectant testing: use of the Bioscreen Microbiological Growth Analyser for laboratory biocide screening. *Letters in Applied Microbiology*, **Vol 26**, pp288-292
- Markwell, M.A.K, Hass, S.M., Bieber, L.L. and Tolbert, N.E. (1978). A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Analytical Biochemistry*, **Vol 87**, pp206-210
- Miles, A.A. and Misra, J.O. (1938). The estimation of the bactericidal power of the blood. *Journal of Hygiene*, **Vol 38**, pp732-749
- Martin, D.J.H, Denyer, S.P, McDonnell, G., and Maillard, J.-Y. (2008). Resistance and cross-resistance to oxidizing agents of bacterial isolates from endoscope washer disinfectors. *Journal of Hospital Infection*, **Vol 69**, pp377-383

President's Fund reports

information

am I eligible - can I apply?

It is not only our student members who require our help. Senior microbiologists often find difficulty in funding attendance at meetings. If you are in this position you are eligible for this fund.

For further information visit:
www.sfam.org.uk/grants.php

Phenotypic and genotypic characterization of *Clostridium difficile*

***Clostridium difficile* is a Gram** positive, anaerobic spore-forming bacillus which forms colonies with a ground glass appearance and typical 'elephant dung' odour. Immunoassays are generally used in the diagnosis of *C. difficile* associated diarrhoea (CDAD). These detect the presence of either Toxin A or Toxin B which are the major virulence factors produced by the organism, and which are responsible for the symptoms of the disease. The organism is not known to produce any other virulence factors and work conducted at Aston University, Birmingham demonstrated that virulence factors such as proteinase, urease, lipase, haemolysins and gelatinase could not be detected in a panel of over sixty isolates obtained from two tertiary referral trusts within Birmingham. The use of immunoassays to detect the presence of the toxins means that phenotypic characteristics of *C. difficile* are rarely seen by scientists in diagnostic laboratories within the UK.

Since the link between *C. difficile* and pseudomembranous colitis was established, research into the organism has been continuous. The rise in the number of cases of CDAD and especially the number of outbreaks has since resulted in more intense research into *C. difficile* and also an increased demand for the characterization and typing of individual isolates. The standard method of typing *C. difficile* isolates is PCR ribotyping where polymorphisms between the 16S and 23S rRNA genes are identified. The increase in demand for typing has lead to the establishment of the *Clostridium difficile* Ribotyping Network for England (CDRNE) which consists of laboratories in six major cities throughout England; this allows isolates to be sent to the relevant regional laboratory rather than the Anaerobic Reference Laboratory (ARL) which is based at University Hospital of Wales in

Cardiff. To date, there have been over 100 different ribotypes of *C. difficile* identified. Types 001, 027 and 106 are the most common in the UK (Brazier *et al.*, 2007). Treatment of CDAD is with either oral vancomycin or metronidazole. The limited treatment options are due to the intrinsic resistance of *C. difficile* to many antibiotics. Due to these limited treatment options and the method by which CDAD is diagnosed, antimicrobial sensitivity testing is not routinely carried out in the UK.

The level of resistance and the antimicrobials to which resistance is displayed can vary greatly between isolates. Minimum Inhibitory Concentrations (MICs) can therefore be useful in predicting genetic differences and can be used to characterize isolates (Green *et al.*, 2008). There are limitations however to using MICs as the sole basis for characterization as there can be slight variations in results, particularly between different laboratories. Differences highlighted through antibiogram profiles are not always reflected following genomic characterization, particularly where there are only minor variations. The use of MICs has however proved useful in other countries where it revealed previously unseen fluoroquinolone resistance in *C. difficile* isolates associated with a higher rate of mortality (Eggerston *et al.*, 2005). This strain was then identified as a hypervirulent strain which produces increased levels of toxin. It was first isolated in Canada (Warny *et al.*, 2005) and is now recognized as ribotype 027 or NAP-1. Since its emergence in Canada it has been reported throughout North America and Europe and is now one of the most frequently encountered strains in the UK (Brazier *et al.*, 2007).

Another technique for the characterization of *C. difficile* is Pulsed Field Gel Electrophoresis (PFGE). When the hypervirulent strain first emerged in Canada, PFGE was one of the genotypic methods used to characterize the strain and was identified by this method as NAP-1 (North America PFGE type 1). When a hypervirulent strain also emerged in the UK and was characterized as ribotype 027, this and the NAP-1 strain were found to be indistinguishable. Although PFGE has a higher discriminatory power than PCR ribotyping due to its capacity to separate large DNA fragments, some *C. difficile* isolates cannot be typed due to degradation of the DNA. There have however been modifications made to the PFGE method to improve it. This has been done successfully and has been shown to be able to subtype within the 001 ribotype (Gal *et al.*, 2005).

Older and now less common methods used for *C. difficile* characterization are toxinotyping and serotyping. Toxinotyping looks at variances within the pathogenicity locus (PaLoc) which is where the genes for toxins A and B can be found. Pathogenic isolates of *C. difficile* produce these two toxins which ultimately lead to fluid leaking into the lumen of the large intestine resulting in diarrhoea; isolates which cannot produce toxin do not cause disease. Other variances in toxin production include those which produce an additional binary toxin, of which the function is unknown and also production of either toxin A or B but not both. Toxinotyping identifies polymorphisms within the PaLoc between isolates that have different patterns in toxin production but also looks for differences within the same group. Serotyping is a phenotypic method of characterizing strains using agglutination techniques to distinguish differences in antigenic properties between isolates. So far, ten major serogroups have been identified (Poilane *et al.*, 2007).

Other methods which have been used to characterize *C. difficile* isolates include S-layer typing which involves extraction of the S-layer proteins followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to denature and visualise the proteins. *C. difficile* has two S-layer proteins which can vary slightly between isolates; these variances can also be detected using genotypic methods by analysing the *slpA* gene which codes for these proteins.

In addition to the methods that have been outlined here there are many other techniques that have also been used, all having advantages and limitations. As with other bacterial species, genotypic methods have a much greater discriminatory power than phenotypic methods when it comes to characterizing *C. difficile* and are favoured particularly in epidemiological and outbreak studies which are important in understanding and trying to control the transmission of CDAD. It could be argued however that there is little relevance in the typing of strains of *C. difficile*, particularly at a local level, as infections within hospitals are often associated with one ribotype. This somewhat limits the value of the information obtained through strain characterization in epidemiological studies.

References

- Brazier, J.S., Patel, B. and Pearson, A. (2007). Distribution of *Clostridium difficile* PCR ribotype 027 in British hospitals. *Eurosurveillance*, **Vol 12**, No 17, pp3182
- Eggertson, L. and Sibbald, B. (2004). Hospitals battling outbreaks of *Clostridium difficile*. *Canadian Medical Association Journal*, **Vol 171**, pp19-21
- Gal, M., Northey, G. and Brazier, J.S. (2005). A modified pulsed-field gel electrophoresis (PFGE) protocol for subtyping previously non-PFGE typeable isolates of *Clostridium difficile* polymerase chain reaction ribotype 001. *Journal of Hospital Infection*, **Vol 61**, pp231-236
- Green, L., Worthington, T., Hilton, A.C. Lambert, P.A., (2008). Antimicrobial susceptibility patterns of *Clostridium difficile* from two tertiary referral trusts within Birmingham, UK. 18th European Congress of Clinical Microbiology and Infectious Diseases, Barcelona, Spain. April 19-22, 2008
- Poilane, I., Humeniuk-Ainouz, C., Durand, I., Janoir, C.,Cruaud, P., Delmée, M., Popoff., M.R. and Collignon, A. (2007). Molecular characterization of *Clostridium difficile* clinical isolates in a geriatric hospital. *Journal of Medical Microbiology*, **Vol 56**, pp386-390
- Warny, M., Pepin, J., Fang, A., Killgore, G., Thompson, A., Brazier, J., Frost, E.,Clifford and McDonald, L. (2005). Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. *The Lancet*, **Vol 366**, pp1079-84

Lauren Green
Aston University, Birmingham

Interactions between foodborne pathogens and protozoa — implications for zoonotic disease

The persistence of foodborne bacterial pathogens in the environment is an essential part of their transmission to food producing animals. The microbial ecology of this process is little understood. In recent years it has been suggested that protozoa,

particularly free-living amoebae, may be important reservoirs for environmental persistence and transmission of three foodborne pathogens of particular significance in the UK: *Campylobacter jejuni*, *Salmonella enterica* and Enterohaemorrhagic *Escherichia coli* such as O157:H7 (Snelling *et. al.*, 2006). The ubiquitous nature of amoebae in soils, water courses and the intestines of many animal species means they have enormous potential as reservoirs for pathogens. The role of amoebae as a pathogen reservoir is best understood in *Legionella pneumophila*, the causative agent of Legionnaires' disease (Swanson & Hammer, 2000). Though survival and replication within amoebae has been shown to occur for a range of bacterial pathogens of animals including *Mycobacterium avium* and *Pasteurella multocida*. *L. pneumophila* is believed to be a natural parasite of fresh water protozoa. Human infection usually occurs following inhalation of aerosols containing *L. pneumophila*. The bacteria are then able to invade and replicate within alveolar macrophages. There are common mechanisms employed in replication within amoebae and macrophages. Furthermore, in experimental animal models disease is exacerbated by infection with *L. pneumophila* and amoebae together and the passage of legionellae through amoebae leads to increased virulence and resistance to antimicrobials.

Recent work has indicated that *C. jejuni* survives well within vacuoles of *Acanthamoeba polyphaga*, and that passage through amoebae increases their fitness (Axelsson-Olssen *et.al.*, 2005). Survival of *C. jejuni* has also been reported within two waterborne protozoa in the drinking water of broiler chickens and their survival within protozoa increases resistance to disinfection (Axelsson-Olssen *et.al.*, 2005). *S. enterica* serovars Typhimurium and Dublin have both been shown to survive within *A. polyphaga* and *Acanthamoeba rhysodes*. Workers at the University of Warwick have described the mechanism of bacterial growth within contractile vacuoles (Gaze *et.al.*, 2003). In common with legionellae, salmonellae are able to survive and replicate within host macrophages and this is an essential process during systemic *Salmonella* infections. One theory is that the ability to survive within phagocytes and the bacterial systems associated with this have evolved from systems involved in surviving predation by amoebae. Some initial descriptions of the role of the *Salmonella* pathogenicity island 1 (SPI1) type III secretion system on invasion and survival in amoebae have been made. The ability of an *S.* Dublin *ΔhilA* strain (HilA is a regulator of expression of this type III system) to enter *A rhysodes* was similar to that of the wild-type (Tezcan-Merdol *et.al.*, 2004). However it could be argued that the SPI2 type III secretion system implicated in survival within phagocytic cells in mammals and birds may play a more significant role.

The main research questions this project proposes to answer are:

1. How well can *Salmonella*, *Campylobacter* and *E .coli* survive in free-living amoebae?
2. Do bacteria associated with amoebae survive better in soil, water and feeds than free-living bacteria?
3. Which bacterial virulence factors are involved in exploitation of and survival within free-living amoebae?
4. Are intra-amoebal salmonellae and campylobacters capable of infecting and colonizing a relevant animal host, and do they show any increase in infectivity?
5. How does passage through amoebae influence the virulence of *Salmonella*, *Campylobacter* and *E. coli*?
6. What role has predation by amoebae had in the evolution of bacterial systems associated with virulence?

At Liverpool we are beginning to address these questions, initially through an *in vitro* system using *A. polyphaga*. This has proved successful in allowing us to determine persistence within amoebae in a range of conditions, to visualise interactions between *Salmonella* and amoebae through live imaging and electron microscopy and to determine expression of *Salmonella* virulence genes within amoebal cells showing that the SPI2 type III secretion system is strongly expressed within amoebae.

Much further work is needed to determine the importance of amoebae as reservoirs of infection. It is true to say that *Salmonella* in particular survives reasonably well in the environment, so why should association with amoebae be necessary? However, amoebae may, particularly as encysted forms, offer a long term haven for pathogens, protected from extremes of temperature and desiccation. The ubiquitous nature of amoebae in the environment means that interactions between bacteria and amoebae are inevitable and are likely to have been a driving force in bacterial evolution. Perhaps the most practical concern is that survival within amoebae offers bacterial pathogens increased resistance to water treatment. Whilst this is unlikely to be a cause of direct transmission to humans from mains water, it has implications in agriculture particularly in more intensive production such as pigs and poultry. Low levels of disinfectant, chlorination or indeed acidification of water may be employed by these industries to control *Salmonella* and *Campylobacter* in water supplies. Survival of the pathogens within amoebae is likely to render these treatments ineffective increasing the risk of transmission to livestock.

References

- Axelsson-Olsson, D., Waldenstrom, J., Broman, T., Olsen, B. and Holmberg M. (2005). Protozoan *Acanthamoeba polyphaga* as a potential reservoir for *Campylobacter jejuni. Appl Environ Microbiol*, **Vol 71**, pp987-992
- Gaze, W. H., Burroughs,N., Gallagher, M.P. and Wellington, E.M. (2003). Interactions between *Salmonella typhimurium* and *Acanthamoeba polyphaga*, and observation of a new mode of intracellular growth within contractile vacuoles. *Microb Ecol*, **Vol 46**, pp358-369
- Snelling, W. J., Moore, J.E., McKenna, J.P., Lecky, D.M. and Dooley, J.S. (2006). Bacterial-protozoa interactions; an update on the role these phenomena play towards human illness. *Microbes Infect*, **Vol 8**, pp578-587
- Swanson, M. S., and Hammer, B.K. (2000). *Legionella pneumophila* pathogenesis: a fateful journey from amoebae to macrophages. *Annual Rev Microbiol*, **Vol 54**, pp567-613
- Tezcan-Merdol, D., Ljungstrom, M., Winiecka-Krusnell, J., Linder, E., L. Engstrand, L. and M. Rhen. (2004). Uptake and replication of *Salmonella enterica* in *Acanthamoeba rhysodes*. *Appl Environ Microbiol*, **Vol 70**, pp3706-3714

Paul Wigley
University of Liverpool, UK

corporate news

The latest news, views and microbiological developments from our corporate members

New year, new look for Lab M's Harlequin™ chromogenic media

Lab M's growing range of chromogenic culture media for the isolation and identification of a variety of important micro-organisms has a new look for 2010. Marketed under the Harlequin™ brand, the range now includes media designed to support the isolation of key organisms of significance to public health such as *Listeria monocytogenes*, *Cronobacter sakazakii*, *Escherichia coli* and *Salmonella* species. A new Harlequin™ logo and separate identity have been created, making it easier for customers to select chromogenic media specifically.

One of the most significant advances in microbiological culture in recent years, chromogenic media provide a rapid, accurate means of isolating and enumerating target micro-organisms based on the detection of specific enzyme activities.

Harlequin™ chromogenic media can help reduce the need for subculture and confirmatory biochemical tests.

further information

Visit: www.labm.com
Tel: +44 (0)161 797 5729
Email: info@labm.com

Neogen Europe introduces a rapid DNA-based test for *Campylobacter* spp.

Neogen Europe Ltd has introduced GeneQuence® Campylobacter, a new DNA hybridization test for the detection of *Campylobacter* spp. GeneQuence Campylobacter qualitatively detects *Campylobacter* spp. in whole chicken carcasses following only 48 hours of enrichment in patented Campy-Cefex culture media.

Campylobacter species are widely recognized as one of the leading identified causes of bacterial infectious disease throughout Europe. According to European Food Safety Authority figures (2005), campylobacteriosis has overtaken salmonellosis as the most reported animal infection transmitted to humans. Various surveys undertaken around the world have found the frequency of *Campylobacter* contamination of commercially available chicken to be between 50-80%.

Neogen's GeneQuence® rapid pathogen detection assays utilize DNA hybridization technology in a microwell format to maximize throughput while minimizing hands-on time. The test system's flexible format allows it to be used manually to run just a few samples, or fully automated to run up to 372 samples at a time. GeneQuence incorporates many features to ensure the quality, reliability, and speed of results—and can run multiple pathogen assays simultaneously.

further information

Visit: www.neogeneurope.com
Tel: +44 (0) 1292 525275
Email: b-reiter-millard@neogeneurope.com

Collection and transport for viruses, chlamydia, mycoplasma and ureaplasma

Included in Medical Wire's new range of preanalyticals is Σ-VCM® for the collection and transportation of key pathogens, including novel H1N1 influenza virus (swine flu). Retaining the well documented qualities of Virocult® medium, VCM® has been adapted to make it suitable not only for viruses, but also for chlamydia, mycoplasma, ureaplasmas, and certain important fastidious bacteria such as *Neisseria gonorrhoeae*. The base medium allows survival and recovery of the target organisms, while a new cocktail of antimicrobials prevents the growth of most contaminating bacteria and fungi in the specimen. Target organisms can be identified by culture or molecular techniques.

Σ-VCM® is available in a range of formats. It is supplied as a sterile device comprising a self-standing conical based vial, choice of fill volume, with or without glass beads, and choice of one or more standard and mini-tip Σ-swabs®, the open cell foam tipped swabs which allow optimum uptake and release of target microorganisms, and complete flow-through of reagents.

further information

Visit: www.mwe.co.uk
Tel: +44 (0) 1225 810361
Email: sales@mwe.co.uk

Microbank celebrates 18th Birthday

Microbank, the original long terms storage and retrieval system for bacterial and fungal cultures is celebrating its 18th Birthday, with over 500,000 units sold worldwide. Available in multiple colours, and a variety of pack sizes, Microbank remains the product of choice for many customer's valuable culture collections, including most reference sites.

Full reference data is available in the "World Wide Microbank Performance Portfolio".

Copies and product samples are available from Pro-Lab Diagnostics.

further information

Visit: www.pro-lab.com.
Tel: +44 (0) 151 353 1613
Email: uksupport@pro-lab.com.

ProtoCOL 2 Zone system speeds up quality testing of antibiotics

ProtoCOL 2 automated zone measuring system offers unique functionality for analysing antibiotic susceptibility testing plates. This can help improve quality testing and speed up the release of new antibiotics, thus reducing the spread of drug resistance.

Using the innovative ergonomically designed ProtoCOL 2 permits precise measurement of inhibition zones on antibiotic susceptibility testing plates (resolution >0.05mm from disc or well's edge), even if the zone's edge is indistinct or if the majority of the plate has been cleared by the antibiotic being tested.

ProtoCOL 2 features integrated sliding doors to eliminate ambient light effects and its unique LED lighting (patent pending) provides exceptional uniform illumination of antibiotic susceptibility testing plates. The system contains a high-resolution CCD camera that automatically generates highly defined zone images in seconds. The new impressive Zone software will assess not just two, but multiple points from the zone to rapidly calculate an average diameter, saving valuable analysis time. The software will also measure and subtract disc and well sizes, making the ProtoCOL 2 the most accurate system currently available for determining an antibiotic's efficacy.

further information

Visit: www.synbiosis.com
Tel: +44 (0)1223 727125
Email: sales@synbiosis.com

Technopath launch of EZAccu Shot™

Technopath are pleased to announce the launch of EZ-Accu Shot™ from MicroBioLogics, a quantitative lyophilized microorganism preparation designed for use in Growth Promotion Tests of microbiological culture media. The Growth Promotion Test is a mandated requirement of the United States (USP,) European (Ph. Eur.) and Japanese (JP) Pharmacopeia.

The purpose of the Growth Promotion Test is to determine the suitability of culture media. The medium is challenged with a small number of microorganisms to assure the nutritive properties. EZ Shot™ quick dissolving lyophilized microorganism pellets are formulated to provide an inoculum of less than 100 colony forming units (CFU). EZ-Accu Shot™ is manufactured with microbial strains derived from the American Type Culture Collection (ATCC®).

The EZ Shot™ kit contains five vials of lyophilized microorganisms and five vials of hydration fluid. The pellet dissolves instantly without the need for pre-incubation or serial dilutions, making it one of the most convenient products of its kind on the market. It does not require freezing and can be easily stored in refrigerated temperatures thereby eliminating the need for dry ice to transport. Following hydration, EZ Shot™ can be used for up to eight hours when refrigerated between uses.

further information

Visit: www.techno-path.com
Tel: +44 (0) 283 0833 808
Email: info@techno-path.com

Web-based temperature monitoring

Still recording temperatures manually? Now you can monitor up to a thousand pieces of laboratory equipment with Labguard, the wireless temperature monitoring system for laboratories.

eviSENSE is the new software that allows web-based monitoring, with significant savings on costly licences and allowing access to an unlimited number of individual PCs.

You can now monitor temperature, oxygen, carbon dioxide, pressure and humidity at multiple locations and multiple sites with one system. You purchase only as many probes and transmitters as you need based on the size of your organization and what you want to monitor, but you can add to the number in the future if necessary.

With the new eviSENSE software, data can be viewed in a variety of formats: tabular reports, graphs, blocks and location maps. It is also possible to create different profiles or provide individual users with different access levels as an added security benefit.

Easy to calibrate and with a full audit trail, Labguard monitors temperatures in real time and is compatible with all accreditation systems.

further information

Visit: www.dwscientific.co.uk
Tel: +44 (0)1274 595728
Email: info@dwscientific.co.uk

Sterilin

Sterilin Ltd, is a leader in premium quality laboratory plastics. The Sterilin range of single-use plastics comprises over 800 products for microbiology and life science laboratories and includes specimen containers, Petri dishes, serological pipettes, multiwell plates and centrifuge tubes.

For more than 40 years, the Sterilin brand name has been synonymous with quality in single-use plastics. This reputation has been established and maintained by the most stringent quality assurance standards in the industry. Branded products are manufactured at the BS EN ISO 9001:2008 accredited purpose-built facility in South Wales, UK, where continuous investment in the latest technology ensures every item is made to the highest quality.

As well as its own proprietary plastics, Sterilin also offers an extensive range of Iwaki cell biology products. This extends from standard flasks and dishes to gridded glass-based dishes designed to monitor individual cells over time in culture using confocal laser microscopy for live cell imaging.

Sterilin Packaging Products offers a range of bottles with an extensive choice of closure options for pharmaceutical and industrial use. Moulded and extruded products, such as medical grade tubing or bespoke silicone products, can be custom manufactured to suit individual requirements by the Sterilin Silicone devices division.

further information

Visit: www.sterilin.co.uk
Tel: +44 (0) 844 844 3737
Email: info@sterilin.co.uk

RapID Kits for Faster and Simpler microbial identifications

The Oxoid RapID range of manual microbial identification systems are easy to use and allow the accurate identification of a comprehensive range of micro-organisms in just four hours, compared to alternative methods which can require 18-72 hours.

The RapID method detects preformed bacterial enzymes and is not dependent on the growth of the organism. This feature allows the panels to be incubated aerobically without the need for oil overlays—resulting in savings in both time and resource.

Unlike alternative methods that require separate inoculation of individual wells, RapID identification panels have a simple, one-step inoculation procedure. A suspension of the colony to be identified is simply added to the inoculation tray and tipped into the test strip, inoculating each well simultaneously.

Each RapID panel has the same simple procedure, so users do not have to remember different procedures for different organism groups. The clearly visible colour reactions are easy to read, and results are interpreted using the user-friendly Windows®-based ERIC® software, with nine comprehensive databases for accurate and reliable results ranked by probability.

further information

Visit: www.oxoid.com
Tel: +44 (0) 1256 841144
Email: oxoid.info@thermofisher.com

information

Are you a corporate member of the Society? If so, this section of *Microbiologist* is for you. Here you can publish short press releases, acquisition notices, news of new staff appointments, technical developments and much more.

Each corporate member of the society may publish ***up to*** 200 words on a topic related to their field of activity in each issue of *Microbiologist*. For further information please contact Lucy Harper by email at: **lucy@sfam.org.uk**

Both corporate members and ordinary members of the Society will find a wealth of useful information and resources in this section.

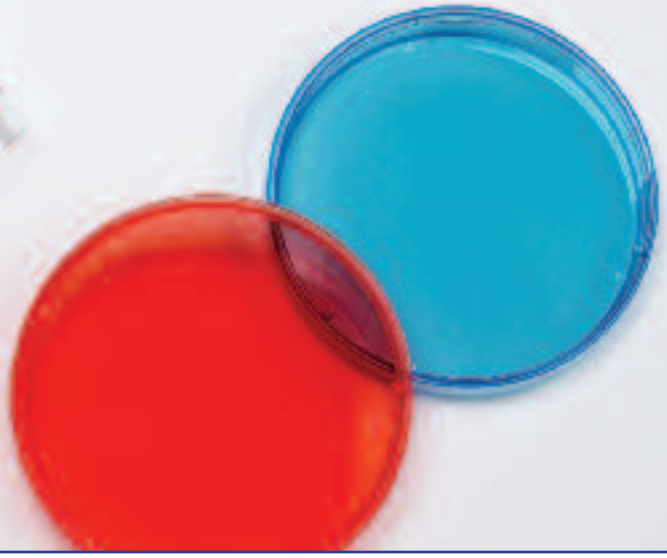
Best in class

As a pioneer of single use plastics, the Sterilin brand has been setting world standards for quality, reliability and user safety for more than 40 years.

Because our products have such a brief and important lifespan, we know they have to be perfect first time, every time. That's why we go to so much trouble to make them reliable, convenient and above all, safe .... in fact to the same quality assured standards only the Sterilin brand name guarantees.

At our ISO 9001:2008 accredited facility in South Wales, UK continuous product development and investment in injection moulding technology, clean room and microbiological testing facilities ensure that every single Sterilin branded product, from petri dishes and pipettes to containers and dishes, is made to the most exacting specifications possible.

Don't compromise with quality. To find out more about our unrivalled range of more than 800 plastic consumables for microbiology and the life sciences, please visit www.sterilin.co.uk



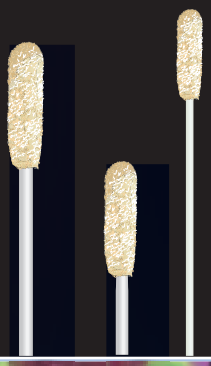
You're safe with Sterilin® ✓

Sterilin

Sterilin Limited, Angel Lane, Aberbargoed, Caerphilly CF81 9FW
Tel: +44 (0)844 844 3737 Fax: +44 (0)844 844 2373
e-mail: info@sterilin.co.uk www.sterilin.co.uk

Supporting UK manufacture

mwe
medical wire



The new generation of preanalyticals

Σ-Transwab®

Σ-Virocult®

Σ-VCM®

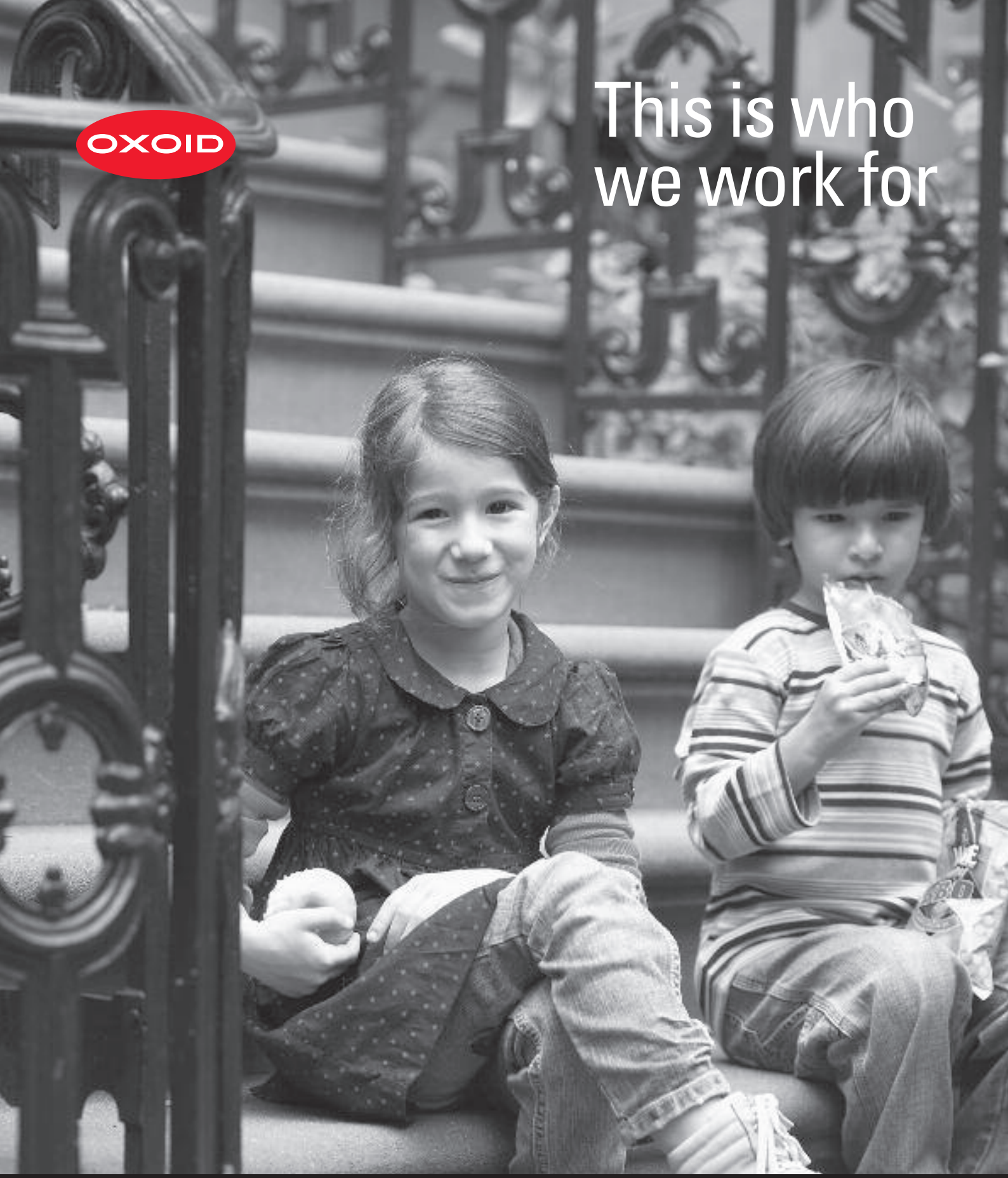
Σ-Swab®

www.mwe.co.uk

Σ-Transwab® all microorganism **Σ-VCM®** viruses, Chlamydia & Mycoplasmas **Σ-Virocult®** viruses

Multipurpose collection and transportation systems

CE 0086



RapID™ kits accurately ID micro-organisms in just 4 hours

Part of Thermo Fisher Scientific

To find out more contact:

Oxoid, Wade Road, Basingstoke,
Hants, RG24 8PW, UK

Tel: +44 (0) 1256 841144
Fax: +44 (0) 1256 329728
Email: oxoid.info@thermofisher.com
www.oxoid.com