Microbiologist

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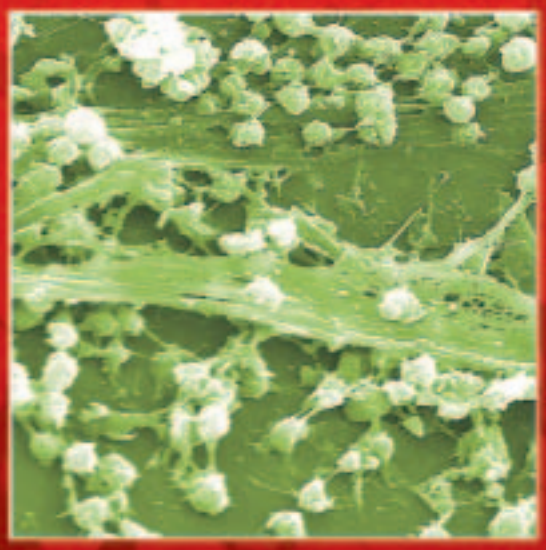

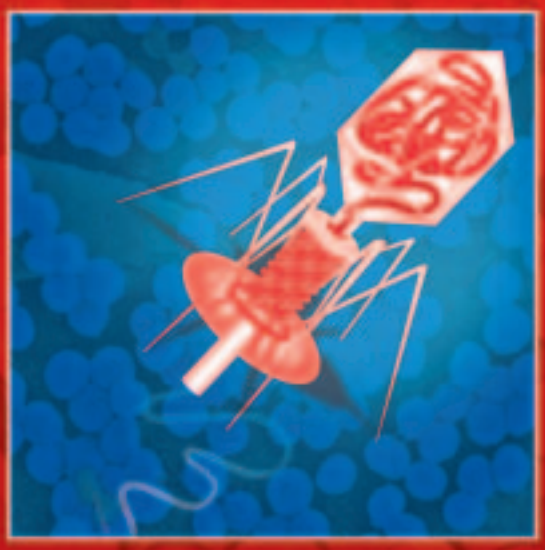

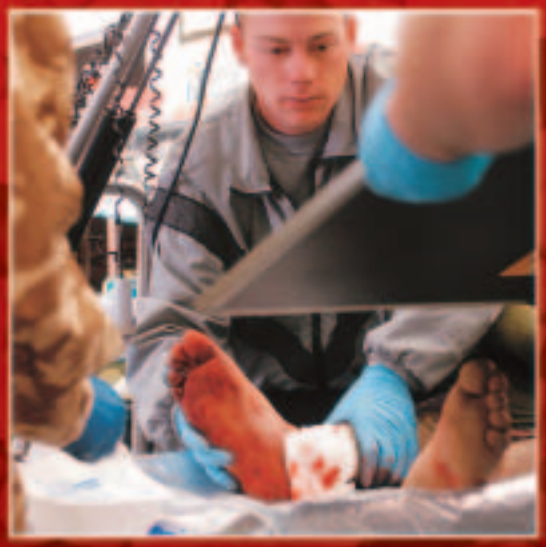

WOUND INFECTION

INSIDE

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December 2011 ■ Vol 12 No 4 ■ ISSN 1479-2699

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editorial

Lucy Harper talks about wound infection — the main theme of this issue of *Microbiologist*

When did you last fall over and cut yourself? For me it was as a child, and I'm happy to say it's been a long time since I encountered an open wound. We're all familiar with the amazing, healing ability of the human body, but if wounds become infected, the formation of biofilms can mean this process is interrupted. Some people encounter wounds as a matter of course as part of their daily routine and on page 26 we hear about the complications of treating wounds of conflict. As the authors say, this isn't new: "*The fight against the development of infection in battle wounds is not a new problem. Sumerian carvings describe the use of beer and hot water to wash wounds which were then dressed with a plaster of herbs or fruits. The ancient Egyptians used honey and sometimes animal faeces to dress wounds and Hippocrates describes the washing of wounds with wine and the drainage of pus to reduce inflammation.*"

Hmmmm… I'm not sure about its efficacy, but I like the idea of treating wounds with wine…

But on a more serious note, the mention of honey leads me nicely on to our second feature on page 30 which describes the use of honey in the treatment of biofilm infections. Again, this is not a new phenomenon: "*Honey is an ancient wound remedy that has been re-introduced into modern medical practice and a range of licensed products that contain honey are available on prescription in Australasia, Europe and North America. They range from sterile medical grade honey in tubes, honey incorporated into ointments or impregnated on to dressings.*"

In the final feature article, we look at a pet love of mine — bacteriophages and their use in the treatment of wound infections. Turn to page 33: "*Bacteriophages are ubiquitous and more abundant than any other nucleic acid-based entities. They display incredible variations in how they adhere to their target host cell, breach the outer defences, replicate, and eventually lyse their host to infect new cells. Added to this complexity are vastly different strategies for phage infections possessed by lytic versus temperate bacteriophages. However, through persistence the therapeutic use of bacteriophages is emerging.*"

In keeping with the approaching festive season, this issue is also one of celebration. We celebrate the winner of this year's Communication Award, Professor Joanna Verran on page 22 and, as with each issue of the magazine, we celebrate the work that's funded by many SfAM grants, including the Sponsored Lecture Grant and the Public Engagement Grant.

All the information you'll need about our grants is available on the new SfAM website http://www.sfam.org.uk/en/grants--awards/index.cfm and we've made it easier for you to apply for grants, by providing a facility to do this online. You can read all about the new SfAM website on page 10 and remember, the site will continue to develop and evolve with the needs of our Members, Non-Member visitors and the development of new technologies. So get in touch with your ideas.

Finally, at this time of celebration, I would like to wish all readers a fantastic festive season and a wonderful 2012.

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

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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.

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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 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.

■ **Retired 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 & Finance 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

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

R. Cavicchioli; D. Mahoney; T. Kostic; A. Loy; M. Wagner

Belgium

V. Castiaux

Canada

J. Foght; L. Y. Stein

Chile

R. T. Espeso

Denmark

M. Kuhl

Germany

M. Friedrich; G. Molinari; V. Muller; B. Tummler

India

N. Desai; D. Kaushik; R. Manickam; H. Panwar; S. Sharma

Ireland

N. Borchert; K. Burgess; M. Friel; A. Grainger; N. Kennedy; M. Lenahan; O. Lynch; M. Martins; E. M. McCabe; M. McCusker; K. McDonald; H. Meredith; D. O'Leary

Italy

F. Nazzaro

Jordan

M. Mustafa

Kenya

M. M. Malenge

Netherlands

B. Lugtenberg

Nigeria

E. O. Adeleke; T. C. Adias; R. Anyanwu; F. V. Daniels; F. Esumeh; N. N. Iwuala; A. I. Obasi; V. A. Oriaku; B. A. Osopale

Spain

A. Jofre; B. Nogales; J. Ramos; F. Rojo

Sweden

U. M. Romling

UK

M. Adnan; Y. Ahad; H. R. Ahmad; S. Akhtar; R. Akthar; S. Ali; A. Ali; F. Alkhaleefah; G. Allardyce; E. Allegra; W. Allison; A. Angel; W. Armour; J. P. Ashton; A. Ashton; N. Astbury; S. M. Athi Narayanan; N. A. Baharin Md Daud; A. Bendall; J. Betts; L. Bishop; B. Blane; S. Bradley; J. Braid; D. Butler; C. Cass; M. Charnick; G. Cole; J. Collins; A. Collins; I. Concepcion; S. Cowper; D. Dadnam; W. Davies; K. Dyson; A. Edwards; A. Fisher; M. Frost; H. Garner; D. Green; N. J. Grover; B. Hadef; M. Hallinan; A. Hammond; K. R. Hardie; R. E. Harris; J. Hawkins; K. Ho; V. Horta de Passo; Z. Hoskins; A. Hunt; R. Ingham; R. Jackson; S. A. James; K. Jenkins; A. Johnson; S. Keane; S. Khalaj; C. Loo; N. Mallon; B. Manku; J. A. Marr; M. D. M. Martin; T. McGenity; A. McIntyre; K. Metson; N. Mistry; A. Mohamudally; R. Monson; J. C. Murrell; S. Nicholson; A. O. Olaifa; I. T. Olonade; C. A. Pallister; B. Pandya; S. Park; K. Patel; H. J. Plant; J. Portman; J. Potrykus; R. Praptiwi; S. Raghvani; B. Reynolds; S. Rubinchik; P. Scanlan; V. Sewell; D. Sharp; N. Stone; B. Suarez Martinez-Falero; R. Swann; C. Swift; D. Taylor; N. Thiru; D. S. L. Thomas; F. Urbas; M. Veses Garcia; C. Waitt; K. Ward; J Wardle; J. M. Warrillier-Grant; S. Weaver

USA

B. Baros; D. Buckley; D. Cherney; R. Chmielewski; T. Crippen; M. Dworkin; R. Gunsalus; J. Handelsman; K. Harper; M. G. Klotz; R. Knight; M. N. Kramer; R. Li; A. Olson; A. Pearson; T. Poole; E. Rose; D. Schaffner; R. Schoenborn; C. Stoltenow; J. Stratton; J. Vornhagen; T. Wood; J. Zehr

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president's column

SfAM's new President, **Professor Martin Adams** talks about peer review, scientific journals and the benefits of membership

It can be irksome when someone fails to recognize your genius, as may happen when anonymous referees reject a paper you have submitted. I have known people (not myself, of course) driven almost apoplectic on such occasions, chewing (in pre-electronic submission days) the corners of the returned manuscript, cursing the referees for their myopia and wishing to see the Editor slowly rotating over a bed of red-hot coals as an interim measure while they decide on a more appropriate fate for them.

In cooler, more rational moments though, we all recognize that the current system has enormous strengths that outweigh these occasional setbacks. If you have failed to convince the referees of the novelty and worth of your submission then perhaps there is a need for redrafting to bring out the key points more clearly, or possibly a need for more supportive evidence. If, despite such efforts, your paper still does not find a home, then there is always the consolation that other great minds have gone unrecognized in their lifetime — *a flower…born to blush unseen*.

Publication supported by the system of peer review remains central to maintaining some form of quality control to smooth the path of scientific progress. Without it, huge resources would be unnecessarily wasted following up erroneous claims and trying to repeat the unrepeatable. The production of scientific journals entails an enormous amount of work on the part of many individuals and we are all heavily indebted to Editors, members of Editorial Boards and individual reviewers for this frequently unrecognized and unrewarded activity. They give of their time, usually outside their normal full-time employment, to do work that is often unheralded and that does not usually provide much grist for the career enhancement and promotion mill.

SfAM gains an enormous amount from its association with a number of excellent journals – our own *Journal of Applied Microbiology*, which goes back to 1938, and, from 1985, *Letters in Applied Microbiology*, as well as those that we publish in partnership with Wiley-Blackwell — *Environmental Microbiology*, *Microbial Biotechnology* and *Environmental Microbiology Reports*. For many microbiologists they are the first choice for publication of research papers and reviews in their respective fields, they are internationally acclaimed and play a significant role in the high international standing of the Society.

There is also a much more tangible way in which the Society and all SfAM members benefit from our association with these journals: the substantial income they generate. Without those funds the Society would be unable to subsidize our three annual meetings, our regional meetings and the whole panoply of grants to anything like the same extent. The Students into Work Grant, the New Lecturer's Research Grant and the Innovative Project awards, to name but three of the many available to members, are enormously successful in promoting and developing applied microbiology. Demand has increased dramatically in recent years but the amounts allocated are generous so there is still a good chance of success. They serve an increasingly important role as sources of funding that are simply not available from many other grant awarding bodies or are increasingly scarce in these financially straitened times.

To close my first President's column, I'd like to thank my predecessor in the Presidency, Professor Geoff Hanlon, for all his excellent work over the last three years and for leaving the Society in such thriving good health. I thoroughly enjoyed working with him and we are all extremely grateful for his work and the way he helped maintain the Society's reputation for its friendly and welcoming character. It has always been an aspiration of the Society back to its early days as the *Society of Agricultural Bacteriologists* to engender "*…an atmosphere of informality and cordiality…*" and I hope and intend to continue that admirable tradition. Finally, may I wish all our Members the very best for the coming season and a very successful and happy New Year.

Martin Adams
President of the Society

ceo's column

Philip Wheat reports on the latest developments within the Society

For many people this time of year is a time for celebration and reflection over the last 12 months. Indeed, this year the Society has reason to celebrate once again. It is pleasing to report that as I am writing this column (late September) membership numbers are at least 100 higher compared to the same time in 2010. A further reason to celebrate is that for the first time ever, applications for our two most popular grants (**President's Fund** and **Students into Work**) were oversubscribed in 2011. If you are thinking of applying for either of these grants in 2012 I would strongly recommend you apply early.

Our scientific meetings were very popular this year. In particular, the Summer Conference in Dublin was extremely successful with record numbers of attendees, including many Student Members who had successfully applied for a studentship to attend. It is also pleasing to report that a large number of abstracts were received. Next year's Summer Conference (Edinburgh, 2 to 5 July, 2012) promises to be popular, so if you are eligible to apply for a studentship please ensure you apply early or indeed if you are a Full Ordinary Member, early registration is recommended.

The Society can look forward with confidence. As I have stated many times membership offers tremendous value for money. We have introduced a further initiative which makes membership subscription even better value. Full Ordinary Members who pay a subscription fee should have recently received a renewal notice and for the first time we are offering a facility whereby if a member pays for two years' membership they will get a third year's membership free. Therefore, anybody taking up this offer will not need to renew their membership until 2015. It will also mean that in effect membership costs just over £34 per year (if paid by credit card) for those three years. If you would like further details please contact either Julie Wright (julie@sfam.org.uk) or Julie Buchanan (julieb@sfam.org.uk).

Another brand new initiative for the coming year is the introduction of a new category of membership — eStudent Member. This category of membership is designed for undergraduates who have an interest in microbiology. It is **free of charge** and applications are welcome from **anywhere in the world**. A PowerPoint slide is available (julieb@sfam.org.uk) should you wish to promote this category of membership in your own institution. Full details, including full benefits and terms and conditions can be found by visiting www.sfam.org.uk. Initial response to this initiative has been very encouraging.

Another new initiative for 2012 is that for the first time the next Spring Meeting (18 April 2012) will be a joint meeting with the Institute of Biomedical Science (IBMS). The meeting forms part of the centenary celebrations of the IBMS. In the morning the meeting will cover "*Historical Perspectives of Microbiology*" and then in the afternoon there will be concurrent sessions covering "*Sepsis and Implants*" and "*Virology — typing and its applications*". A full programme will be available online in 2012.

As I've referred you to the website a couple of times in this column, it seems appropriate to mention the new S*f*AM website which went live on 7 October 2011. I hope you have enjoyed using the new site and if you've not yet visited I would encourage you to go to www.sfam.org.uk and have a browse. And do get in touch with us at communications@sfam.org.uk with any ideas or suggestions you have for the site. To read more about the new website, see page 10.

All that remains is for me to wish you all a happy festive period and a prosperous New Year.

Philip Wheat
Chief Executive Officer

Statistical Analysis in Microbiology: StatNotes

By Richard A Armstrong and Anthony C Hilton.
Published by Wiley-Blackwell/S*f*AM, 2010

StatNotes has been designed specifically for microbiologists who are involved in experimental research and need to draw accurate conclusions from their findings. It features 28 StatNotes that together enable you to understand the basic principles of statistics, choose the correct statistical methods to analyse your experimental data, and work with a variety of commercially available statistical software packages. Written specifically for microbiologists, StatNotes enable you to choose which statistical methods should be applied to analyse and draw correct conclusions from your experimental data.

New S*f*AM website

membership matters

As you will know by now, the new S*f*AM website went live on 7 October 2011! You will have received an email reminding you of your username and password which you'll need to access the Member's area of the site. Do feel free to browse the site and let us know what you think. We hope you like the new site which has so much more to offer both Member and Non-Member visitors. In response to the Member's Questionnaire we distributed in 2009, and taking on board what Members and Non-Member stakeholders have told us via a specific questionnaire and interviews about the website, we've added new functionality to the site and put in place a new navigation system which means you'll find it easier to find what you're looking for. There are news listings, events booking and links to our social media pages on *Facebook* and *LinkedIn*. You can also keep up with the latest microbiology news on our *Twitter* feed, which appears on the home page.

Members will find a new improved 'Member's area' which includes new online grant application and a new forum for our Special Interest Groups.

But it doesn't stop there. We will continue to develop the site with the latest information and using the latest technological developments. So contact us (communications@sfam.org.uk) with your ideas and tell us what else you'd like to see on the S*f*AM website. Happy browsing!

Congratulations

S*f*AM Honorary General Secretary **Mark Fielder** has been conferred with the title Professor of Medical Microbiology by Kingston University. Congratulations to Mark on this very well deserved achievement.

S. Peter Borriello has been appointed as Chief Executive of the Veterinary Medicines Directorate. All at S*f*AM would like to congratulate him on his new appointment.

bio**Focus**

Mark Downs reports: legislation from the European Union

Like it or not, all biologists need to be aware of legislation. It creates the framework for the operation of organizations, places responsibility individually or corporately and creates our ethical framework. Regulation is something we all want less of, but can't do without. Despite all the rhetoric from successive Governments, legislation is a growth area. Everyone knows it is important and can have a dramatic impact on the way we work and run our lives, yet no single person has the capacity to follow it all and "do the day job". This is where professional bodies come in — identifying priority areas, summarizing the key issues, consulting experts and representing members' interests wherever possible. At the Society of Biology we are trying to do this for both our individual and organizational members focussing on the cross-cutting and generic issues such as science funding, education, training, skills and ethical frameworks.

Dependent upon how you define new legislation, 70 to 90% now originates from the European Union. The most common type of legislation is a Directive. These are proposals put forward by the EU's civil servants (the Commission) and then debated and amended by the Member States (the Council) and the European Parliament. The Member State negotiations take place behind closed doors and (for the UK) are led by home department officials and their colleagues from the "Embassy" to the EU (UKREP). In parallel through one or more committees, and then typically two readings in Parliament, MEPs shape their own text through public debate. At this point there are two sets of different text for the same purpose! After typically months of negotiation, publicly and privately, the end is then almost in sight, well sort of: agreement between Member States and the Parliament is often still a distant dream. To resolve disputes a bizarre process known as conciliation is invoked whereby the EU Parliament and Member States delegations (led by the rotating Presidency) argue it out privately on a time limited basis until a consensus is reached, often through the night. If there is none, the legislation fails to become a Directive. This might often be the best outcome but given all the work everyone has put in there is a danger that it will seem more attractive to have bad regulation than none at all.

All clear? Probably not! It is far from a transparent process. Once a Directive is published Member States usually have 24 months to implement ("transpose") it into their domestic law. Directives set minimum requirements seeking to harmonize law across the EU driving down costs for business and increasing common standards for EU citizens.

My experience from working within the system is that it is pretty much basic horse trading. Forget evidence based policymaking — important though it often is. This is political in every sense. There are red-lines between ministers, between departments, differing Member State views, domestic and EU lobby groups and the need to get agreement from an almost non accountable European Parliament.

OK, so I'm being harsh. But how many reading this article can name their MEP let alone comment on what stance they have taken on key political issues? Do they support good science or understand the breadth, impact and value of biology? The truth is they are largely anonymous and when that happens accountability is less obvious. The EU focus for science is often on the huge Framework research programmes. But, the wider regulation agenda must never be ignored.

Influencing the outcome of the EU decision-making process is not straightforward. But it can be done. Large, broad spectrum groups with a clear, well-argued and balanced message are difficult to ignore domestically or at an EU level. But, there needs to be recognition of the differing issues and interests across the EU, and timing of lobbying has to be right. For example, months of hard fought changes to draft text can be lost or changed in an instant by last minute lobbying of the EU equivalent of a party whip.

In my view, the overall legislative burden is not set to change, and this Government's and Parliament's appetite for consultation on both the legislative and policy agenda seem at an all-time high both for Westminster and the devolved administrations. Since the formation of the new coalition Government the Society has dealt with dozens of consultations having also considered responding to many more!

We strive to represent the views of biologists and your expert knowledge, and your opinions are vital ingredients in this. Please remember to have your say and get involved with the policy agenda through S*f*AM colleagues or directly. For weekly updates on general science policy issues subscribe to our free Science Policy Newsletter (email: policy@societyofbiology.org) and visit the website's policy pages to see some of our work.

Dr Mark Downs, PhD, FSB
Chief Executive, Society of Biology

British Science Festival

Forging networks for collaboration

The Society of Biology and Stempra held an event at the British Science Festival this year. The event was to launch a report written by Jenna Stevens-Smith of the Society of Biology (SB) which represents the culmination of over six months work in fostering collaboration between members of staff at the Member Organizations (MOs) of the SB.

The Science Communication Project involved relevant staff members of MOs, as well as external representatives from advisory bodies such as: the Department for Business Innovation and Skills (BIS), Nature, and the Science Media Centre. Each meeting focussed on a different area of science communication, and members shared ideas and best practice as well as useful hints and resources in:

- Interacting with the media.
- Communicating science policy.
- Public engagement.
- The Internet as a means of communication.

On Tuesday 13 September the great and the good in the world of Science Communication gathered in elegant surroundings of the Great Victoria Hotel in Bradford. We all heard a few introductory words from Society of Biology Chief Executive Mark Downs, followed by the SBs Media and Events Executive Jenna Stevens-Smith who described how the project formed, how it developed and her vision for the future. Next we heard from Jen Middleton who welcomed all Stempra members — especially those based in the North of England and had the nice job of asking people to eat canapés and drink wine. I'm sure a lot of successful networking went on that night — the best way of launching a report which fosters the formation of networks and collaboration.

To download the report and listen to a podcast of my interview with Jenna Stevens-Smith about the report, visit http://www.sfam.org.uk/en/news/index.cfm/sbscicommreport/ and click on the link.

Lucy Harper
Communications Manager

The following articles were the most downloaded articles from the Society for Applied Microbiology's journals between January and September 2011.

Journal of Applied Microbiology

Antimicrobial agents from plants: antibacterial activity of plant volatile oils. H. J. D. Dorman and S. G. Deans, **Vol. 88, No. 2.**

Antimicrobial activity of essential oils and other plant extracts. K. A. Hammer, C. F. Carson and T. V. Riley, **Vol. 86, No. 6.**

A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. R. J. W. Lambert, P. N. Skandamis, P. J. Coote and G.-J.E. Nychas, **Vol. 91, No. 3.**

journal**Watch**

News about the Society's journals

Letters in Applied Microbiology

Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. A. Nostro, M.P. Germanò, V. D'Angelo, A. Marino and M.A. Cannatelli, **Vol. 30, No. 5.**

Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. S.A. Burt and R.D. Reinders, **Vol. 36, No. 3.**

Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris* pathovars. S. Satish, K. A. Raveesha and G. R. Janardhana, **Vol. 28, No. 2.**

Environmental Microbiology

Referees' quotes — 2010. **Vol. 12, No. 12.**

Fresh fruit and vegetables as vehicles for the transmission of human pathogens. C. N. Berger, S. V. Sodha, R. K. Shaw, P. M. Griffin, D. Pink, P. Hand and G. Frankel, **Vol. 12, No. 9.**

Global patterns in the biogeography of bacterial taxa. D. R. Nemergut, E. K. Costello M. Hamady, C. Lozupone, L. Jiang, S. K. Schmidt, N. Fierer, A. R. Townsend, C. C. Cleveland, L. Stanish and R. Knight, **Vol. 13, No. 1.**

Environmental Microbiology Reports

Environmental reservoirs of *Vibrio cholerae* and their role in cholera. L. Vezzulli, C. Pruzzo, A. Huq and R. R. Colwell, **Vol. 2, No. 1.**

Alkane degradation under anoxic conditions by a nitrate-reducing bacterium with possible involvement of the electron acceptor in substrate activation. J. Zedelius, R. Rabus, O. Grundmann, I. Werner, D. Brodkorb, F. Schreiber, P. Ehrenreich, A. Behrends, H. Wilkes, M. Kube, R. Reinhardt and F. Widdel, **Vol. 3, No. 1.**

Powering microbes with electricity: direct electron transfer from electrodes to microbes. D. Lovley, **Vol. 3, No. 1.**

Major accolades for Microbial Biotechnology

Journal now indexed in PubMed, Scopus and Thomson Reuters-ISI, and awarded an SJR Indicator!

Microbial Biotechnology, published jointly by Wiley-Blackwell and S*f*AM, has been accepted for inclusion in widely read databases PubMed, Scopus and Thomson Reuters (ISI). It has also received a SCImago Journal Rank (SJR) Indicator of 0.234 in recognition of the excellent quality of its papers.

Microbial Biotechnology is a sister of the premier microbiology journals *Environmental Microbiology*, *Molecular Microbiology* and *Cellular Microbiology*. It publishes papers of original research reporting significant advances in any aspect of microbial applications.

"*More than 90% of microbial diversity still remains to be discovered. It is this new biodiversity that will become the treasure chest of new and improved biotechnological developments and applications in the sectors of chemicals, pharmaceuticals, energy, mining, materials, agriculture, food, and environmental protection,*" said Editor-in-Chief Professor Ken Timmis. *"This journal harnesses original research reporting advances belonging to the upper 25% in the field in any aspect of microbial applications.*"

The journal invites potential contributors to be part of the growing success of *Microbial Biotechnology*, submit their article online and take advantage of the author benefits offered. These include:

- Broad, inclusive scope representing all current and emerging topics in applied microbiology.
- Regular Special Issues specifically targeting topical themes.
- Editorial team who are leaders in the various fields of microbial biotechnology.

- Online submission and review process, leading to faster decision times.
- Early View — article by article publishing.
- Exceptional exposure to researchers and institutions worldwide.
- Indexing in Thomson Reuters (ISI), PubMed and Scopus.

Recently published Special Issues include:

Extremophiles, Lactic Acid Bacteria, Streptomyces, Marine omics, Biocatalysis, Microbiology of energy biotechnology, Life of microbes that interact with plants, and Bioremediation.

Forthcoming Special Issues will cover:

Microbial vaccines and immunomodulators, Microbial resource management, and Biodefence.

The most cited papers in the journal to date are:

Metabolic engineering to enhance bacterial hydrogen production. T. Maeda, V. Sanchez-Torres and T. K. Wood, **Vol. 1, No. 1.**

Identification of furfural as a key toxin in lignocellulosic hydrolysates and evolution of a tolerant yeast strain. D. Heer and U. Sauer, **Vol. 1, No. 6.**

T-DNA insertion, plasmid rescue and integration analysis in the model mycorrhizal fungus *Laccaria bicolour*. M. Kemppainen, S. Duplessis and A. G. Pardo, **Vol. 1, No. 3.**

The most read papers in 2010 were:

Bioactive compounds from marine bacteria and fungi. A. Debbab, A. H. Aly, W.H. Lin and P. Proksch, **Vol. 3, No. 5.**

Prokaryotic whole-transcriptome analysis: deep sequencing and tiling arrays. R. J. Siezen, G. Wilson and T. Todt, **Vol. 3, No. 2.**

Microbial fuel cells. L. P. Wackett, **Vol. 3, No. 2.**

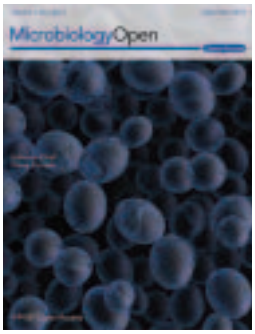
Microbial Biotechnology is available to institutions and individuals via a paid subscription fee. To recommend the journal to your librarian today, please visit http://bit.ly/recommendmbt and complete the short online form.

For further information on the journal, please visit www.microbialbiotech.com.

Felicity Howlett
Wiley-Blackwell

MicrobiologyOpen: a special Society for Applied Microbiology Member offer

"I am pleased and honoured to introduce MicrobiologyOpen, a new journal from Wiley-Blackwell." *MicrobiologyOpen* is a peer-reviewed journal delivering rapid decisions and fast publication of microbial science, a field which is undergoing a profound and exciting evolution in this post-genomic era. The journal's Editor-in-Chief, Pierre Cornelis, explains the concept:



"Why then a new journal? The term 'open' has two meanings: first, MicrobiologyOpen is an open access journal, but, more importantly, it also means that it is open to all aspects of microbiology."

The journal gives priority to reports of quality research, pure or applied, that further our understanding of microbial interactions and microbial processes.

"MicrobiologyOpen launches specifically to serve the broad microbiology community…[it] is a response to the growth of research being undertaken and the data now available. It is clear that the community will benefit from the increased flexibility provided by the online only, open access format."

All articles published by *MicrobiologyOpen* are fully open access: immediately freely available to read, download and share. To cover the cost of publishing *MicrobiologyOpen* charges a publication fee. **SfAM Members** benefit from a 10% discount on the article publication charge for *MicrobiologyOpen*, when they submit a paper directly to the journal. The usual price for publishing in the new open access journal is $2,175 / £1,400 / €1,650. As a SfAM member, you pay just $1,957.50 / £1,260 / €1,485. To take advantage of this special offer, simply enter the Society Member Account Code, which can be obtained from the Society office by contacting either **julie@sfam.org.uk** or **julieb@sfam.org.uk**, when presented with payment options when submitting to the journal www.microbiologyopen.com.

Ellie Key
Wiley-Blackwell

SfAM events in 2012 — save the dates!

Wednesday 11 January 2012

Winter Meeting

- **Microbiological safety of imported food**
- **Microorganisms and climate change**

■ Including the Denver Russell Memorial Lecture

The Royal Society, London, UK

Wednesday 18 April 2012

Spring Meeting

6th broadening microbiology horizons in biomedical science meeting

■ Including the Procter and Gamble Applied Healthcare Microbiology Award Lecture

The Stratford Q Hotel, Stratford-upon-Avon, UK

2 - 5 July 2012

Summer Conference

● Microbial resistance to antibiotics and biocides ● Natural and experimental adaptation in bacteria ● Bioremediation

■ Including the Lewis B Perry Memorial Lecture: Globalization of antimicrobial resistance. *Didier Pittet, University Hospital in Geneva*

The George Hotel, Edinburgh, UK

For further information on these events please visit sfam.org.uk or contact Sally Cryer
■ Email: sally@sfam.org.uk ■ Telephone +44 (0)1933 382191

Summer Conference 2011 report
Food Microbiology

Monday 4 - Thursday 7 July 2011 Clontarf Castle, Dublin, Ireland

Message from the Honorary Meetings Secretary

Firstly, I would like to thank all of you who attended this year's Summer Conference at the Clontarf Castle Hotel, Dublin and I hope you enjoyed it as much as I did. This Summer Conference was one of the most successful we have organized in many years. Although there is a comprehensive report describing the meeting in this issue of *Microbiologist* I did want to make a few more general comments.

This summer we welcomed over 230 delegates in total representing 33 countries spanning all five continents which reflects how "international" the Society has become. We also had to close our registration to delegates several weeks before the meeting due to us reaching the capacity of the lecture rooms. This is an action I can't recall ever happening at a *Sf*AM Summer Conference before. We also awarded 29 studentships to enable our Student Members to attend and present their research work and we would encourage students to apply for studentships again in the future. The number of abstracts submitted for poster presentation was well over 100 plus; four student oral presentations were selected to compete for the "Best Student Oral Presentation".

A lot of work goes into organizing the Summer Conference and I would like to thank all of the *Sf*AM staff and Committee for all of the hard work they put in to make it such a success.

In summer 2012 the Summer Conference moves to The George Hotel in the centre of the beautiful city of Edinburgh where we will have another excellent program addressing Microbial resistance, Natural and experimental adaptation in bacteria and Bioremediation.

We hope to see you all there and remember to book early to avoid disappointment!

Andrew Sails
*Sf*AM Honorary Meetings Secretary

Clontarf Castle Hotel in Dublin provided a very grand setting for four fascinating days of lectures on 'Food microbiology'. On the Monday a number of delegates attended the 'risk assessment workshop' with Deon Mahoney, FAO, Bangladesh and John Bassett, Unilever, UK. This gave delegates an insight into risk assessment in the food industry and the level of detail required. After lunch a teamwork exercise got everyone thinking about the detail and appropriate information needed in a risk assessment. What seemed like a 'good' risk assessment to begin with, by the end of the afternoon, and after much critical discussion, turned out to be in fact a really 'bad' risk assessment. The key points from this exercise were that risk assessments in the food industry are complicated and have to be conducted

throughout the food chain (from farm to table).

In the evening all delegates had the chance to tour the Guinness Storehouse and see what makes the perfect pint of Guinness before the Lewis B Perry Memorial Lecture. Alan Reilly (Food Safety Authority of Ireland) gave a fascinating talk about 'food safety in a global environment' and how communication is key. He also praised social media, such as Twitter, emphasizing how it influences the communication between scientists, journalists and the general public.

After the lecture a Guinness style buffet was prepared for delegates, along with a complimentary pint of Guinness.

Samantha Price PECS Events Officer

Chris Low from the Scottish Agricultural College started the session on 'Pathogen updates' with an informative update on verotoxigenic *E. coli*. Verocytotoxigenic *E. coli* (VTEC) were first recognized in 1977 becoming recognized as significant pathogens during the 1980s. Most often associated with serotype O157:H7 (although not exclusively), this organism was elevated up the political agenda following several significant outbreaks in Scotland and England. Understanding the pathogenesis of these strains is pivotal to successful implementation of control measures. Infection is usually by direct or indirect contact with ruminants, though waterborne and foodborne sources are also well documented. Despite this, most infection is sporadic. In cattle infection is without clinical consequence, with the organism typically colonizing the distal rectum being shed for up to three weeks. The H antigen (H7) is important for attachment and some success has been seen in reducing colonization among orally dosed cattle, but is not licensed for UK use. The toxin is encoded by bacteriophage with VT2 being correlated with greatest disease severity. Among cattle it is believed that carriage of the bacteriophage endows a selective advantage, but in man this correlates with pathogenic potential. More recently another VT positive novel strain has emerged in Germany associated with 810 cases and 27 deaths. This is a different serogroup, 104, and shows greatest homology with an enteroaggregative *E. coli* isolated from Africa. Bean sprouts have been incriminated as the likely source of infection in Germany. The timing of this outbreak with this presentation ensured that this talk was truly a "hot topic" without a spare seat left in the lecture theatre.

This was followed by Simon Park, University of Surrey, talking on *Campylobacter*. Being the leading cause of foodborne infection their reduction is of paramount importance. Despite their ability to result in such an enormity of human infection, the organism remains restively "fragile" with little in the way of resistance, or ability to survive extreme environmental stresses. Of particular interest are their strict demands for a microaerophilic atmosphere. This could offer a means of controlling *Campylobacter*. Similarly, 5% ethanol is inhibitory for *Campylobacter*. Potentially this could be combined with oxygenation as a means of reducing *Campylobacter*. What the clinical consequences are of using an oxygenated diet remains to be explored?

Marion Koopmans from RIVM, in the Netherlands went on to describe many foodborne viruses, including the headline stealing, norovirus, followed by hepatitis A and hepatitis E viruses. Despite the highest levels of infection, norovirus has the least clinical impact, with much higher mortality being associated with the hepatitis A virus. In order to recognize outbreaks of infection and gain insights into their epidemiology, a global network, "noronet" has been established. Through such a resource, levels of foodborne versus person-to-person infection can be monitored together with new or emerging genotypes.

When looking at levels of virus in food-handling establishments, it was no surprise that during an outbreak of infection; the toilet was highly contaminated, with 61% of samples being positive. This dropped to 3.1% in the absence of outbreaks, with the kitchen only yielding 1.6% of positive samples.

Correlations have been made between genotypes and virulence, with new waves of infection emerging through

antigenic variants every couple of years. Epidemiological analysis can be hampered by the ability of these viruses to remain infective after frozen storage of foods.

Hepatitis A virus, in contrast to norovirus above, shows significantly more geographical and food-related clustering. Current oyster die-offs occurring near France are being associated with an influx of oysters from other producers such as China, Japan and Korea. Whether this will result in changed patterns of infection is under scrutiny.

Taking us back to bacterial pathogens, John Threlfall went on to describe how *Salmonella* could be considered as multi-flexible pathogens. We were then enlightened by the diversity and often unpredictable changes being witnessed in *Salmonella* spp. causing human infections. Not only have we seen the acquisition of increasing levels of chromosomal encoded resistance, typically associated with chromosomal islands, but also plasmid encoded resistance traits. Outbreaks of infection have occurred with different phage types associated with diverse food vehicles ranging from pork sausages, eggs (poultry and duck), bean sprouts, to reptile food such as "pinkies" (suckling mice mass-produced for reptile consumption). Furthermore, an upsurge in infections with monophasic variants of *Salm. enterica* with chromosomally-encoded resistance to ampicillin, streptomycin, sulphonamides and tetracyclines (ASSuT) have been seen in Europe.

Furthermore, changing patterns of food-production might give rise to changing opportunities for infection, such as organic versus conventional farming. Reduction of *Salmonella* requires a multifactorial approach including prudent use of antimicrobials; stringent biosecurity; and good farm hygiene levels.

Initially known as "sausage poisoning", Mike Peck went on to describe the history of the neuroparalytic intoxication known as botulism from foodborne; infant botulism; to wound botulism. Although production of potent botulinum toxin unifies *Clostridium botulinum* species, the microorganisms producing this toxin are microbiologically heterogeneous belonging to four distinct groups. Of these, *Cl. botulinum* Group I producing a proteolytic toxin and the non-proteolytic *Cl. botulinum* toxin Group II toxins are responsible for most episodes of botulism. More recently, other species names have been attributed to botulinum toxin producing strains.

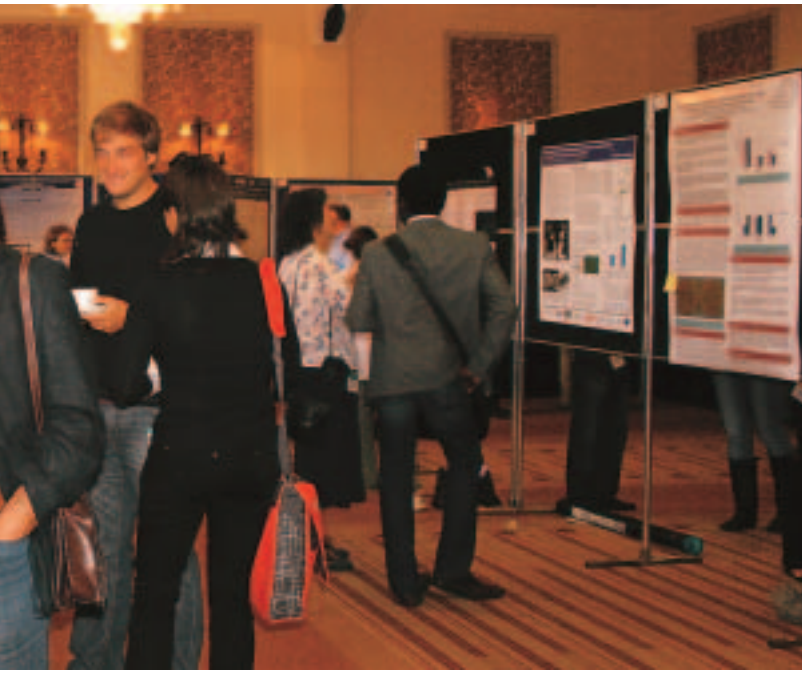
The toxin itself is astoundingly toxic with just 3g being enough to kill all humans in the UK, and 400g enough to kill mankind worldwide! Despite this, therapeutic applications of the toxin are now becoming commonplace. Though clinical cases of botulism are rarely encountered, the implications both for the individual and the economy are huge. Survivors of infection may require years to regain mobility. For food-producers, recovery is often impossible, as seen with the demise of hazelnut yoghurt producers in the UK. The "botulinum cook" of 121°C for 3 minutes or for minimally heated chilled foods, around 90°C for 10 minutes are essential to prevent this notorious toxaemia.

Sally Cutler SfAM Main Committee

The afternoon's session on 'Pathogen updates' commenced with Seamus Fanning from University College Dublin giving an overview of *Cronobacter* spp. Ireland produces 15% of the global supply of infant nutritionals including powdered

formula and due to the nature of *Cronobacter* spp. being able to withstand dry environments this organism is a health concern within these products, with 42% of cases of meningitis caused by *Cronobacter* spp. being fatal. The FDA method for identification was updated in 2009 with the original protocol being linked to PCR for more accurate identification. The control of *Cronobacter* spp. in the manufacturing environment is vital and thus quality control processes have been put in place including PFGE, to identify persistent strains and mapping transmission to define the ecology of the organism in the production environment and thus be able to produce an evaluated risk model. The second talk was given by Niall Logan from Glasgow Caledonian on *Bacillus* spp. and its relatives. Niall began by giving us the history of an arctic venture in the 19th century which led to *Bacillus subtilis* being isolated in the arctic in 1936. *Bacillus* spp. cause opportunistic infections in wounds, abscesses, the ocular system to name a few; however it is also prominent in food, with *B. cereus*, *B. licheniformis*, *B. subtilis* and *B. pumilus* often being isolated. *Bacillus* spp. can cause disease in two forms; diarrhoeal where the vegetative organism sporulates and produce enterotoxins and emetic illness where symptoms are induced by heat stable toxins. Food related outbreaks are often associated with milk, ice cream, sandwiches and reheated rice from Chinese restaurants. The biggest worry though is that *Bacillus* spp. are often used in probiotics!

The 'Epidemiology of foodborne disease' session was kicked off by Hilde Kruse from the WHO, Italy, discussing the current challenges to microbial food safety, highlighting the increasing cases of emerging diseases in the last 60 to 70 years with 335 new infectious diseases being identified and 95 of these being associated with food, this is thought to be due to changes in agriculture and food processes and also climate change. To combat this, a new food safety initiative was put in place in 2007 called the Foodborne Disease Burden Epidemiology Reference Group (FERG). To date some of the achievements of this task force include, assessing the true



estimate of mortality of children in Africa and South East Asia from foodborne diseases and determining the actual number of the population that have peanut allergies (1 to 2%).

Katie Laird *Sf*AM Main Committee

Following a tea break and an opportunity to visit the trade show, Marion Wooldridge from the AHVLA talked about 'Climate change and the challenge of new pathogens'. Marion gave evidence of how climate change has impacted vectors resulting in changes in pathogen prevalence, incursion and persistence. Marion also highlighted that such pathogens are emerging in new areas as a result of changes in host ranges and susceptibility as well as through mutations. One example of the impact of climate change on vectorborne viruses is the mutation of a vial envelope in *Aedes aegypti* (yellow fever mosquito) to the *Aedes albopictus* responsible for the CHIKV outbreak.

This talk was followed by Alec Kyriakydes, the Head of Quality, Safety & Supplier Performance at Sainsbury's who spoke about the 'Retailer's perspective on food safety'. In his talk he discussed the changing trends in food choices and factors affecting shift in infection and disease, this included agricultural and industrial factors as well as the impact of sourcing and supply. A decline in research base, funding and food scientists, coupled with the emergence of new hazards were discussed as was the importance and influence of social media in food microbiology.

Last thing on Tuesday was the student session, a popular and key highlight for Student Members at the S*f*AM Summer Conference. The focus of these sessions was career development for PhD students and early career scientists. This year's theme was titled 'Enhance your employability'. During this session S*f*AM Communications Officer, Clare Doggett, talked about interview skills and transferable skills which will increase employment potential after which S*f*AM Communications Manager and Editor of *Microbiologist*, Lucy Harper, talked about communication skills and the importance of communicating clearly and correctly as "poor communication causes problems". This talk was followed by Professor Mark Fielder who, as always, provided an entertaining but informative talk on presentation skills. Finally, Sorcha Mulcahy, Career Development Adviser at University College Dublin, spoke about getting the most from your CV.

The student session served as a reminder to the participating PECS members on important but occasionally neglected aspects of 'student development' however, as expected it was a light-hearted affair with great feedback.

Emmanuel Adukwu PECS Events Officer

The first talk on Wednesday morning concluded Session 2. Hilde Kruse, WHO, Italy, spoke about 'The threat of antibiotic resistance in the food chain for human health'. Antibiotic resistance doesn't respect phylogenetic, geographical or ecological borders and any use of them can select for resistance. Two types of hazards are recognized: direct hazards from ingestion or handling of food containing antibiotic resistant bacteria and indirect hazards associated with transfer of antibiotic resistance genes.

After giving examples of specific organisms with well-documented resistance to antibiotics, Hilde described the WHO European strategy for tackling antibiotic resistance from a food safety perspective. She cited seven action areas and seven key messages for countries. A copy of a WHO publication on this topic was included in the delegate packs. It was stressed that an intersectoral, multi-faceted response is required at the national and international level.

Microbiological risk assessment was the theme for Session 3 which was chaired by Louise Fielding. The session was opened by Sarah Cahill, Food and Agriculture Organization of the United Nations, Italy, discussing 'Recent global risk assessments and impact on Codex standard setting'. She began by providing some background information about bodies such as Codex Alimentarius, the World Trade Organization and the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA).

The use of risk assessments and scientific advice in the setting and adoption of standards has improved over the last five years. Sarah used the example of *Cronobacter* spp. in powdered infant formula to show the improvements from the time of alerts in 2003 through to adoption of microbiological criteria for follow-up formula by Codex in 2009.

A website www.mramodels.org which allows real-time modelling has been successful in making people less afraid of risk assessments. Sarah described a risk management tool which has been developed for control of *Campylobacter* and *Salmonella* spp. in chicken meat. It is undergoing pilot testing currently and should be available by the end of the year as a support tool for risk managers. This new approach is less prescriptive and allows more flexibility than previous approaches and so encourages risk assessment at the national level.

The final presentation of the morning was given by Maarten Nauta, DTU, Denmark, on 'Recent developments in *Campylobacter* risk assessment'. Such risk assessments provide estimates of absolute risk, relative risk and added value and are needed for risk-based food safety target setting. MedVetNet Workpackage 24, which entailed a comparison of

Campylobacter quantitative microbiological risk assessments (QMRAs) from four EU countries, was summarized. Despite many differences between the models used, similar conclusions were drawn. For example, high concentrations of bacteria pose the largest risk and logistic slaughter has little effect.

After commenting on the recently issued EFSA Scientific Opinion on *Campylobacter* control, Maarten devoted the rest of his talk to the use of risk assessment in defining microbiological criteria as an instrument to control food safety. He remarked on the difficulty of modelling the consumer phase and presented the results of a comparison of eight such models for *Campylobacter*. Finally, further simplification of QMRA modelling was described as an ongoing challenge.

Louise Hill-King Grants Editor

The afternoon session was opened by Pirkko Tuominen (Food Safety Authority, Finland) who discussed '*Salmonella* Risk Assessment in Finland'. Pirkko spoke about the history of *Salmonella* control in Finland and how the risk has been assessed for over 10 years. She explained how the national *Salmonella* control programme (SCP) is in place to ensure that *Salmonella* prevalence shall not exceed 1% at any stage of beef, pork, broiler, turkey or egg production. If, during sampling, *Salmonella* is detected within the SCP, follow-up action must be taken. Pirkko also explained how production chain quantitative microbiological risk assessments (QMRAs) have also been exploited e.g. cost-benefit analysis, sampling procedure comparison and planning of food safety management metrics. She explained how the QMRAs had been converted from independent model chains into one combined Bayesian network. The aim of the Bayesian approach is to allow risk managers to make revisions on an annual basis, follow the *Salmonella* situation, respond quickly to changes and guide food operators and inspectors efficiently. Pirkko concluded that the SCP had been estimated cost effective and although *Salmonella* cases in Finland had remained the same, human cases acquired from Finland as well as from foods under SCP had decreased over the last 10 years.

Phil Voysey (Campden BRI, UK) continued on the topic of microbial risk assessment by talking about '*Listeria* risk in butter'. Phil described how butter is not normally regarded as hazardous; however there have been a number of issues relating to listeriosis which has promoted their research into why *L. monocytogenes* should pose a risk in butter. Initially, the work concentrated on the requirements for growth of *L. monocytogenes*, the parameters of butter and butter-containing spreads (e.g. salt levels, temperature, water droplet size, pH) and how these conditions allowed the growth of *L. monocytogenes*. The second phase of this project focused on the characteristics of the butters and strains of *L. monocytogenes* that were associated with listeriosis outbreaks and the methods of manufacture, formulation and handling of butters. Phil went on to tell us the final phase of the practical work which involved testing of hypotheses gathered from the first two phases of the project. Once the methods of inoculating butter with *L. monocytogenes* had been investigated the effect of water droplet size, salt concentration and tempering and temperature on the behaviour of *L. monocytogenes* was assessed. It was concluded that the

crucial factors were salt levels and water droplet size. Phil highlighted that these factors should be taken into consideration by butter manufacturers and how good manufacturing technique will prevent problems from the growth of *L. monocytogenes*.

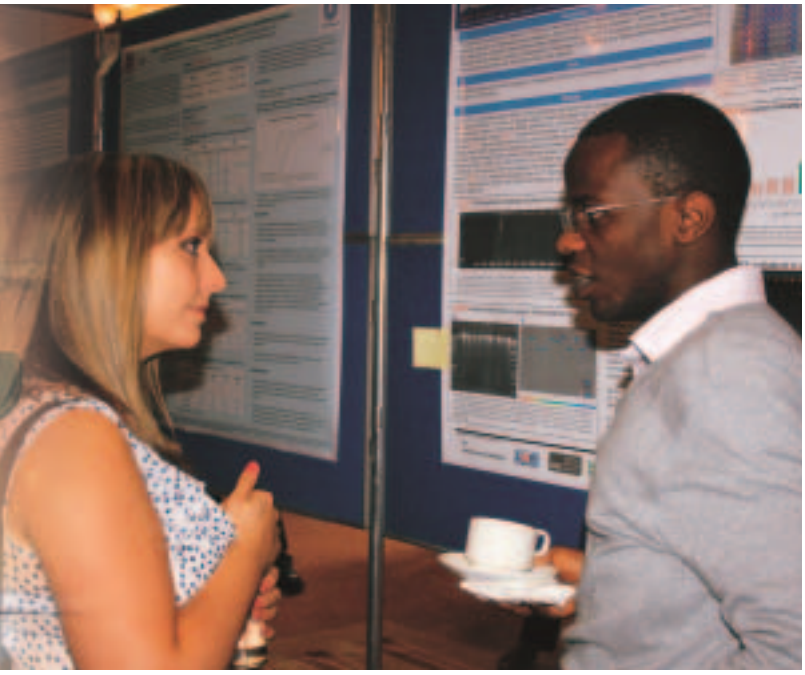
Samantha Law *Sf*AM Main Committee

After a tea and coffee break we all settled back to watch the student presentations.

First up was Phillip Humphryes. Phillip gave an introduction to the world of *Leptospira* vaccine proteomes, with a thorough discussion of the infection process and sparing none of the gruesome details of vaccine testing on hamsters. Phillip expressed his desire to find an alternative to hamster testing, which was shared by the audience. Next he explained the various investigations he had undertaken in analysing a variety of currently available vaccines. These included assays of the components, comparing the protein content and composition and separating various fractions to better understand commercial products.

Following on from Phillip, Samantha Price gave a presentation focused on an investigation into the mycobactericidal and bactericidal properties of a novel catalyst, which enhances the effects of hydrogen peroxide. She discussed the uses and mechanisms of hydrogen peroxide and the properties of the catalyst. There were a plethora of graphs, which showed the catalyst's activity against aerobic *Bacillus* spp., *Mycobacterium* spp. and *Staphylococcus aureus*. Interestingly, Samantha has found the leachate from the catalyst also has a residual effect on the organisms exposed to it. Further work will include re-testing in the presence of soiling. Samantha concludes that this catalyst could certainly be useful in industries such as healthcare, where infection control is a constant battle.

Next Patience C. Obinna-Echem presented work which investigated the bacterial and yeast communities which may be responsible for fermentation of starchy-meal and which can



make it unsafe. There was a thorough background discussing the importance of the fermented starchy-meal which is a staple food in Africa. Current production does not involve a starter culture and so the fermentation process is spontaneous and relatively inefficient. The main part of this presentation focused on culture techniques and identification of species which may be responsible for fermentation and spoilage. A number of lactic acid bacteria were commonly found.

Finally Steffi Bough gave a presentation on her work investigating the association between multi-drug efflux pumps and *Salmonella* biofilms. She gave a comprehensive background of the various themes of the work including *Salmonella* biofilm formation, the impacts of biofilms and efflux pump physiology. There were stunning visuals in the form of scanning electron microscope pictures of biofilms. She went on to discuss the contribution of different components of the pumps to biofilm production. Finally the talk covered the relationships between expression of regulators for biofilm production and the structure and longevity of biofilms.

Next we had two lectures from recipients of S*f*AM awards and grants.

Firstly Clare Taylor of Edinburgh Napier University, UK, a recipient of the S*f*AM New Lecturer Research Grant gave a presentation on 'Microbes and metal: Metal transport during bacterial infection'. Clare discussed her progress investigating the role of different gene regions thought to be involved in the transport of cobalt, iron and other metals. Her work has shown some interesting results, highlighting potential targets for novel anti-virulence therapies.

Finally the W H Pierce Prize Lecture was given this year by Brian Jones, University of Brighton, and was on exploring the human gut mobile metagenome.

Brian's talk focused on investigations into the mobile genetic elements (MGE) and bacteriophages found within the gut microbiota. This has been called the mobile metagenome and can be likened to a virtual organ. The exciting possibilities for scientific and pharmaceutical value are as yet unexplored.

The Conference continued well on into the evening with the AGM followed by the Summer Conference dinner, which was held at Jameson's Distillery. Delegates got a tour of the old distillery followed by a delicious dinner and some Irish entertainment including Irish dancing and a live band.

Joanna Tarrant PECS Events Officer

The Thursday morning session, 'Novel technologies to control safety and stability', began with an overview of Novel technologies from Bala Balasubramaniam (Ohio State University, USA). Bala began by outlining how alternative food preservation techniques, that can preserve heat sensitive functional components of food whilst still extending shelf life and being microbiologically safe, are becoming more important as consumer interest in healthy eating increases. He then went on to detail how these novel techniques could be grouped into two categories, advanced thermal and non-thermal food processing. Advanced thermal techniques mentioned included ohmic, microwave, and radio frequency heating which have the benefit of being very fast but the disadvantage that slow cooling of the food can affect its quality. Non-thermal approaches discussed included high pressure, pulsed electric fields, irradiation and ultrasound, in general these approaches preserved the quality of the food but were not without their own disadvantages. He concluded by discussing how the increasing resistance of bacteria would play a key role in the future of this field.

Stefan Toepfl (DIL German Institute of Food Technologies, Germany) continued the session by speaking on 'Pulsed Electric Fields'. Stefan began by giving a brief overview of the technique and its history, detailing how its application in the food industry has been investigated. He continued by describing ongoing efforts to scale this technique up to be used in an industrial setting and explained how this could be optimized.

Following this, Frank Devlieghere (Ghent University, Belgium) discussed 'Use of packaging for food preservation'. To start he examined how the current trend in food manufacture is to reduce the number of preservatives added to food whilst retaining its shelf life and microbial safety. He then went on to discuss how maintaining certain gaseous atmospheres within food packaging could be used to reduce bacterial numbers and prevent spoilage. Frank concluded by mentioning how the optimal compromise between preserving the quality of food whilst maintaining shelf life would be to use a combination of mild inactivation of the bacteria alongside packaging in a modified atmosphere.

The last presentation of the session, and the conference was given by Alejandro M. Amezquit (Unilever, UK) on 'Pressure assisted thermal sterilization'. Alejandro described how the use of high pressure could raise a temperature of 80 to 90^{o}C to levels high enough to initiate sterilization, in a manner similar to classic thermal processes, with the advantage that once the pressure is removed the temperature of the food returns to normal levels. Alejandro went on to discuss his efforts in validating this method using *Clostridium botulinum* before concluding that further work was required.

Phillip Humphryes PECS

Science Communication Award winner announced

about this award

The SfAM **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 SfAM. The award will be for £1000 and nominations must be from Full Ordinary or Student Members with a deadline in April each year.

More information about this award can be found on the SfAM website at: http://www.sfam.org.uk/en/grants--awards/sfam-communications-award.cfm together with an application form in Adobe PDF format.

Professor Joanna Verran (right) with SfAM Communications Manager, Lucy Harper

Professor Joanna Verran, was this year's winner of the Society for Applied Microbiology (SfAM) Communications Award in recognition of her vital work conveying the principles of applied microbiology to the general public. She has used a range of different activities, in particular reading and discussing works of popular fiction to make the subject easily understood by non-scientists.

She has been innovative in her approach to microbiology education and public engagement and is the founder of the "Bad Bugs Book Club" the first of which was held during the SfAM Summer Conference in Manchester, 2009. Her down-to-earth attitude and friendly disposition make her an engaging speaker. Jo's warmth and accessibility combine with a sharp wit to impress and communicate sometimes complex information effectively to a lay audience. This was evident at the Summer Conference dinner, in Dublin, where she was presented her award. She gave us a rundown of three experiences of science communication, all delivered with skill, fun and a great deal of humour.

When asked about her achievement, Professor Verran said: "*I am absolutely thrilled to have received this award. I love microbiology, and have always tried to encourage similar passion and interest amongst lots of different audiences, so it is great to have received this recognition for my work. In my opinion, applied microbiology is a subject of importance and fascination to everyone, and* SfAM *does a great job keeping it in the public eye (or ear?!) as 'the voice of applied microbiology'.*"

Jo is a regular contributor to TV news and features programs including BBC's *The One Show*, and she has been quoted in many newspaper articles. She has worked with SfAM and other organizations on countless public engagement events at science festivals across the UK, including SfAM's own "*Outbreak: engaging the public in infectious disease*" for the British Science Festival. She's also worked at Manchester Science Festival, Cheltenham Science Festival, and most recently she has talked about her public engagement work at the Federation of European Microbiology Societies Congress in Geneva, Switzerland.

According to our President, Professor Martin Adams: "*Jo's wide knowledge and infectious enthusiasm have made her an outstanding public communicator and ambassador for applied microbiology. This award is well deserved recognition of her innovative work in this area. Long may it continue.*"

Lucy Harper
Communications Manager

Environmental Microbiology Lecture 2011

Professor Willem de Vos (centre) receiving a commemorative plaque from SfAM President Martin Adams (left) and Chief Editor of Environmental Microbiology Ken Timmis (right)

This year's *Environmental Microbiology* Lecture once again took place at the Royal Society of Medicine in London a fitting venue for this year's talk, 'Microbes inside'.

The evening started with tea and coffee and a chance to catch up with members of the Society and supporters of S*f*AM before the talk began. Professor Willem de Vos, Professor at Helsinki and Wageningen Universities was this year's very worthy speaker on the topic of the intestinal microbiome.

Willem began by giving us a potted history of the microbiology of the intestines starting with Leeuwenhoek's animalcules (little animals), some of the earliest references to microbes. Leeuwenhoek discovered animalcules after becoming ill to a diarrhoeal disease. Willem continued by giving us all a little perspective explaining that not only did intestinal microbes outnumber our own cells by one or more orders of magnitude, but that one microbial metagenome (the genomic material present in one host's microbiome) is 150 times larger than the human genome! It was enough to make us feel very insignificant.

Willem then moved on to discuss the different approaches that are being used to understand the intestinal microbiota. Willem summarized some of the most recent papers covering many aspects of the intestinal microbiota including comparisons of the pili of lactobacilli (the bacteria commonly used in probiotics) and the pathogenic vancomycin-resistant *Enterococcus faecium*. The pili in these bacteria are very similar and allow both probiotic and pathogenic strains to bind to intestinal mucus. Recent research found that the *Lactobacillus* strain can outcompete the pathogenic bacteria as an effective infection control method.

Willem went on to discuss the complexity of the gut microbiota. The majority of microbes within the intestines have not been cultured and the majority of studies are now based on an "omics" approach. Willem explained that whilst there are common networks of microbes, everyone's microbiota is individual. Many influences alter or determine the microbes in our gut, including blood group and conditions such as irritable bowel syndrome (IBS). Willem highlighted one particular study which demonstrated that people with IBS have similar microbiota which is distinctly different to that of healthy individuals.

Willem then focussed on one particular organism *Akkermansia*, which belongs to the verrucomicrobia phylum, the only intestinal bacteria to do so. This small organism degrades the intestinal mucus and is present in most people's intestinal microbiome. Interestingly, Willem pointed out, *Akkermansia* is abundant in individuals with a healthy appendix, but is only present at very low levels in individuals with appendicitis. This trend is also true of healthy individuals compared with those who have irritable bowel disease (IBD). Willem raised the possibility that *Akkermansia* could, therefore, act as a biomarker for a healthy intestine.

Willem went on to discuss health implications of the intestinal microbiota including the effect of extremely low calorie diets on the composition of the microbiota and the fascinating results of studies on faecal transplantation in *Clostridium difficile* infection and people with insulin resistance. Willem ended his talk by reminding us "*we feed our microbes, they talk to us, we benefit… we just have to understand and exploit this*".

Professor de Vos was presented with a commemorative plaque by S*f*AM President Martin Adams and Chief Editor of *Environmental Microbiology* Ken Timmis. The evening finished with a wine reception and a chance to discuss the fascinating talk.

If you would like to watch the lecture it is now available at: www.yada-yada.co.uk/Blackwell/SFAM2011/SFAM2011.html

Clare Doggett
Communications Officer

Wednesday 11 January 2012

Winter Meeting

- **Microbiological safety of imported food**
- **Microorganisms and climate change**

■ Including the Denver Russell Memorial Lecture

The Royal Society, London, UK

CPD ACCREDITATION APPLIED FOR

Programme

10.00 – 10.30	**Tea, coffee and registration**
Chair:	**Martin Adams**
10.30 – 11.15	**The Denver Russell Memorial Lecture:** To be confirmed
11.15 – 11.50	**Monitoring the microbiological safety of imported foods** Caroline Willis, HPA, Southampton, UK
11.50 – 12.25	**www.spatialepidemiology.net — tools for mapping infectious disease epidemiology** To be confirmed
12.25 – 13.30	**Lunch**
Session A	**Microbiological safety of imported food**
Chair:	**Andy Sails**
13.30 – 14.05	**From the banal to the bizarre — microbiological hazards and imported foods** Sue Jones, HPA, Southampton, UK
14.05 – 14.40	**Salad days — foodborne outbreaks due to imported fruit and vegetables: hazards, vehicles & sources** Christine Little, HPA, Colindale, UK
14.40 – 15.00	**Tea and coffee**
15.00 – 15.35	***Salmonella* and imported eggs and poultry** Sarah O'Brien, University of Liverpool, UK
15.35 – 16.10	**Safety of imported foods — a commercial perspective** Kaarin Goodburn, Chilled Food Association, UK
Session B	**Microorganisms and climate change**
Chair:	**Mark Fielder**
13.30 – 14.05	**Microbes as climate engineers** Dave Reay, University of Edinburgh, UK
14.05 – 14.40	**Climate change and communicable disease: what are the risks?** Andrew Nichols, University of Plymouth, UK
14.40 – 15.00	**Tea and coffee**
15.00 – 15.35	**Assessing the impact of climate change on vector-borne viruses in the EU through the elicitation of expert opinion** Paul Gale, AHVLA, UK
15.35 – 16.10	**Antibiotics and climate change** Marion Wooldridge, AHVLA, UK
16.10 – 16.45	**Shifting trends in pathogen dynamics on a changing planet** Paul Hoskisson, University of Strathclyde, UK
16.45	**Close**

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

2012 WINTER MEETING BOOKING FORM and INVOICE

SfAM WINTER MEETING WEDNESDAY 11 JANUARY 2012

Only ONE person per form please. CLOSING DATE FOR REGISTRATIONS: Wednesday 4 January 2012
EARLY BIRD DISCOUNT of £30.00 is applied to all bookings made before **Friday 16 December 2011**

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.

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*ADD MEMBERSHIP TO YOUR BOOKING

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

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The evolving challenge of war wounds

Surgeon Lieutenant **Andrew Matheson**, Major **Emma Hutley** and Group Captain **Andrew Green** detail the historical developments in managing infection in combat wounds and discuss the current issues faced by British forces treating wounds sustained in Afghanistan, elaborating on some of the more unusual infections seen and the management strategies developed to deal with them

The very nature of conflict is to inflict wounds which, if they do not kill directly, have the potential to become infected, causing further injury and death. The approach to the challenge of preventing and treating combat wound infections has changed throughout the centuries, however the challenge continues to evolve and so must the management.

Background

The main focus of current British military operations is Afghanistan where there are about 9,500 troops. Current work is with the Afghan Security Forces to maintain the hard won gains from the Taliban over the last 10 years and to train the Afghan Army and Police as they start to take control of their national security. As of June 2011, 331 British personnel have been killed due to hostile action in Afghanistan. In 2010 alone, 806 members of the Afghan National Army and 1,250 members of the Afghan National Police lost their lives. However, this loss of life is only part of the picture; severe injuries and loss of limb(s) are a daily occurrence. In 2010, 518 UK personnel were wounded in action

(http://www.mod.uk/DefenceInternet/FactSheets/OperationsFactsheets.htm2011) and the survival of triple amputees is not unusual.

The UK medical services in Afghanistan consist of teams of medics and doctors, highly trained in battlefield trauma resuscitation, providing pre-hospital care in the Forward Operating Bases (FOBs) and on every patrol. These teams are supported by a rapidly deployable Medical Emergency Response Team (MERT) from the field hospital which includes specialists in anaesthetics or emergency medicine. The MERT flies as close to the point of wounding as possible to collect the casualty who is stabilized and treated en route back to the hospital. The deployed hospital at Camp Bastion provides immediate life and limb saving treatment for any injured personnel, be they coalition forces, insurgents, Taliban or local civilians. UK personnel are aeromedically evacuated back to the UK, usually within 24 hours, on a Critical Care in the Air Support Team (CCAST) aircraft which provides an 'intensive care' level of care. On return to the UK, all patients go to the role four hospital at University Hospitals Birmingham where they receive definitive care; usually involving multiple reviews of wounds, further debridements and intensive care support. Rehabilitation is conducted at the defence medical rehabilitation centre Headley Court.

The history of wound infections

The fight against the development of infection in battle wounds is not a new problem. Sumerian carvings describe the use of beer and hot water to wash wounds which were then dressed with a plaster of herbs or fruits. The ancient Egyptians used honey and sometimes animal faeces to dress wounds (Molan, 2006) and Hippocrates describes the washing of wounds with wine and the drainage of pus to reduce inflammation. The Ancient Greeks felt there were different types of pus and the encouragement of benign pus was central to therapy (Pikoulis *et al.*, 2004), a practice continued by Galen.

The late Middle Ages brought a new focus on cautery of wounds with hot irons or oil to prevent infection. In the 16th century a French surgeon, Ambroise Paré, introduced wound debridement. The Napoleonic Wars meant French surgeons remained at the forefront with amputation becoming the standard of care. Dominique-Jean Larrey, Surgeon-in-Chief of the Napoleonic armies and favourite of the emperor, could perform hip and shoulder joint amputations in 15 seconds and 11 seconds respectively with a 75% success rate in preventing infection (Pruitt, 2006).

The first descriptions of war wound bacteriology came from Fleming during The Great War. Initially, after injury, wounds were infected with anaerobes, streptococci and other faecal pathogens. By day seven, streptococci were predominant and after 20 days a mixture of staphylococci and streptococci existed. In particular, Fleming noticed *Clostridium welchii* (now *Cl. perfringens*) in 81% of wounds from 1 to 9 days after injury, but only 34% of wounds from 8 to 20 days (Fleming, 1915). World War 2 saw the introduction of antibiotics with topical sulfanilamide powder, and then penicillin. These, along with further surgical developments such as delayed primary closure, quicker evacuation to improved hospital facilities and improvements in infection control, reduced mortality from most wounds in World War 2. An exception was gas gangrene in the Middle East where MacLennan noted an increase in mortality of 25% compared to World War 1. He felt the underlying cause was less extensive wound debridement than performed in Flanders in 1917/18. He concluded that antimicrobials could not replace surgery as the primary method of wound treatment; a principle which is still important today (MacLennan, 1943).

In Korea, wound infections again declined, with improved evacuation times following the introduction of helicopter ambulances. The side effects of administration of antibiotic prophylaxis following injury were also documented in Korea.

Surgeons in a receiving hospital in Tokyo observed a high incidence of infection with organisms resistant to penicillin and streptomycin in soldiers given those drugs following wounding. Resistance to penicillin was demonstrated in 48 of 58 cases and to streptomycin in 49 of 58 cases (Wannamaker & Pulaski, 1958). Prophylactic antibiotics leading to colonization with resistant organisms remains a concern today.

Vietnam highlighted the importance of good wound management and several studies of wound bacteriology showed an initial mix of Gram-positive and Gram-negative organisms, with an increasing proportion of Gram-negative (*Enterobacter* spp., *Pseudomonas* spp. and *Proteus* spp.) from day five onwards.

The harsh terrain of the Falklands conflict, combined with the nature of the fighting and limited evacuation assets, led to long delays before initial debridement could be performed. Where prophylactic antibiotics were not administered and surgery was delayed by over three hours the infection rate was 33% (Jackson, 2007).

The challenges of Afghanistan

Most front-line troops work in the green zone, a stretch of fertile ground along the Helmand River Valley. The Forward Operating Bases (FOBs) and Patrol Bases are often based within the towns and villages of this area and much of the daily work is in the surrounding fields and wadis. This not only makes evacuation of injured troops challenging, but means wounds are heavily contaminated with mud and foliage.

Seriously injured casualties arriving in the deployed hospital undergo Damage Control Resuscitation (DCR), which aims to reverse the lethal triad of coagulopathy, acidosis and hypothermia resulting from their trauma. Surgery is focussed on arrest of haemorrhage, wound debridement, temporary stabilization of fractures and removal of contamination (clothing, dirt, foliage and potentially tissue from other casualties). Initial wound management aims to "*remove all foreign and non-viable material from the wound and so create the right conditions for wound healing or reconstruction without infection*" (Guthrie *et al.*, 2011). The challenge is correctly determining how much tissue to debride in severely contaminated wounds; too much debridement removes viable tissue which is essential for reconstruction at a later date while too little leaves dead tissue and foreign material as a focus for infection. Furthermore, severely injured casualties should spend the minimum possible amount of time in theatre to prevent further acidosis, hypothermia and bleeding due to the ensuing coagulopathy. This means debridement as radical or thorough as the surgeon would like, may not be possible. Additionally, patients are immunosuppressed due to the massive blood transfusions required to keep them alive at a time that their wounds are still contaminated. The combination of all these factors means that casualties have a high risk of wound infection. Patients with these severe injuries (Injury Severity Scores of greater than 60) are now described as 'unexpected survivors' and some have gone on to develop unusual late wound infections with invasive fungi including zygomycetes.

The challenges facing the deployed laboratory staff are also different to those faced in the NHS. Military laboratory staff must multitask as microbiologists, biochemists, haematologists and transfusionists. The ability to automate is limited by the environment, need for mobility and logistic

challenges, so staff must be able to deal with a variety of isolates with the traditional tools of microscopy, culture and simple biochemical tests. Additionally, the deployed laboratory is usually diverted to providing the huge numbers of units of packed red cells and platelets required whenever a severe trauma case arrives.

Microbiology of Afghan wounds

The most common isolate seen in infected wounds is *Staphylococcus aureus*, followed by *Pseudomonas aeruginosa*, *Enterobacter cloacae* and *Bacillus* spp. This data is from the Wound Infection Surveillance Program, which records data on all wounds in UK soldiers evacuated to Birmingham. The diagnosis of infection in these wounds is based on the CDC 1992 criteria that are also used in the national Surgical Site Surveillance Program (Horan *et al.*, 1992).

An interesting organism seen in some patients injured whilst patrolling in the green zone is *Aeromonas hydrophila*. Infection following injury in similar conditions, immersed in water or wet muddy fields, has been previously reported. Zygomycetes and *Aeromonas* spp. were isolated from injuries sustained in waterlogged environments during the Asian tsunami (Hiransuthikul *et al.*, 2005; Andresen *et al.*, 2005).

Multiple resistant *Acinetobacter baumannii-calcoaceticus* complex (ABC) was a significant issue during operations in Iraq. Much debate occurred as to the source of the organism and the clinical sequelae. The UK and US experiences and outcomes varied markedly. From a UK perspective, no significant infections occurred in UK service personnel despite many patients being colonized. This may be explained by the more narrow spectrum antibiotic policy used by the UK medical chain and the significant attention given to infection control.

As mentioned earlier, a number of the 'unexpected survivor' group of patients from Afghanistan have developed zygomycete infections. This is thought to be linked to the type and severity of injury in these individuals (Evriviades *et al.*, 2011). Zygomycetes such as *Rhizopus* spp., *Apophysomyces* spp., *Mucor* spp., *Saksenaea* spp. and *Absidia* spp. have caused infection. The classical zygomycosis is invasive rhinocerebellar disease in poorly controlled diabetic patients who develop sinusitis following inhalation of fungal spores. The infection then spreads into adjacent tissues causing thrombosis and local necrosis. Metabolic acidosis and immunosuppression following cytotoxic or radiation treatment are also recognized as risk factors for zygomycosis.

Zygomycete spores are common in the environment, and inoculation of spores can result in invasive infection following severe trauma with heavy soil contamination. Invasive fungal infection with zygomycetes has been reported in traffic and farming accidents (Vitrat-Hincky *et al.*, 2009), following the tsunami in 2004 and more recently following a tornado in Missouri in 2011 (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6029a5.htm). Our 'unexpected survivors' are at particular risk of invasive fungal wound infection due to the extensive soil contamination and microvascular effects from blast wounds, metabolic acidosis from muscle death and immunosuppression following large blood transfusions. These infections have been reduced by pre-emptive use of antifungal agents in patients fulfilling a specific set of risk criteria (personal communication). Burn wounds are predisposed to infection due to the loss of the protective layer of the skin, and zygomycosis of burn wounds has been reported in Afghanistan (Hospenthal *et al.*, 2011).

Diagnosis of fungal disease is challenging in the deployed environment. Ideally, histological examination of tissues is required to demonstrate tissue invasion and infection, and culture of the fungi is needed for identification and susceptibility profiles (Cuenca-Estrella *et al.*, 2011). Tissue removed during debridement of wounds is stained with fluorescent calcofluor white stain to look for fungal hyphae, and it may be possible to identify the organism from the morphology (Evriviades *et al.*, 2011).

Conclusion

The challenge of treating war wounds is constantly changing, depending on the nature of the conflict. In Afghanistan, improvements in pre-hospital care mean previously unsurvivable blast injuries are pushing the limit of our medical and surgical ability. The optimum management of these 'unexpected survivors' is still unknown, with alterations to surgical technique and the use of novel pre-emptive antimicrobials under consideration in the face of delayed wound debridement. The Wound Infection Surveillance Program which has been running for three years will hopefully allow us to learn more and adapt further to these new combat wound infection challenges.

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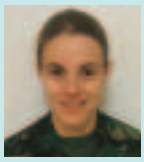
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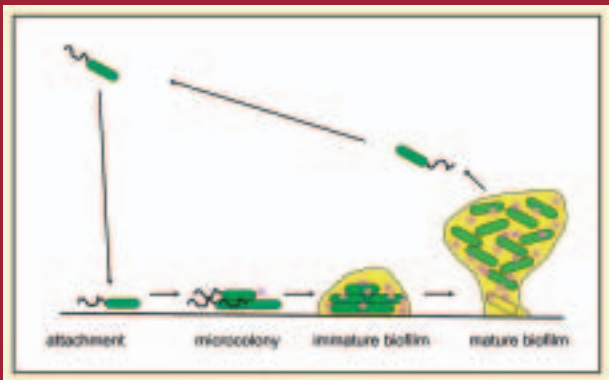
Surgeon Lieutenant Andrew Matheson[1], Major Emma Hutley[2] and Group Captain Andrew Green[3]
Royal Centre for Defence Medicine

Is the use of honey for the treatment of biofilms a sticky subject?

Figure 1. *Biofilm imformation*



Traditionally most bacteria were thought to exist in nature as independent, free-living or planktonic cells. The recognition that most species exist in biofilms has revolutionized our perception.

Biofilms are three dimensional structures firmly attached to surfaces. They are comprised of either single or multiple species organized into complex communities and encased in a matrix of mutually secreted slimy extracellular polymeric substances (EPS). Although many different signals can elicit biofilm formation, the way in which they develop is well established (Figure 1). Initially, cells are attracted to an interface where reversible attachment of planktonic cells is mediated by surface appendages (such as pili and flagella) or polysaccharide and protein cell surface adhesins. Growth and division leads to the formation of a localized microcolony. The continual emission and detection of signalling molecules allows individual cells within the microcolony to estimate the number of similar cells within close proximity by chemical communication or quorum sensing. When a critical number is exceeded, differential gene expression initiates biofilm formation, which results in irreversible attachment, loss of motility, increased virulence and EPS secretion. Gradually, the immature biofilm matures into complex three dimensional or mushroom-like structures with fluid filled channels for the exchange of nutrients and waste products. Cells in established biofilms exhibit slow growth rates and their susceptibility to antimicrobial agents is at least 500 times less than that of planktonic cells (Stewart & Costerton, 2001). Fragments of mature biofilm can become detached by shear forces and dispersal of planktonic cells from the mature biofilm is influenced by nutritional triggers.

Biofilms in human disease

Biofilms are widely distributed throughout the environment and can give rise to either beneficial or detrimental effects. Their role in the corrosion of submerged metal structures, or occlusion of pipelines is well described. Biofilms that become established within the human mouth, gut or vagina may provide protection against infection. However, biofilms have been implicated in 65% of human infections (Potera, 1999) and that estimate is probably too low. Whereas acute

infections that respond to antibiotics are considered not to involve biofilms, persistent infections such as cystic fibrosis, osteomyelitis, periodontal disease, prostatitis, sinusitis and infections associated with indwelling medical devices have been associated with biofilms. Recently, chronicity in wounds was linked to the presence of biofilms (James *et al.*, 2008). Difficulties in treating biofilm infections stem not only from their recalcitrance to antibiotics and antiseptics, but also to their ability to suppress the normal host immune responses (Bjarnsholt *et al.*, 2008). Innovative treatment strategies are urgently required.

Control of biofilms in wounds

Although routine methods of diagnosing biofilms in human tissues are not yet available, the financial and social burden of chronic wound infections on healthcare resources has prompted searches for effective antibiofilm agents. The problem of how to manage biofilms in wounds is currently receiving much attention. Two approaches have been identified: biofilm prevention and biofilm disruption. The first clinical study to employ strategies designed to disrupt biofilms in ischaemic leg ulcers utilized sharp debridement and topical application of lactoferrin and xylitol. This approach was called biofilm-based wound care (BBWC) (Wolcott & Rhoads, 2008). Removal of tissue from the wound bed aimed to disrupt the biofilm and reduce its biomass. Lactoferrin was used to prevent biofilm formation by sequestering iron and stimulating bacteria to adopt a specialized form of motility, which precluded the formation of biofilms (Singh *et al.*, 2002) and xylitol was used to block the adherence of Gram-positive bacteria to epithelial cell surfaces and tissue surfaces (Tapiainen *et al.*, 2004). Combination therapies seem to be more effective than single interventions.

Other possible treatments for biofilms in wounds include maggots, garlic (Rasmussen *et al.*, 2005), silver (Bjarnsholt *et al.*, 2007) and honey. Honey is an ancient wound remedy that has been re-introduced into modern medical practice and a range of licensed products that contain honey are available on prescription in Australasia, Europe and North America. They range from sterile medical grade honey in tubes, honey incorporated into ointments or impregnated on to dressings. One non-sticky, flexible sheet has also been developed for irregularly shaped wounds. The floral origin of the honeys utilized are not always declared on the labels of these products, but include manuka (*Leptospermum scoparium*), buckwheat, chestnut or they can be multifloral.

Inhibition of bacteria by honey

Honey is a broad spectrum antimicrobial agent that has been shown to inhibit a wide range of microbial species *in vitro*; more recently honey has been reported to inhibit antibiotic-resistant and multi-drug resistant wound pathogens such as MRSA, vancomycin resistant enterococci (VRE), *Acintetobacter* and *Pseudomonas aeruginosa* (Cooper, Molan & Harding, 2002; George & Cutting, 2007; Kwakman *et al.*, 2008; Blair *et al.*, 2009). The chemistry of honey is complex and varies with floral origin, species of bee, harvesting and storage conditions. Antibacterial activity of undiluted honey is derived from its high sugar content, low water content and low pH. In diluted honeys, antibacterial activity may be attributed to the generation of hydrogen peroxide (Bang, Buntting & Molan, 2003), the presence of methylglyoxal (Mavric *et al.*, 2008; Adams *et al.*, 2008) or bee defensin (Kwakman *et al.*, 2010). Not all honeys possess all of these antimicrobial components (Kwakman *et al.*, 2011). Manuka honey from New Zealand has been shown to prevent cell division in planktonic cultures of MRSA (Jenkins, Burton & Cooper, 2011) and to cause cell surface changes and lysis in *Ps. aeruginosa* (Henriques *et al.*, 2010).

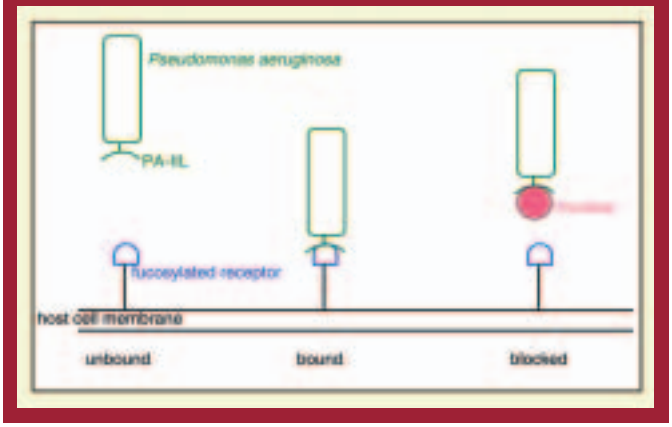
Inhibition of biofilms by honey

Laboratory investigations into the effect of honey on biofilms indicate that planktonic cells are more susceptible to honey than biofilms. Also, lower concentrations of honey are required to prevent biofilm formation than those required to disrupt established biofilms (Merckoll *et al.*, 2009; Cooper, Jenkins & Rowlands, 2011). Inhibition of *Ps. aeruginosa* biofilms was found to be dependent on exposure time and honey concentration (Okhiria *et al.*, 2009).

A study to determine the effectiveness of honey in inhibiting biofilms of *Ps. aeruginosa* and *Staphylococcus aureus* grown in the Calgary biofilm device showed that Sidr honey from the Yemen and manuka honey from New Zealand were more effective than commonly used antibiotics. Since these test organisms have been implicated in chronic rhinosinusitis, the authors suggested that nasal lavage with solutions of honey could have a role to play in treating patients with recurrent and persistent infections (Alandejani *et al.*, 2009). Typically, manuka honey contains higher levels of methylglyoxal (MGO) than other honeys (Mavric *et al.*, 2008; Adams *et al.*, 2008). It was recently demonstrated that honeys containing at least 0.53 mg/ml MGO had biofilm-cidal activity against *Staph. aureus*. Furthermore the antibiofilm activity of a non-MGO honey supplemented with MGO was observed to mimic that of manuka honey, but MGO did not account for all of the activity of manuka honey (Jervis-Bardy *et al.*, 2011). It is possible that further antibacterial components in manuka honey may yet be discovered.

It has been demonstrated that honey prevents biofilm formation in *Ps. aeruginosa* because molecules of fructose (which is the most abundant sugar in honey) competitively block the PA-IIL lectin that mediates adhesion of the bacterium to fucosylated receptors on the membranes of potential host cells (Figure 2) (Lerrer *et al.*, 2007). Failure of *Ps. aeruginosa* to bind to target host cells precludes infection and biofilm formation.

Figure 2. *Blocking of lectin PA-IIL in Pseudomonas aeruginosa by fructose molecules in honey (after Lerrer et al., 2007)*



One other interesting attribute of honey is its ability to inhibit quorum sensing. In a survey of 29 unifloral honeys a pigmented reporter bacterium (*Chromobacter violaceum*) was employed to detect quorum sensing agonists. Chestnut honey and linden honey showed the highest quorum sensing inhibition activity (Truchado *et al.*, 2009a).

The capacity of chestnut honey and its aqueous and methanolic extracts to inhibit quorum sensing controlled events in one plant pathogen and two human pathogens was further investigated by quantifying N-acetyl-L-homoserine lactones (AHL) produced in supernatants of test bacteria exposed to honey. The aqueous extract of chestnut honey was found not only to inhibit AHL production in each of the three test bacteria but to cause degradation of AHLs. The quorum sensing inhibitory activity was thought to be contained within the carbohydrate fraction (Truchado *et al.*, 2009b). These findings suggest that chestnut honey may be used to control pathogens in plants, animals and humans by attenuating virulence and preventing biofilm formation and infection. A wound dressing containing chestnut honey is already available in Slovenia, but whether it controls biofilms in wounds is not yet known.

Conclusion

At the present time the industrial and clinical implications of biofilms in unwanted situations are far-reaching. The reduced susceptibility of biofilms to antimicrobial agents confounds the use of conventional antibiotics and antiseptics and requires some novel interventions. The ability of honey to interrupt quorum sensing provides a means to prevent and disrupt biofilms in diseased plants and animals, and the eradication of antibiotic-resistant strains from wounds by topical application of honey might also help to reduce cross infection in healthcare establishments. There is a need to characterize the active component(s) in honey and to determine how quorum sensing is inhibited. The development of a new class of antimicrobial agent might be possible. Honey no longer seems to be just a quaint folk remedy for wounds that has no place in modern clinical practice.

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Bacteriophages' function in wound healing

When we encountered the idea of using bacteriophages to eradicate human infection, the epiphany was exciting and almost overwhelming. Imagine, with antibiotics failing, an entity almost as old as time itself being harnessed by humans to eradicate infectious disease. Bacteriophages may be the most important "post antibiotic era" treating agent that medicine can utilize to intercede in this growing global crisis. This gives us hope; yet our hope is counterbalanced by the complexity of chronic infections.

Bacteriophages possess incredible potential; however, we now realize the nature of infectious diseases is much more complex; so a large amount of hard work remains to be done to harness their potential.

Clinical Microbiology is dominated by Koch

Clinical microbiology continues to be dominated by the early work performed by Robert Koch in the late 1800s. Koch's famous postulates, especially the pillar of clinical microbiology that "*one organism produces one infection*", have led to the clinical bias that only "*a single organism*" that is causing the infection must be targeted, and all other microorganisms are just contaminants. When Koch first started his quest for pure culture (predicated on the idea of one organism causing one disease), he looked for culture media which would select for the one organism. Other scientists of his day found, "*No matter how ingenious the machinery, how careful the researchers, they kept ending up with beakers of mixed bacteria. The inability to get anything but mixed cultures led many scientists to believe that bacteria had to be in mixed groups in order to thrive*" (Hager, 2006). Now we can say with almost certainty that this is a foreshadowing of the biofilm infections we understand today.

The genius of Robert Koch was to "*make sense out of the chaos*" (Nobel Foundation, 2007). For episodic planktonic

diseases, Koch was mostly right because planktonic bacteria compete and tend to be monoclonal infections. However, chronic infections tend to have a complex, polymicrobial diversity, something which is inherent in biofilms. It is this complexity which challenges bacteriophage therapy. But the use of bacteriophages can still play an important role in managing human infection.

Bacteriophages and biofilms

Bacteria developed the ability to organize into biofilms 3.25 billion years ago. A well-vetted and widely accepted theory is that bacteria selected for biofilm phenotype mode of growth in the environment mainly to protect themselves from UV light and bacteriophages (Stoodley *et al.*, 2002). The ancient dance between the predator bacteriophages and their bacterial prey is billions of years old with the bacteria mutating (dropping out epitopes, developing restriction enzymes, etc.) and the bacteriophages exerting countermeasures (depolymerase, adhesin mutations, etc.). The colony defences of biofilms limit the predatory effectiveness of bacteriophages. So the narrow host spectrum of the bacteriophage in the context of the polymicrobial diversity of biofilms makes it impossible for a single bacteriophage to eradicate the biofilm.

Bacteria pursue two different strategies for infection

Costerton *et al.*, (1999) first suggested that bacteria can infect human tissue through a biofilm strategy. The molecular underpinnings of this theory have been elucidated, culminating in the observation by Kim *et al.*, (2010) that bacteria infect host tissue in two very different ways. Bacteria can choose to infect in a planktonic mode of growth, in which the bacteria will upregulate virulence factors killing the host tissue. The bacteria then digest the dead tissue to obtain nutrition and propagate in the host environment. This most closely correlates with acute infection. The second method of infection described by Kim *et al.*, (2010) is for bacteria to organize in a biofilm phenotype mode of growth, downregulating virulence factors and causing host cell senescence to produce a sustainable host niche. These biofilm infections then obtain nourishment through host inflammation, yielding plasma which percolates through the biofilm community. In Kim's words, "*Bacterial pathogens use a variety of mechanisms that balance breaching the epithelial barrier with maintaining the epithelium in order to promote bacterial colonization. These complex strategies represent a new paradigm of bacterial pathogenesis*."

Wound bioburden

In collaboration with the Center for Biofilm Engineering in 2008, we were able to establish that biofilms are present in chronic wounds and not in acute wounds. Once we understood that biofilm-forming bacteria on the surface of the wound was the *sine qua non* of chronic wounds, it became important for us to understand the importance of the wound bioburden in non-healing wounds. In other words, is the wound biofilm a primary or secondary factor in the recalcitrance of chronic wounds to healing?

One of the first and most exciting findings in the evaluation of wound biofilms, using molecular methods, was the incredible microbial diversity in chronic wounds. The diversity added a robustness to the wound bioburden which made healing these wounds much more challenging. But it also became clear that it wasn't just the species present, but how these species coordinate with each other to act upon the host to maintain their niche (Wolcott *et al.*, 2008).

Our group has produced large cohort studies (level 2 evidence) which demonstrate that when wound biofilm is suppressed, chronic wounds improve their wound healing trajectory: 48% of wounds heal in six months without targeting biofilms, whereas 90% heal when a biofilm is specifically targeted (Dowd *et al.*, 2011). This is sufficient evidence to suggest that the biofilm is a primary barrier to wound healing.

Bacteriophages and/or their components have long been known to demonstrate a power against many of the individual bacteria which constitute a wound biofilm. Now it is up to scientists and clinicians to try to exploit this power to suppress wound biofilm formation to a level which allows host healing.

Bacteriophages

Bacteriophages are ubiquitous and more abundant than any other nucleic acid-based entities. They display incredible variations in how they adhere to their target host cell, breach the outer defences, replicate, and eventually lyse their host to infect new cells. Added to this complexity are vastly different strategies for phage infections possessed by lytic versus temperate bacteriophages. However, through persistence the therapeutic use of bacteriophages is emerging.

The therapeutic use of bacteriophages was popularized by Felix d'Herelle in the early 1900s. Soon after the discovery of the bacteriophage, d'Herelle, Frederick Twort, George Eliava and others utilized bacteriophage's predatory propensities towards a variety of human pathogens. This was met with only limited success and, with the advent of antibiotics, bacteriophage therapy decreased in popularity across the developed world, except for in Eastern European countries. Bacteriophages produced at the Eliava Institute in Tbilisi, Georgia, have been used to treat tens of millions of patients with conditions as diverse as wound infections, prostatitis, trauma, osteomyelitis, pharyngitis, dysentery, and many other infectious diseases. Bacteriophages seem to have had some success, yet it is only in recent years that this efficacy has been scientifically documented.

As with any new agent, there have been concerns within the US FDA as to the safety of bacteriophages. Our group pursued a Phase I randomized control trial to establish the safety of bacteriophages in an acceptable randomized control trial format (Rhoads *et al.*, 2009). After a significant amount of negotiation with the FDA, we were permitted to pursue a study protocol which utilized a cocktail of lytic bacteriophages targeting *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The protocol limited the number of study patients to just 40 (20 in the treatment group) with careful observations at each visit to rule out any adverse events. In the 20 patients that were treated with bacteriophages, there were no adverse events. Since only a small number of patients could be included, no information about the efficacy of phage therapy could be obtained. However, utilizing the currently accepted study method of a randomized control trial, our study did demonstrate conclusively to the FDA that lytic phage cocktails are indeed safe in treating human patients.

The FDA has imposed two significant requirements for the

use of bacteriophages in human treatments. First, only lytic phages can be used as they have a very low incidence of transferring potentially dangerous genetic material to the targeted host bacterium. This transferred genetic material could result in increased pathogenicity. It is our opinion that the risk of using lysongenous phages is minimal and could actually offer some advantages over lytic phages.

The second requirement of the FDA is that all bacteriophages be sequenced and must demonstrate safety and efficacy through all three phases of clinical trials. This adds significant cost to the use of bacteriophages. This is probably the most significant regulatory hurdle that must be overcome for the general use of bacteriophages as human therapeutic agents. It is our view that it would be more appropriate to treat bacteriophages like vaccines: that is, once a bacteriophage is proven safe and effective, any new treatment for phage should be approved by an administrative process rather than clinical trials.

Phage therapy

We began utilizing phage therapy in 2005 after a visit to Tbilisi, Georgia. Our colleagues in Tbilisi verified the safety of lytic bacteriophages along with case-after-case demonstrating the therapeutic efficacy of phages. We first started treating chronic wound patients with a commercially available preparation from the Eliava Institute. Piobiophage is a phage cocktail that is poorly defined but is mainly comprised of bacteriophages isolated from chronic wounds. The clinicians in Tbilisi, Georgia, have the advantage of being close to a very cheap source of large volumes of this cocktail, and as a result, they have achieved outstanding clinical results.

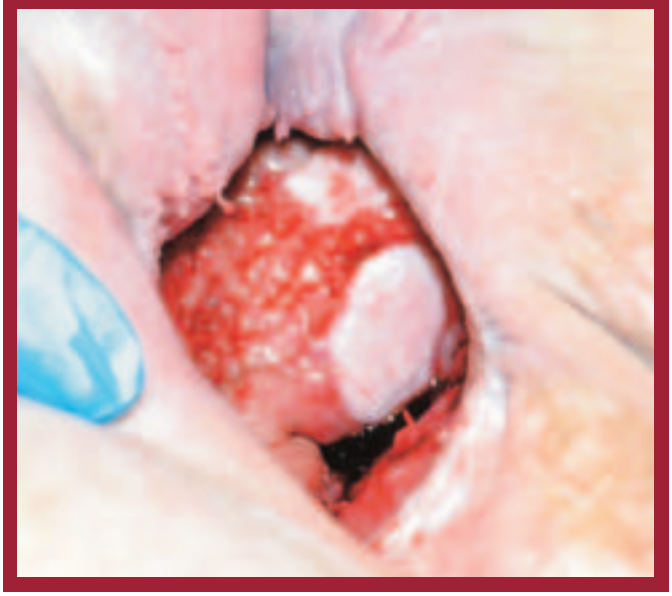
Despite the limited supply of this cocktail, we have seen some clinical response. Several patients showed rapid and complete clearing of the slough of their wounds within 24 to 48 hours, followed by rapid progression of their wounds to complete healing. A second group of patients showed a very rapid but incomplete response to the treatment. By the end of two weeks the wound healing had stalled and a thick, waxy type of slough had developed on the wound. However, the majority of wounds treated with Piobiophage had no response at all.

This seems puzzling in that we specifically chose wounds that routine clinical culture information indicated bacterial species would be targeted by the cocktail. We had only been working with biofilm phenotype bacteria for about two years at this time, but made the partially correct assumption that biofilm colony defences must be playing some role in this incomplete efficacy of phage therapy.

We started exploring bacteriophages relative to biofilms and found that bacteriophages indeed struggle with infecting their host bacterium when it is encased by a biofilm matrix. In the laboratory, bacteriophages were more successful when the matrix of the biofilm was disrupted or any of the other colony defences were degraded prior to application of the bacteriophage. Yet when we applied the bacteriophages to chronic wounds post-debridement, while concurrently specifically targeting the biofilm defences, there was no significant improvement in clinical outcome.

It was during this time that we developed molecular techniques for identifying and quantifying the bacteria present in wound biofilms. We found that wound biofilms contain highly diverse bacterial species, often more than 40 species in a single wound, and this was the reason for the lack of efficacy of our bacteriophage therapy. We now know that the major constituents of the wound bioburden seem to vary between different geographic locations. The bacteriophage cocktail from Tiblisi is tailored towards their most common organisms, and we were probably trying to treat different organisms. But not all wound biofilms are highly diverse. There are a small percentage of wounds which are primarily a single species such as *Ps. aeruginosa*, *Staph. aureus*, *Serratia marcescens*. Those occasional wounds that showed dramatic improvements in wound healing, associated with our use of bacteriophage therapy, were comprised of a single susceptible bacterial species. But bacteriophage treatment can be successful against some biofilms, so we must assume that bacteriophages possess effective strategies for infecting biofilm bacteria.

Figure 1. *Phage epitheleal island*



After our sputtering start into the clinical use of bacteriophages, we realized that bacteriophage therapy will need to become much more sophisticated to be useful in everyday clinical wound care practice. Because bacteriophages have such a narrow host range, it is necessary for wounds to be fully diagnosed not only in terms of the bacterial species present, but also how much of each species is present.

We currently use a combination of PCR and sequencing technologies to fully diagnose wound bioburden. When wounds are identified with a preponderance of *Ps. aeruginosa* or *Staph. aureus* we include bacteriophage therapy with other simultaneous treatments. We found that by frequently debriding the wound this not only degrades the protective biofilm matrix (EPS) but it also forces the biofilm to reconstitute itself, becoming more metabolically active and therefore more sensitive to treating agents (Wolcott *et al.*, 2010). With frequent debridement, we include targeting the wound biofilm with anti-biofilm agents (quorum sensing inhibitors, etc.) and other agents such as antibiotics and/or biocides. Theoretically, there is some question regarding the use of antibiotics alongside bacteriophages, but in the laboratory as well as clinic use there seems to be a synergy

between the concurrent uses of these multiple strategies.

Occasionally, the bacteriophages are extremely successful, rapidly collapsing the predominant *Ps. aeruginosa* or *Staph. aureus* populations. This allows the host to regain its advantage. Once the wound bed has been freed of the dead, devitalized, and senescent tissue, new blood vessel formation and wound contracture rapidly occurs with the wound healing more like an acute wound than a chronic wound and in some cases; the healing can be quite dramatic (Figure 1).

The future of bacteriophage therapy

For phage therapy to become clinically useful in the management of chronic wounds, a large library of bacteriophages must be developed that address the most prevalent bacteria identified in the wound biofilm. For example, anaerobes are a major component of wound biofilm, and it will be a difficult task to develop bacteriophages for these microorganisms. Also, phage components have the potential to be developed into adjunctive therapies which degrade biofilm defences. For example, phage enzymes, which break down specific bonds of the complex sugars which makeup the EPS of a biofilm, could cause disruption of chronic infections not accessible to debridement. Another strategy is to utilize the adhesins that target different specific bacteria as a drug delivery system. Several researchers are already producing large quantities of phage lysozymes (lysins) against various bacterial species. Lysins work on either side of the cell membrane but they do still maintain their very narrow host range.

There is a lot of work left to be done, but with our current tools and a clear vision of what can and needs to be accomplished, bacteriophage therapy could soon be a very important part of managing chronic wounds and indeed all chronic infections caused by biofilms.

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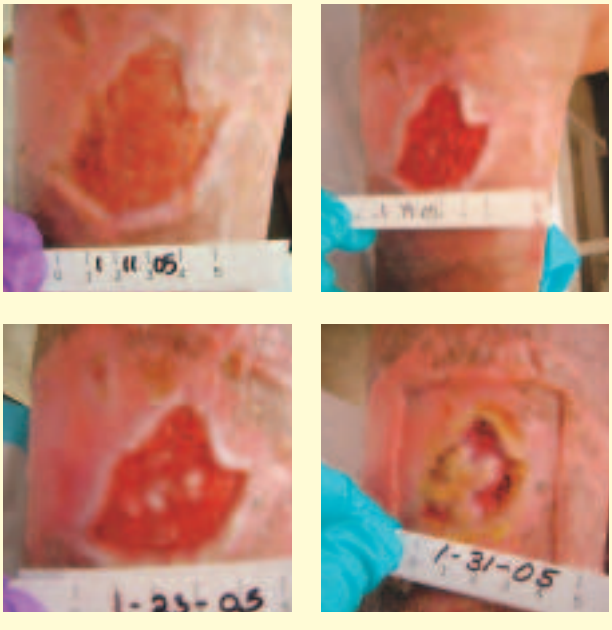
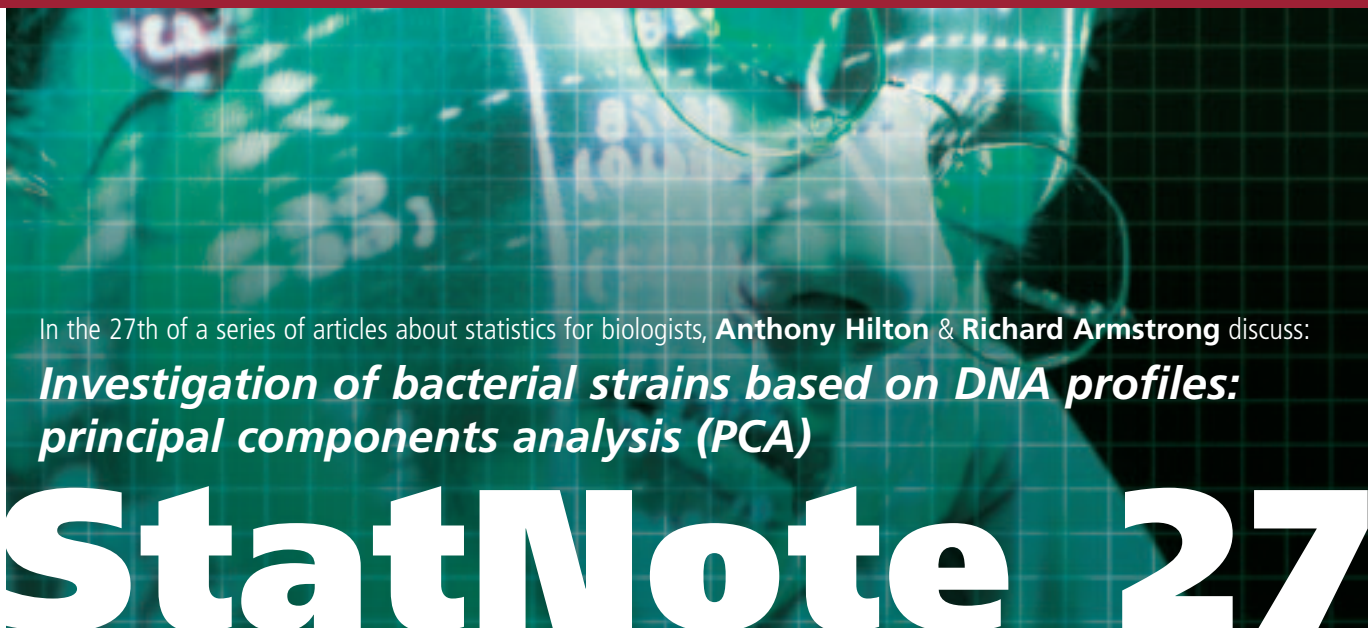
case history



The patient is a very pleasant 60-year-old white male with lymphedema in the bilateral lower extremities, uncontrolled non-insulin dependent diabetes mellitus and non-healing wounds on his lower extremities for many years. The patient's first visit to our clinic was 26 June 2002 with a very painful highly exudative wound. He failed to respond to comprehensive wound management. The patient was readmitted in May of 2004 with a dramatic increase in pain. The wounds were much deeper and more exudative. The patient had multidrug resistant *Ps. aeruginosa* bacteria intermediately sensitive to Amikacin and resistant to all other antibiotics. He was depressed, and ended up losing his job. The patient was started on biofilm based wound management along with bacteriophage therapy with specific phages against *Ps. aeruginosa*. The patient showed dramatic improvement in his wounds over the course of four weeks.

Teaching Point: The patient has multidrug resistant *Ps. aeruginosa* and only responded to phage therapy. Of interest, his wound developed epithelial islands throughout the centre of the wound, which coalesced and filled the wound in, which is a different healing pattern than normally seen.



In the 27th of a series of articles about statistics for biologists, **Anthony Hilton** & **Richard Armstrong** discuss:

Investigation of bacterial strains based on DNA profiles: principal components analysis (PCA)

StatNote 27

Introduction

In StatNotes 24 (Hilton & Armstrong, 2011a) and 25 (Hilton & Armstrong, 2011b), multiple linear regression, a statistical method that examines the relationship between a single dependent variable (*Y*) and two or more independent variables (*X*), was described. The principle objective of such an analysis was to determine which of the *X* variables had a significant influence on *Y* and to construct an equation that predicts *Y* from the *X* variables. 'Principal components analysis' (PCA) and 'factor analysis' (FA) are also methods of examining the relationships between different variables but they differ from multiple regression in that no distinction is made between the dependent and independent variables, all variables being essentially treated the same. Originally, PCA and FA were regarded as distinct methods but in recent times they have been combined into a single analysis, PCA often being the first stage of a FA (Norman & Streiner, 1994). The basic objective of a PCA/FA is to examine the relationships between the variables or the 'structure' of the variables and to determine whether these relationships can be explained by a smaller number of 'factors'.

In StatNote 26, (Hilton & Armstrong, 2011c) the application of classificatory methods (cluster analysis) to the analysis of the DNA profiles from a sample of eight unknown isolates of MRSA was described. The most commonly employed hierarchical clustering method is the un-weighted pair group method using arithmetic averages (UPGMA) (Clifford & Sokal, 1975). The result of the analysis is a dendrogram (tree diagram) which classifies the bacterial strains into clusters. A major problem with this approach, however, is whether or not classification or clustering of the data is appropriate. The strains may consist of a number of different distinct groups or species or they may merge imperceptibly into one another because their DNA profiles actually vary continuously. An alternative to cluster analysis is to examine the spatial relationships between the strains using a 'non-hierarchical' method of analysis. This StatNote describes the use of PCA/FA in the analysis of the differences between the DNA profiles of different MRSA strains introduced in StatNote 26.

Scenario

We return to the scenario described in StatNote 26 in which the genetic relationship between eight unknown isolates of MRSA was studied. Bacterial strains were incubated for 18 to 24 hours at 37°C in Brain-Heart Infusion (BHI) broth. Following incubation, the bacterial cells were harvested and 20 milligrams (wet weight) of cells were re-suspended in 1ml NET-100 (0.1 M Na_2EDTA (pH 8.0), 0.1M NaCl, 0.01M Tris-HCl (pH 8.0)) and mixed with an equal volume of molten low melting point chromosomal grade agarose (0.9% (w/v) in NET-100; BioRad,UK). The prepared blocks were incubated for 24h at 37°C in 3ml lysis solution (6mM Tris pH 7.6, 100mM EDTA pH 8, 100mM NaCl, 0.5% lauroyl sarcosine and 1mg/ml lysozyme) with 20 units of lysostaphin (Sigma, UK). The initial lysis solution was removed and the blocks were incubated for 48h at 50°C in 3ml ESP (0.5M EDTA pH 9, 1.5 mg/ml proteinase K (Sigma, UK) and 1% lauroyl sarcosine). The blocks were washed at room temperature twice for 2h followed by two 1h washes using TE buffer (10mM Tris and 1mM EDTA, pH 8). A portion of each agarose block (1×1×9 mm) was digested with 20 units of *Sma*I (Roche, UK) in 0.1ml buffer for 16h at 25°C. The digested DNA samples were subjected to PFGE (CHEF Mapper system, BioRad, UK) under the conditions outlined by Bannerman *et al.*, 1995. Gels were stained with 1μg/ml of ethidium bromide for 45min and destained for 45min in distilled water. Gels were visualized under UV illumination and photographed using the GeneGenius Bio Imaging System (Syngene, UK).

Data

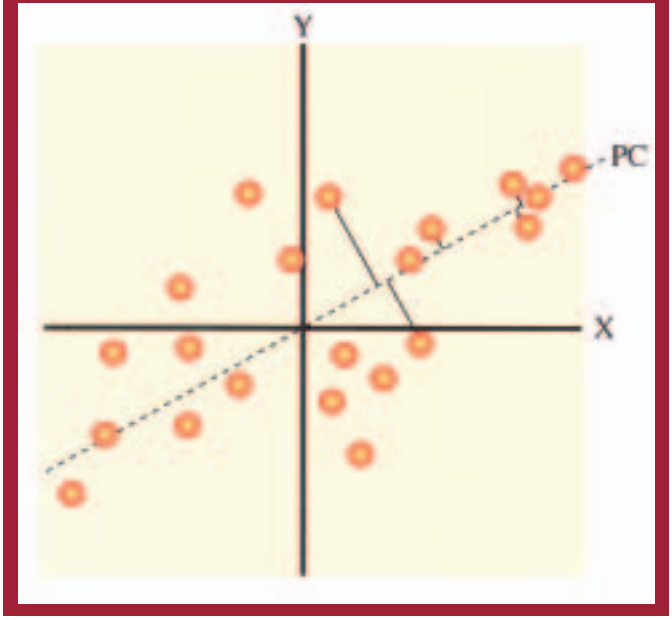
The data comprise the band distances migrated in millimeters from the origin of the gel for each of the DNA preparations and are presented in Table 1 of StatNote 26. Hence the data comprise a series of *X* variables (bacterial strains) and there is no dependent *Y* variable.

How is the analysis carried out?

Theory

The 'variables' in a PCA/FA are defined by a number of 'criteria'. If the variables are bacterial strains, called a 'Q-type analysis' (Pielou, 1969), the criteria could be the distance travelled by the various DNA fragments on the gel. Hence, if there are 's' criteria, each strain can be represented by a point plotted in a Euclidean space defined by 's' dimensions, i.e., each criterion can be considered as an axis orthogonal to all the other criteria and each strain as a point in this s-dimensional coordinate frame. Conceptually, in a PCA/FA, the original 's' dimensional geometric frame is reduced to two (2D) or three dimensions (3D) such that the original spatial relationships between the strains are preserved as far as possible. Strictly PCA is just one method, albeit the most

Figure 1. *A hypothetical example in which variables are defined by only two criteria (X,Y), resulting in a two-dimensional (2D) scatter plot. In theory we could reduce the 2D display of the patients to 1D by projecting the points so that they lie on a single line (PC)*



common, for selecting these fewer dimensions.

A simple geometric model of the procedure is shown in Figure 1. Imagine that each of 21 variables were defined by only two criteria and the data plotted as a 2D scatter plot. Now suppose that we wish to simplify this 2D display by projecting the points on to a single line. In carrying out this procedure, there is an inevitable loss of information regarding the spatial relationships between the variables. The loss of spatial information would be minimized if the line was orientated through the cluster of points to preserve as much of the original spatial variability as possible. In PCA/FA, if the number of original axes is 's', a smaller number of axes or 'factors' are extracted which account for significant proportions of the original variance in the data. If the extraction procedure is PCA, then the extracted factors are called principal components (PC). The first principal component (PC1) accounts for the maximum variance while the remaining PCs account for decreasing proportions of the remaining variance. The PC is analogous to the single line drawn through the points in Figure 1. Individual variables such as strains could then be plotted in relation to these extracted PCs. Such plots summarize the similarities and differences that exist between the variables; variables close together in the plot being more similar in the measured criteria than those furthest apart. Such an analysis can indicate whether variation between bacterial strains was continuously distributed or clustered into subgroups. In other uses of PCA/FA, the variables may not be bacterial strains but the criteria used to define them (sometimes called a R-type analysis) and the intention may be to determine whether the variables (distances of the DNA bands) can be grouped into a smaller number of underlying 'factors' which can best explain the patterns of variation among the strains.

Most of the major statistical packages such as SPSS and STATISTICA will offer FA and PCA often as part of a 'multivariate analysis' option. We will illustrate the analysis of our data using STATISTICA software.

Analysis

The original 'raw' data set comprises the variables (the columns or strains) defined by a number of criteria (the rows or band distances). The first stage in a PCA/FA is the calculation of the correlation of each variable with all of the other variables. If the measured variables are clearly related to a smaller number of underlying 'factors', then this may be apparent by inspection of the correlation coefficient matrix. This is because all the variables that measure one factor would correlate strongly with each other and not with the variables associated with another factor. In practice, however, it is very difficult to extract the actual factors by visual examination of a correlation matrix since all variables are likely to show some degree of correlation with other variables.

The next stage of the analysis involves checking whether it is worth carrying out a PCA/FA with the existing data, i.e., are there sufficiently strong correlations between the variables to analyse them factorally? The simplest of these tests involves examination of the 'strength' of the correlations within the matrix. If there are few correlations greater than about r = 0.30, then it is probably not worth proceeding any further (Tabachnick & Fidell, 1989), i.e., the correlations between variables are too weak for them to be combined into fewer factors. A second test involves examination of the 'partial correlations' between the variables. If there are only three variables in the study (X_1, X_2, X_3), the partial correlation coefficient between any two of them, say X_1 and X_2, is the correlation between them in a cross-section of individuals all having the same value of X_3. In other words, the effect of X_3 is removed from a test of the correlation between X_1 and X_2. In the case of a PCA/FA, if the variables correlate with each other because they are related to a smaller number of underlying factors, the partial correlations should be small (Guttman, 1954). Another statistical test employed is 'Bartlett's Test of Sphericity' which uses χ^2 as a test statistic. If the value of χ^2 is not significant ($P > 0.05$), then no correlations are present and the analysis should not proceed. A final test is that of 'sampling adequacy' and is a measure of the degree of correlation within the data set as a whole and of the individual variables. If the sampling adequacy for the whole data set is <0.50, it is better not to proceed with the analysis. Similarly, if the sampling adequacy for an individual variable is <0.50, that variable is unlikely to show much correlation with the other variables in the study and it may be better to proceed without it.

The PCs are extracted so that first, they are uncorrelated with each other, and second, that each successive PC accounts for a decreasing proportion of the remaining variance. Hence, PCA/FA tries to 'explain' the variance of a group of variables in terms of a smaller number of uncorrelated PCs.

The analysis will extract as many PCs as there are variables included in the analysis. An important question is how many PCs should actually be extracted from the data and retained for examination? There is no objective statistical method available which can determine the number of PCs. Instead, most statistical programs employ one or more rules, termed 'stopping rules', to determine how many PCs should be retained. In a PCA/FA, each PC is associated with an 'eigenvalue' which is the per cent of the total variance in the

Table 1. Simple correlation matrix (Pearson's correlation coefficient 'r') between the bacterial strains. There are eight *Sma*I genomic digests of Meticillin-resistant *Staphylococcus aureus* (MRSA) while wells C and H carry a *Sma*I chromosomal digest from *Staph. aureus* strain NCTC 8325 as a control and molecular weight marker. The majority of the correlations exceed 0.30 suggesting that the data are suitable for PCA/FA

	Bacterial strains									
	A	B	C	D	E	F	G	H	I	J
A	1									
B	1	1								
C	0.98	0.98	1							
D	1	1	0.98	1						
E	1	1	0.98	1	1					
F	0.68	0.68	0.62	0.68	0.68	1				
G	0.48	0.48	0.52	0.49	0.48	0.20	1			
H	0.98	0.98	1	0.98	0.98	0.62	0.52	1		
I	0.70	0.70	0.63	0.70	0.70	0.99	0.24	0.63	1	
J	0.70	0.70	0.63	0.70	0.70	0.99	0.24	0.63	1	1

Table 2. Unrotated factor loading matrix: the correlation between each of the bacterial strains and the extracted principal components (PC) and the percentage of the total variance explained by each PC

	Extracted PCs	
Strain	PC1	PC2
A	-0.977435	-0.161167
B	-0.977435	-0.161167
C	-0.952375	-0.255144
D	-0.977486	-0.166691
E	-0.977435	-0.161167
F	-0.804656	0.572954
G	-0.498039	-0.489264
H	-0.952375	-0.255144
I	-0.823068	0.549259
J	-0.823068	0.549259
% Variance	78.861	14.069

Table 3. Correlations between the band distances and the factor loadings of the bacterial strains on PC1 and PC2 (** P < 0.01, ***P < 0.001)

	Extracted PCs	
Band	PC1	PC2
1	0.26	0.54
2	0.51	-0.08
3	0.39	-0.18
4	0.66**	0.40
5	-0.13	0.32
6	-0.04	0.13
7	-0.16	0.28
8	-0.21	0.38
9	-0.42	0.14
10	-0.57	0.22
11	-0.69**	0.26
12	-0.80**	0.55
13	-0.31	-0.96***
14	0.88***	-0.44

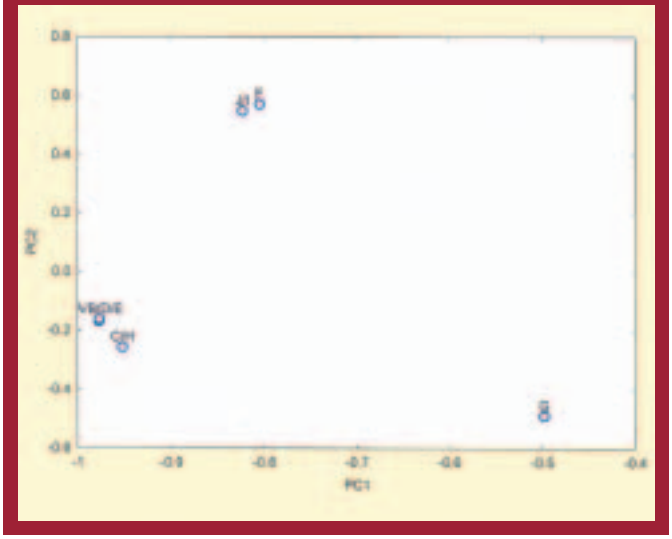
data explained by that PC. First, the 'Kaiser' criterion selects all eigenvalues greater than 1. This criterion has two disadvantages: (1) it is essentially arbitrary and (2) it tends to select either too many or too few PCs depending on the number of variables in the study. Second, in 'Cattell's Scree Test', the eigenvalues for each factor are plotted in descending order. The change in the eigenvalue is rapid at first and then levels off and the last factor is chosen before the flat portion of the curve. Third, the 75% stopping rule selects all PCs whose variance when added up sums to 75% and is also likely to extract many PCs. Whichever method is used, it is commonly observed in a PCA/FA that the first PC will contain a disproportionate amount of the variance, and the others relatively small amounts. Hence, in many studies, it is often not worthwhile to examine more than the first three PCs.

The next stage involves determining which of the measured variables are associated with each PC and this is strictly where the factor analysis part of the method begins. PCA/FA calculates the correlations between each variable and the extracted PC and these are known as the 'factor loadings', a high value indicates a strong relationship between the variable and the PC. Ideally, each of the variables should load onto only one PC, i.e., the loadings should be close to unity or zero. In practice, variables are often complex and load onto more than one PC and this can make their interpretation more difficult. Another problem is that some variables may have positive loadings and others negative loadings on a PC. In some types of PCA/FA, rotation of the PCs is often advocated to produce a solution that is easier to interpret. Rotation can be carried out in such a way that the PCs remain uncorrelated (an 'orthogonal' solution) or this condition can be relaxed and some degree of correlation between the PC can be accepted (an 'oblique' solution). Rotation of the PCs will often produce a more equitable distribution of the variance between the extracted PCs and also result in individual loadings that are closer to unity or zero.

Interpretation

An important step in a PCA/FA is to attempt to interpret what the extracted PCs actually mean with reference to the problem or hypothesis posed. The first stage of this analysis involves determining which variables load significantly onto each PC. A simple procedure would be to accept as significant

Figure 2. *The resulting pulsed-field gel electrophoresis (PFGE) patterns of the eight SmaI genomic digests of Meticillin-resistant Staphylococcus aureus (MRSA); wells C and H carry a SmaI chromosomal digest from Staph. aureus strain NCTC 8325 as a control and molecular weight marker plotted in relation to PC1 and PC2*



any variable whose loading was larger than a certain value, e.g., >0.30 or >0.50 but this is an arbitrary procedure and does not take into account sample size. A more rigorous method is to test the loadings statistically using 'Stevens method' (Norman & Streiner, 1994) and is given for a sample size of 'N' by the equation:

$$\text{Critical value} = 5.152/\sqrt{(N-2)} \quad (1)$$

Hence, any variable whose factor loading exceeds this critical value may be regarded as being significantly correlated with a PC.

In the present application of PCA/FA, bacterial strains are the variables (Q-type analysis) and the result is a scatter plot of the strains in relation to the extracted PC. The objective is twofold: (1) to describe the pattern of variation between bacterial strains and (2) to identify those features of the DNA profiles that best correlate with the distribution of the strains.

The matrix of correlations between the MRSA strains is shown in Table 1. The majority of the correlations exceed 0.30 suggesting that the data are suitable for PCA/FA. Two PCs were extracted from the data, using Cattell's Scree Test, accounting for approximately 93% of the total variance in the data (Table 2). Hence, reducing the original 10D frame to 2D has resulted in the loss of approximately 7% of the original spatial information. A plot of the bacterial strains in relation to PC1 and PC2 is shown in Figure 2. The data suggest that the data from wells I/J, C/H, and A/B/E immediately form three groups which are each identical according to band distances. Furthermore, D is the strain most closely related to E/B/A and F is most closely related to J/I. Strain G appears to be the most unrelated to the others. In addition, the correlations between the band distances for each strain and the factor loadings of the strains on PC1 and PC2 are shown in Table 3. Bands 4 and 14 were positively correlated with PC1 and bands 11 and 12 negatively correlated with PC1 and hence, these are the DNA band distances which are the most important in determining the distribution of the strains. Moreover, band 13 was negatively correlated with PC2.

The interpretation of the PCA/FA data in this example is in close agreement with that of the dendrogram analysis described in StatNote 26. However, the PCA has a number of advantages over that of classification: (1) no assumptions are made that the data are actually classifiable, (2) the relationship between strains and clusters of strains is spatially displayed which facilitates discussion of the implications of the analysis, and (3) the analysis identifies the criteria, in this case the band distances, which best differentiate between the strains.

Conclusion

PCA/FA are methods of analysing complex data sets in which there are no clearly defined *X* or *Y* variables. They have multiple uses including the study of the pattern of variation between individual entities such as bacterial strains and the detailed study of descriptive variables. In most applications, variables are related to a smaller number of *factors* or PCs that account for the maximum variance in the data and hence, may explain important trends among the variables. No assumptions are made before the analysis that the variables can actually be classified and this may be a considerable advantage in the analysis of more complex data sets such as DNA band data of bacterial strains which may be more continuously distributed.

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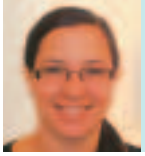
writing up

For the latest PECS pages we wanted an insight into writing up so PECS Communications Officer, Irene Freire-Martin posed some questions to PECS Secretary **Amara Anyogu** about her experiences

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

PECS NEWS

We would like to announce that Simon Gould has stepped down as Chairman of the PECS Committee and thank him for all his work. Congratulations to Emmanuel Adukwu on his new role as Chairman. We would also like to welcome two new members of the PECS Team, Danny Sewell and Jenni Drever-Heaps. If you would like to let us know about a fellow student or early career scientist who has recently been awarded a PhD/prize/award please email pecs@sfam.org.uk.



Irene Freire-Martin
PECS Communications Officer

Q How did you prepare before starting to write up?

A One of the best things my supervisor did for me was to insist I write a transfer report about halfway into my PhD even though at the time it wasn't a requirement. This allowed me to experience 'writing up' before the main 'writing up'. It also provided a very robust 'skeleton' on which I could build the thesis on. With hindsight, I would encourage PhD students to always be writing something. Sometimes when reading a completed thesis and comparing the clarity and structure to your writing we forget that it took a lot of drafts to get there. It doesn't have to be perfect, just written. Leaving any writing for the final year is never a good idea in my opinion. Many institutions now insist on progressing students submitting lengthy pieces of work at least once a year to ensure that they are always writing.

When I write, I tend to start with what I consider the 'easier' parts of the thesis. So for each chapter, I would put in the methods section, followed by the results. The sense of accomplishment from seeing the chapter develop encouraged me to write more. The writing process can be difficult, it calls for organizational skills. You must plan your work and set goals and targets for yourself. Discussing these targets with your supervisors gives you that extra kick to stick to your plan. The thesis itself is a big project but it can be managed if it's broken into smaller projects and you can celebrate when you pass each milestone. I found taking short breaks from writing helped. Reading a section after a few days (well hours sometimes) gave me some distance that enabled me to spot any errors, ask myself questions and rewrite with more clarity.

I learnt that the writing process usually takes longer than you estimate in your head. You have to give time to send your drafts to your supervisory team, receive their comments and make those corrections. Remember that you are not their only priority. These were things I didn't really consider and contributed to the writing taking longer than I actually thought it would.

Q What is your advice regarding managing your references?

A I'll say do what works best for you. Most students I know use referencing systems like EndNote. I'm not 'techy' so I managed mine manually. I don't think this is the best way, it certainly isn't the easiest for most people but it worked very well for me. Each chapter had its own folder with the papers I was using and I constantly edited the reference list at specific writing times to ensure that all my cited references ended up on the reference list. So far, I haven't noticed any mistakes!

Q What kind of preparation are you doing for the viva?

A I discussed with students who had recently completed their PhDs and survived the viva. I've been told to make sure I really understand what I've written in my introduction section! I've been reading through the thesis and trying to anticipate any questions I may be asked. Also trying to answer the question, 'What is the contribution this body of work has made in your field?' Making notes on concepts/mechanisms I have briefly discussed but may require further explanation. Creating a list of any mistakes e.g. spelling mistakes, figure annotations that I observe as I am reading the thesis and submitting this to the examiner during the viva.

Q Any other advice?

A In addition to the questions you've asked, I would say that students should be aware of the guidelines of their institution regarding submission and the oral examination. What forms need to be filled prior to submission/to arrange your viva? How many copies of your thesis need to be submitted? These are little administrative things but can lead to unnecessary delays if not dealt with.

Lastly, I would say all the best. It is a daunting but very achievable task. The work has been done already, but you need to write it up in a way that gives you and the work credit.

Amara Anyogu
PECS Secretary

Public Engagement Grant: Germ Wars

DUNDEE SCIENCE CENTRE
sensation

The Germ Wars initiative began in 2010 with a small pilot project with three Dundee schools, engaging 80 Primary 6 (age 10 to 11) pupils with microbiology. This project was very successful with pupils becoming fascinated by the subject, and teachers building up their confidence to teach microbiology in the future. It was clear that this project was a success, and thanks to the kind support of *Sf*AM it was rolled out to 10 Dundee City Council schools this year, with 250 pupils taking part!

'Germ Wars' is a highly engaging book in which the author, Gill Arbuthnott, uses fun illustrations and a sound scientific knowledge to introduce children to the sometimes mysterious world of microbiology! The book has four fabulous sections: What are Germs?, Diseases you never want to meet!, Fighting back! and The future… what happens next? While this book mainly deals with the horrors of microbiology, including Black Death, small pox, and the terror of biological weapons such as Anthrax, it also tells the story of Edward Jenner, which is a proven favourite with the pupils! The book not only tells the story of small pox eradication, but also gives the science behind vaccination, giving children a basic understanding of our immune system. The book then goes on to tell the story of Alexander Fleming,

Howard Florey and Ernst Chain and the race to make and mass produce penicillin during wartime. The book ends with a section on the future of antimicrobial medicines, introducing the hot topic of antibiotic resistance, and future possibilities such as phage medicine and wallaby milk. Gill is a fantastic writer, and has a knack of knowing exactly what the children will engage with, always managing to tell a great story while including sound science knowledge.

The project ran under the same format as last year's, starting with a CPD evening for teachers before Easter. The session introduced them to the project, allowing familiarization with our microbiology workshop, which would later be delivered to their class, and also provided them with an activity pack full of active learning ideas and a list of resources to support them further. Teachers were given a copy of the 'Germ Wars' book, under which the project is based, to allow them time to explore the book and plan out their lessons.

The kind funding from the Society allowed all 250 children to be given their own copy of the book to keep as a memento for life, and these were handed out after the Easter holidays. The classes were able to either dedicate their term to microbiology as a main topic or have it as an extra, and feedback this year shows that three of the classes devoted their term to the project!

In June, Dundee Science Centre staff visited each class and delivered our 'Befriending Bacteria' workshop which aimed to show children the good side of microbes, their importance for our everyday health and well-being and how we can look after our good bacteria through our diet, helping to build our immune system and stay healthy! Additionally, we used art to help children understand the differences in shapes and sizes of different microbes, and let them design their own to conclude the session.

Finally, classes visited Dundee Science Centre to meet Gill Arbuthnott. In this session she talked to the children about the book and told some more fun science stories before signing their books.

Full evaluative techniques, including Likert scales were used to evaluate this project. At the start of the CPD, teachers told us that they did not feel confident about teaching microbiology: when asked to rate their confidence on a Likert scale (in which 1 is lowest and 8 is highest), an average of 5.6 was cited, however, by the end of the project, average teacher confidence had increased to 7.3. This is fantastic, with comments that they would definitely approach microbiology in future and had learned lots of new knowledge and skills through the project.

At the end of the project pupils filled out a short feedback form, which provided a fantastic insight into the children's feelings about, not only the project, but microbiology as a whole. Pupils were asked to score the overall enjoyment of the project, with a final average score of 7.3 out of 8! A small number of pupils did not enjoy it, with just 2% of pupils scoring below 5. When asked if they would like to learn more about microbiology, 22% scored lower than 5, with an average score of 6 out of 8. These results highlight how well children can engage with this subject and that with increased training for teachers this subject can be taught very effectively in the primary classroom.

Pupils were also asked a range of questions such as favourite and worst parts of the project. 54% highlighted their visit to meet Gill as a favourite part, while 23% commented that they loved using the UV light box to learn more about epidemiology and how far a sneeze can travel. 60% of pupils said they enjoyed all of it and did not have a worst part, while the remainder either didn't like all the gruesome bits, or said the book was too short.

The project also had some major impacts and extensions that were unexpected, including a group of girls from one school taking Germ Wars to their Brownie pack. When Dundee Science Centre staff went out to the school they presented a big poster made by the pack about Millennium Development Goal 6, detailing all the information they'd found out about malaria. Some children had worked at home with their parents to research an area they were interested in, taking the learning outside the classroom and demonstrating the far reaching impacts this three-month immersive project can have.

Dundee Science Centre has immensely enjoyed the opportunity to engage 250 children with microbiology, and see the long-term impacts this project has on not only pupils understanding, but teacher's knowledge, skills and confidence to teach this subject throughout future years!

We hope you enjoyed the read, and will leave you with some amazing comments from both contributors and learners.

"I just wanted to say thank you for another terrific set of sessions in Dundee. It's obvious that the pupils have found the work you have done with them really inspiring, and I'm honoured to be part of such a great team! I'm already looking forward to next year..."

Gill Arbuthnott, author of '*Germ Wars*'

"…after the outreach visit from DSC I thought one of my pupils had been captured and replaced by a new boy! He was completely engaged and had enjoyed the session so much it had positively impacted on his behaviour which was normally not that great."

"Children learned how easily germs can spread, that there are good and bad bacteria, and science is fun!"

"I asked the children to vote on their favourite topic of the whole year, and the resounding favourite was Germ Wars!"

"I learned MILLIONS that my brain is actually tired, it's that full!"

"This was a great project, I learned all about small pox, bacteria and wallaby milk! I would definitely do it again!"

"I learned that there are thousands of bugs in your gut!"

"I learned about bacteria I never even knew about!"

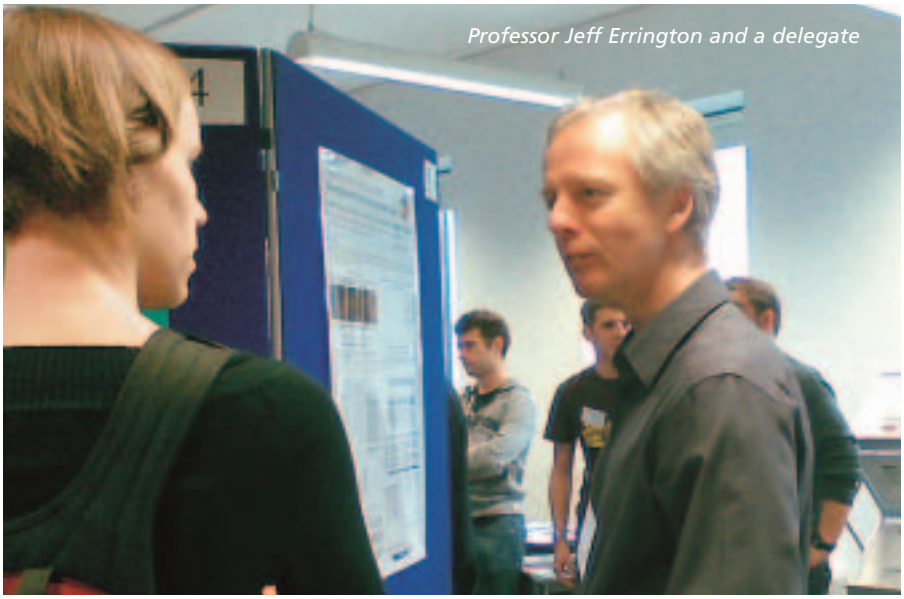
"I learned that you do get good germs!"

Katie Blackett
Dundee Science Centre

Sponsored Lecture Grant

UCC Hosts Young Microbiologist Mini-Symposium: Microbe Signalling, Organization and Pathogenesis – 2009

Picture by kind courtesy of Ms Phrueksa Lawongsa, BIOMERIT Research Centre, University College Cork



Professor Jeff Errington and a delegate

The University College Cork hosted the first Young Microbiologist Mini-Symposium on Microbe Signalling, Organization and Pathogenesis (21 to 22 April 2009). The Conference took place in Brookfield Health Sciences Complex UCC. This two-day international conference was intended to bring together graduate students, postdoctoral and young principal investigator researchers from leading laboratories around the world to discuss their current research. Over 110 scientists gathered for the conference where young microbiologists shared their research findings. This event was organized by Dr Robert Ryan (University College Cork) and Dr Sarah Coulthurst (University Of Dundee). The symposium was funded under the British Councils programme for International Networking for Young Scientists.

There were four seminar sessions, covering the following highly topical areas of prokaryotic molecular microbiology:

1) Gene regulation and intracellular signalling.
2) Structure, biogenesis and transport across membranes.
3) Microbe-microbe interactions.
4) Host-microbe interactions — pathogenesis and commensalism.

Each of these sessions was chaired by distinguished academics from around Europe but the main core of the talks were given by young researchers for leading laboratories around the world.

As part of this conference the Society for Applied Microbiology awarded funding to Professor Paul Williams from the University of Nottingham to speak on "Quorum sensing and *Pseudomonas aeruginosa*: a tale of regulatory networks and multifunctional signal molecules".

During his lecture Professor Williams described how bacteria employ sophisticated cell-to-cell communication or 'quorum sensing' (QS) systems for promoting collective behaviours that depend on the actions of one or more chemically distinct diffusible signal molecules. He went on to describe that many bacteria have multiple QS systems which are often integrated with each other and within global regulatory networks and subject to the prevailing environmental conditions as well as the presence and activities of other organisms. QS signal molecules, although largely considered as effectors of QS-dependent gene expression, are also emerging as multifunctional molecules that influence life, development and death in single and mixed microbial populations and impact significantly the outcome of host-pathogen interactions.

Professor Williams went on to mention that in pathogenic bacteria, QS represents an exciting target for novel antibacterials: the inhibition of QS in bacteria such as *Ps. aeruginosa* attenuates virulence and renders biofilms more susceptible to antimicrobial agents and host defences. The development of potent, safe QS inhibitors offers considerable scope in the battle against multi-antibiotic-resistant bacteria and chronic biofilm-centred infections.

The conference also had two other keynote lectures given by eminent scientists during the meeting. The EMBO lecture given by Professor Jeff Errington FRS (Newcastle University) was entitled "Life without a wall or a division machine in *B. subtilis*: implications for the origins of life" and the ASM Lecture given by Professor Tony Romeo (University of Florida) was entitled "The Csr system: A global regulatory circuit that governs bacterial biofilm development".

During the conference, senior delegates adjudicated on the short student talks and posters. Prizes for these were sponsored by the Journals - Biochemical Journal, Molecular Microbiology and Nature Reviews Microbiology. Javier López Garrido (University of Seville) a PhD student from Professor Josep Casadesús' laboratory was awarded the 1st Poster Prize: "Regulation of *Salmonella* pathogenicity island 1 by DNA adenine methylation: Evidence for posttranscriptional control by the RcsBCD signalling system". Natalia Tschowri (Freie Universität Berlin) a PhD student from Professor Regine Hengge's laboratory was awarded 1st Prize Young Scientist Award for her talk entitled: "The BLUF-EAL protein YcgF acts as a direct anti-repressor in a blue light response of *Escherichia coli*."

Robert Ryan
University College Cork

Students into Work Grant reports

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 below.

For further information visit: **http://www.sfam.org.uk/en/grants--awards/students-into-work-grant.cfm**

Identification of *Bacillus* spp. causing rope formation in wheaten bread

Prior to entering my final year of study for a BSc degree in Food Quality, Safety and Nutrition at Queen's University Belfast, I was given the opportunity to work in a Containment Level 2 Pathogen Laboratory within the Institute of Agri-Food and Land Use. My interest in food microbiology came about after completing a module in this area during my degree course, and as the opportunity arose to undertake a 10 week summer placement in food microbiology research I was very keen to get started.

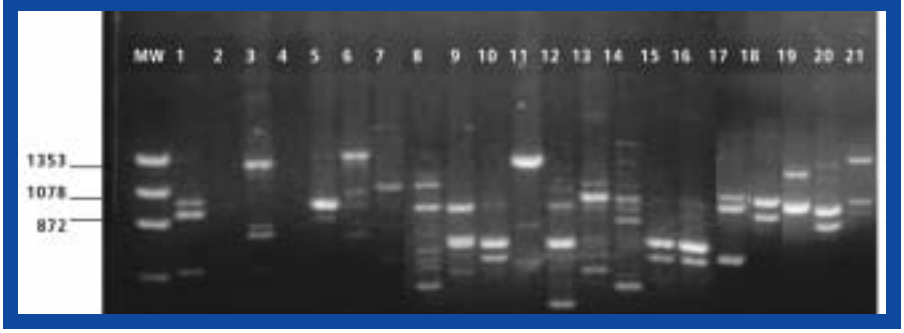
I had just completed a year-long industrial placement in a local bakery company and during my time there problems were occurring concerning rope in wheaten bread. Rope is a common quality issue in bread products and has been known about for many years. Rope is visible as clear, thin, thread-like strands and is caused by growth of *Bacillus* spp. It is accompanied by unpleasant odours, followed by enzymatic degradation of the crumb causing it to become soft and sticky due to the production of extracellular polysaccharides. Three different shaped wheaten loaves were tested to see if there was a difference between: the bacterial species present in each loaf, pH changes during shelf life and if their shape had an impact on the bake core temperature of the loaves, and hence on *Bacillus* spp. remaining viable in the wheaten bread. My main objective during the summer placement was to determine the species of *Bacillus* causing rope in the three different types of wheaten bread. The wheaten bread samples were tested on the day of production and then four days later to compare which *Bacillus* spp. were present immediately after baking and which dominated at the end of the products shelf life. Data generated during the summer placement project would complete my final year honours project when I started back at university.

A series of experiments were completed which gave me the experience of working with several new methods and technologies. Around 120 bacterial isolates were obtained from the wheaten breads sampled. These were initially grouped according to: colony size and appearance, Gram reaction, presence of spores, and oxidase, catalase and amylase test results. This resulted in 38 different bacterial 'types' and DNA was extracted from a representative of each of these types using a freeze-boil method and DNA concentration was measured using a biophotometer. A presumptive identification of *Bacillus* spp. was then confirmed using *Bacillus*-specific primers and PCR. The API® 50 CH system was used to identify some of the isolates on the basis of results of 50 biochemical tests studying carbohydrate metabolism.

Speciation of the *Bacillus* isolates was then attempted by RAPD, which is another type of PCR technique that amplifies random DNA sequences which result in a banded profile for each isolate. The isolates were also subjected to 16S PCR and denaturing gradient gel electrophoresis (16S PCR-DGGE) using primers EC1055+GC clamp and EC1392. The 16S PCR-DGGE method allowed the separation of PCR products on a 35 to 70% urea gradient gel which generally yielded a single band per isolate. UVP gel analysis software was used to help group the wheaten bread isolates with similar 16S PCR-DGGE or RAPD profiles, in comparison to profiles of reference *Bacillus* strains. Unfortunately, RAPD-PCR using primer OPA3 was of little help in identifying the majority of isolates. 16S PCR-DGGE resulted in fewer different profiles and greater correlation with reference *Bacillus* profiles. Instead of there being a dominant *Bacillus* spp. present when rope occurred in wheaten bread, between four and six different *Bacillus* spp. were present, including *B. licheniformis*, *B. pumilus* and *B. subtilis*.

Aside from working on the *Bacillus*

Figure 1. *RAPD-PCR profiles of Bacillus isolates obtained at the end of product shelf life from different shaped wheaten breads: lanes 1-7 rectangular, lanes 8-16 square and lanes 12-21 oval*



project, I was also given the chance to participate in other experiments carried out in the lab. This included working with MAP (*Mycobacterium avium* subsp. *paratuberculosis*) cultures. I gained an insight into methods such as phage amplification assay, phage titre, immunomagnetic separation (manual and the automated Bead Retriever method), and the ELISA technique. I enjoyed learning about these methods as I had never heard of them before. It was difficult at the start getting to grips with these procedures but with practice I have mastered these new skills.

This experience in a food microbiology research laboratory has given me the opportunity to work closely with PCR and other techniques that I would not have gained experience of during my degree course due to their complexity. This insight into food microbiology research has made me determined to pursue a career in this area and I hope to undertake a PhD after graduation next year. I am very grateful for having been given this opportunity to work in this area of research and would like thank S*f*AM for a Students into Work Grant. I would also like to express my appreciation to my supervisor, Dr Irene Grant, for her time in teaching me new skills and for all her help and support throughout.

Elaine Emo
Queen's University Belfast

Surveillance of human viruses by wastewater sampling

Following my honours year studying Infectious Diseases at The University of Edinburgh, I was provided with the opportunity to spend the summer researching the incidence of viruses in wastewater and I was delighted to have a S*f*AM Students into Work Grant to make this possible.

There is a long history of wastewater being used to screen for human pathogenic viruses, most especially for the monitoring of poliovirus. My project was designed to develop a methodology suitable for screening wastewater which would enable the identification and genetic characterization of a range of known and recently discovered enteric viruses that may be circulating within the community.

Four positive sense RNA viruses were chosen for screening, all of which are members of one of the major families of enteric viruses, the *Picornaviridae*. Human enteroviruses (HEVs) and human parechoviruses (HPeVs) are frequently associated with asymptomatic infection but can cause serious conditions such as aseptic meningitis, respiratory and enteric disease and, in former times before universal poliovirus immunization, poliomyelitis. We also screened for human cardiovirus (HuCV) and human cosavirus (HCoSV), both recently discovered viruses with largely unknown epidemiologies and infection frequencies in the UK or elsewhere.

The *Enterovirus* genus is comprised of four different human species (A-D) and has recently been expanded to include the rhinoviruses (A-C). Many well-known and well characterized viruses belong to this genus, for example polioviruses, coxsackieviruses and echoviruses.

Parechoviruses were originally classified as enteroviruses, but were re-classified after sequence analysis showed them to represent a distinct genus within the picornavirus family. There are currently 14 genotypes recognized through the sequence divergence they show in the capsid region of their genome. Type 1 was originally recognized through an enterovirus screening programme in the 1960s and has been found throughout the world. Other genotypes that are frequently detected in Western countries are types 3 and 6, the former associated with neonatal sepsis and severe CNS disease; nothing is known about the circulation and clinical impact of type 6 infections.

Identification of serotypes of HEV and HPeV is regularly undertaken in hospitals and permits epidemiological surveillance, monitoring of the emergence of more pathogenic variants and investigating infection sources. However, it is not known whether or not the genetic variants infecting individuals asymptomatically in the community correspond with those found in diagnostic samples from symptomatic patients.

The *Cardiovirus* genus consists of two species, encephalomyocarditis virus (EMCV) and theiloviruses. EMCV consists of a single serotype whereas there are four theiloviruses, including the recently discovered HuCV. The even more recently discovered cosaviruses are a highly heterogeneous group of viruses classified into a new genus within the picornaviruses, currently having five highly divergent species (A-E) recognized. It was originally identified in 2008 from the stools of Pakistani children with acute flaccid paralysis, discovered through a poliovirus screening programme, although its possible link with severe neurological or other disease has yet to be clearly established.

Samples were collected from the Veolia Wastewater Treatment works just outside Edinburgh city centre, which treats sewage from the Midlothian area along with run-off from roads. Sampling of solid waste was performed weekly for 10 consecutive weeks. In the lab, phosphate buffered saline (PBS) was added to the samples and vortex mixed. The liquid portion was filtered three times through progressively smaller pores to remove large particles and concentrate any viruses present. RNA was extracted from the filtrate, the RNA was reverse transcribed and the cDNA screened for the four viruses of interest using a sensitive nested amplification procedure with specific primers. Positive samples were sequenced, aligned with relevant sequences from Genbank using the Simmonics computer programme and phylogenetic trees were constructed. Twelve separate reverse transcriptions and PCRs were performed on each RNA sample. Not all of the PCRs were positive and analysis of the sequences indicated that a single sequence was present in each case, thus circumventing the need for cloning.

This methodology proved quite successful in detecting the viruses of interest with positive samples being found every week, most of which were sufficiently divergent from each other to indicate that they were from different infected individuals in the community.

HEVs were screened using primers which amplify the VP4 genome region of the viral capsid. This region can be used to assign isolates into different species and serotypes. Over the course of the study, 39 different positive samples were found and phylogenetic analysis showed they were comprised of species B and C enteroviruses. Amplicons were predominantly identified as species C (35/39) and clustered most closely with coxsackievirus A1 (26/35), A19 (6/35) and A22 (3/35). The three species B

isolates were most closely related to echoviruses 6 or 11. The VP4 primers also amplify rhinoviruses and a single rhinovirus species C sequence was identified.

The presence of HPeV was determined using primers which amplify the VP3/1 region, enabling genotype identification. In total, 35 HPeV sequences were found, all of which were from genotypes 1 or 6.

For HuCV a region of the conserved polymerase was amplified. This cannot be used to assign isolates into different genotypes as picornaviruses frequently undergo recombination in this genome region. However, analysis strongly suggested the sequences belonged predominantly to Saffold Virus (SAFV) type 1. In addition, two distinct clusters were identified, one of which is 5% divergent from SAFV-1 (5/25) and the other of which is 8% divergent from SAFV-2 (8/25). This level of divergence indicates that these sequences belong to a new polymerase clade and it is likely that they represent new genotypes of SAFV. In addition to HuCV, we identified three sequences closely related to another species of *Cardiovirus*, Thera virus, which has, to date, only been found in rats and mice. This highlights a potential drawback of this type of community surveillance in that viruses shed by rodents and other animals may also be detected in the tested samples.

A region of the 5' untranslated region was amplified for HCoSV and 36 sequences were found. The cosavirus sequences from the current study allowed the variants to be identified as species A (20/36) and D (16/36). However this genome region can only be used for species identification, not serotyping which requires sequences from the VP1 region to be amplified.

This summer project has enabled the development of a methodology for collecting wastewater and determining the types of picornaviruses within it, as well as increasing the number of sequences of each virus type. The high detection frequency of both cardioviruses and cosaviruses was unexpected and the first evidence for the extensive circulation of these newly discovered viruses in the UK. Finally, the surveillance data will be of considerable long-term value in providing the opportunity to compare and contrast variants causing disease with those that are present in the community. From a personal perspective, I have gained valuable experience within the lab which I will be able to draw upon as I begin a research Masters in Medical Microbiology studying broader aspects of public health in the same department. I wish to thank Dr Carol McWilliam Leitch for the opportunity to study wastewater, Professor Simmonds for the opportunity to work in the Viral Evolution Group and SfAM for a grant enabling me to have this opportunity.

Joseph Calvert
Viral Evolution Group, Centre of Infectious Diseases, The University of Edinburgh

President's Fund reports

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: **http://www.sfam.org.uk/en/grants--awards/presidents-fund.cfm**

The effect of soil pH on crenarchaeal communities

Archaea represent one of the three primary domains of life. Although many archaeal species resemble bacteria when viewed using a microscope, they are evolutionarily distinct and are actually more closely related to eukaryotes. Archaea were considered to be exclusively 'extremophiles', restricted to environments extreme in temperature, salinity or anoxia. However, since the early 1990s, molecular methodologies have revolutionized our understanding of these organisms and they are now recognized as a significant proportion of microbes in most, if not all, 'non-extreme' environments.

The archaeal domain has been traditionally divided into two kingdoms: the *Euryarchaeota* and the *Crenarchaeota* (although other kingdoms have been proposed such as the thermophilic *Korarchaeota*). Although once considered to be exclusively sulfur-dependent (hyper) thermophiles, a distinct but related lineage ('Group 1' Crenarchaea) is now recognized as one of the most abundant (if not the most abundant) kingdom of prokaryotes in the biosphere, where they may constitute around 6% of all microbial biomass in aquatic and terrestrial environments. Despite their ubiquity however, the ecological function of these organisms remained elusive until very recently.

A number of ground-breaking studies indicated that these organisms have the potential ability to oxidize ammonia (the first step in nitrification) and therefore may have a major role in the global nitrogen cycle. Metagenomic analyses in soil and the oceans found genes encoding putative ammonia monooxygenase subunit homologues within crenarchaeal genome fragments. Archaeal ammonia oxidation activity was subsequently confirmed by the characterization of an ammonia oxidizing crenarchaeon, *Nitrosopumilus maritimus*, isolated from a marine aquarium. This organism grows chemolithoautotrophically, using ammonia and inorganic carbon as the sole energy and carbon source, respectively. These characteristics indicate that these organisms are similar to ammonia-oxidizing bacteria such as

Nitrosomonas europaea. Intriguingly however, quantitative studies have shown that these 'ammonia-oxidizing archaea' are numerically dominant over their bacterial counterparts in most environments studied to date. It is therefore possible that archaea, and not bacteria as previously assumed, are the central players in ammonia oxidation in the environment.

In most soil systems, crenarchaeal communities are dominated by one specific evolutionary lineage, the ubiquitous Group 1.1b lineage. This is one of the evolutionary lineages that has been implicated as capable of ammonia oxidation. A number of molecular studies have indicated that this ubiquitous group is conspicuously absent from, or present in relatively low numbers, in some acidic soil environments, including coniferous forest soils. In these habitats, crenarchaeal communities can be dominated by organisms with 16S rRNA gene sequences placed within the related but distinct Group 1.1c. Unlike the Group 1.1b, the ecological role of this group is unknown with no evidence to suggest that it is involved in nitrogen cycling.

Previous studies have indicated that soil pH may be a major factor in determining microbial community composition and experiments have been performed to investigate whether the abundance and diversity of lineage 1.1c crenarchaea are also pH-dependent. To determine the impact of soil pH, I have been examining crenarchaeal communities across an established gradient ranging from pH 4.5 to 7.5. The abundance of the different crenarchaeal populations was assessed by quantitative PCR (qPCR) targeting the 16S rRNA gene, revealing that soil pH has no effect on the overall numbers of crenarchaea or bacteria. In contrast, the 16S rRNA gene abundance of crenarchaeal 1.1c organisms declined with increasing pH. These observations strongly support the hypothesis that pH is a major driver of 1.1c crenarchaeal abundance. The results are intriguing and raise a number of questions regarding the physiology and ecological function of these organisms. For example, the presence of the ammonia monooxygenase genes has not been verified in Group 1.1c crenarchaea. Also, as nitrification is typically restricted at low pH due to low availability of ammonia, it is possible that these organisms do not oxidize ammonia to generate energy, as this would require adaptation by lineage 1.1c to very low concentrations of ammonia. My current research is attempting to understand the ecological function of Group 1.1c crenarchaea, and whether they are involved in nitrification or other important ecological functions.

I am grateful to S*f*AM for awarding me this grant from the President's Fund, which allowed me to attend the 12th International Symposium of Microbial Ecology in Cairns, Australia in August 2008. This has enabled me to communicate my research findings to the science community as well as learn from other leading research groups in my field.

Laura Lehtovirta
University of Aberdeen, UK

Illustrating the importance of good hygiene practice

A report from the CDC indicates that little progress has been made in controlling foodborne illness in the USA since 2004 and that enhanced measures are required to educate consumers about infection risks and prevention measures. In an effort to better understand consumer kitchen sanitation and food hygiene practices, we compared consumer questionnaire responses with behaviours observed on video, in a descriptive study of 30 households. The results highlighted the differences between individuals' reported beliefs and actual practice. For example, whilst 100% of the participants reported that handwashing after handling ground beef is an important practice, 30% of them were not compliant in practice. Of those who did wash their hands, 40% washed for 10 seconds or less, although 70% of all participants reported 20 seconds as effective. When asked, 67% of participants thought it was important to use a meat thermometer but only one participant actually used a thermometer when cooking a hamburger. While 90% of participants were aware of the link between *E. coli* and ground beef, 70% had never heard of *Campylobacter*. When asked about risky behaviours, 90% attached little or no risk to preparing food at home, while at the same time 30% indicated that thawing frozen beef at room temperature for 12 hours was only moderately risky. We believe that information from this and other similar studies can inform consumer food safety education.

Another topic of great current concern is the emergence of MRSA. In a bacterial study of 32 hand and food contact surfaces in 35 healthy homes, we isolated MRSA from a variety of surfaces in 9 of these homes. We analysed for a number of factors that might serve as predictors of MRSA and a positive correlation was found between the isolation of MRSA from surfaces and the presence of cats in the home. This provides further evidence for the potential for infection transmission through inanimate surfaces in our homes.

In 2007, two of our students were fortunate enough to benefit from President's Fund student travel awards, allowing them to attend the annual S*f*AM conference in Cardiff and present their research posters on the bacterial load in pre-packaged, ready-to-eat spinach (Asjeric & Scott, 2007; Bruen & Scott, 2007). The following year, a new project we worked on was an assessment of different patterns of consumer use and the impact of the effectiveness of the low technology, slow-sand water filters that Simmons students are building and installing in a rural community in Nicaragua. To date (2008) we have constructed a slow-sand filter in our laboratory and we are in the early stages of priming it with local river water to encourage the formation of a biofilm layer of *Schmutzedecke*, which provides a biological treatment layer, supported by underlayers of sand and gravel. Well managed slow-sand filter systems are capable of reducing the bacterial contamination in water by 99%. We will be testing for faecal coliforms in the river water, the filtered river water and the filtered river water after storage under different patterns of usage. For example, we will compare the water quality when the filter is used daily as opposed to intermittent use. In addition, we are preparing a questionnaire survey tool to establish patterns of use of these filter systems in the community in Nicaragua. For this reason, I was very grateful to the S*f*AM President's Fund for giving me the opportunity to attend the 2008 annual conference in Belfast, on the topic of 'Water in Work, Rest and Play'.

Elizabeth Scott
Simmons College Center for Hygiene and Health in Home and Community

Physiological and functional diversity among ammonia oxidizing bacteria

Soil autotrophic ammonia oxidizing bacteria (AOB) are a monophyletic group within the β-subgroup of the division Proteobacteria whose main ecological function is the oxidation of ammonia to nitrite: an important, rate-limiting stage in the nitrogen cycle. Nitrogen is fundamental to all life on earth as a major constituent of protein and nucleic acid and is assimilated and excreted in different forms by different organisms. Successful crop production is dependent on the presence of sufficient bioavailable nitrogen in soil and the activities of soil microorganisms largely dictate this. Ammonia oxidizing crenarchaea also contribute to the oxidation of ammonia to nitrite, which is further oxidized by nitrite oxidizers to nitrate, a combined process referred to as nitrification. Excessive rates of nitrification to the more soluble nitrate form lead to leaching of nitrogen from soils and a decrease in agricultural productivity. A good understanding of the rate-limiting ammonia oxidation step is therefore an important requirement for agriculture, particularly in terms of nitrogen fertilizers addition and conversion.

During the last few decades advances in molecular technology and development of new techniques have revolutionized many aspects of the life sciences. Application of community profiling tools in microbial ecology has revealed huge diversities of previously unseen organisms responsible for important processes including nitrogen cycling (Morgan, 1991). Techniques include PCR amplification of community DNA biomarkers with subsequent resolution by DGGE or terminal restriction fragment length polymorphism analysis (T-RFLP). Studies utilizing such techniques have shown evidence of diverse AOB communities in soils exhibiting changes in structure in response to pH and other conditions (Stephen *et al.*, 1998). The recent emphasis in microbial ecology has been on molecular characterization and observation of natural communities but, despite the knowledge of high molecular diversities, it is difficult to resolve the physiological and functional diversity within a community. Without this knowledge it is difficult to predict how a particular community will respond to changes caused by land management regimes. AOB strains isolated in pure culture provide an opportunity to characterize this physiological diversity towards a better understanding of the ecological dynamics in soil communities and the significance of their diversity.

The majority of reports in the literature have characterized physiological aspects of particular strains. The aim of my work is to quantitatively characterize various ecologically relevant physiological traits of six soil AOB, two *Nitrosomonas* and four *Nitrosospira* strains, previously isolated to pure culture, and to assess their diversity. Physiological responses relevant to soil ecology include: ammonia oxidation rate at different ammonia concentrations, tolerance of high levels of ammonia and the ability to regain ammonia oxidation activity quickly after periods of starvation. Many enzyme-mediated processes follow the same type of saturation kinetics whereby the increase in reaction rate responds less as the substrate concentration increases until the rate approaches a theoretical maximum, at which point saturation has been reached. This has been modelled by Michaelis and Menten (1913) amongst others and can be applied to specific enzymes in whole cells whose substrates are acquired externally providing two useful parameters with ecological implications. The first is the Michaelis constant (K_M) corresponding to the substrate affinity of an enzyme or population and the second is the maximum reaction velocity (V_{MAX}) per enzyme or unit biomass. Under most nitrogen based fertilizer regimes the dominant members of the community would be expected to have a high V_{MAX} whilst fallow or pristine land may be dominated by organisms with low K_M, an adaptation to low ammonia concentrations.

An organism may gain certain adaptations in evolving to fill an ecological niche. However, in becoming well suited to one niche it may become less suited to others, because of physical or chemical constraints (Bohannan *et al.*, 2002). This concept could help explain how members of diverse communities, such as AOB communities, can co-exist. With respect to ammonia concentration, a hypothetical trade-off could be adaptation to either high or low ammonia concentrations because of physiological constraints. Two ecological strategies would result: low K_M and V_{MAX} equivalent to an 'autochthonous' lifestyle or a high K_M and V_{MAX} equivalent to a 'zymogenous' lifestyle. If certain groups, identified by molecular techniques to be present within a soil community, are associated with particular physiological traits, predictions about the overall community function and potential response to land use could be made. Such predictions could help adaptation of land use strategies for mitigation of soil nitrogen loss through nitrification. In a soil dominated by AOB with a high V_{MAX} and rapid recovery following starvation, a fertilizer regime in which large amounts of ammonia quickly enter the soil may be liable to loss by leaching through rapid nitrification. A slow nitrogen-release fertilizer may therefore be preferable.

In this study some significant differences were found between ammonia oxidation recovery potential following starvation and K_M and V_{MAX} values of the six AOB strains. Molecular community analyses have shown close relatives of these strains to be dominant under different conditions (Webster et al., 2005). These observations suggest the physiological traits characterized have ecological relevance.

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corporate news

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Both Corporate Members and Ordinary Members of the Society will find a wealth of useful information and resources in this section.

New primary collection tubes for automated urinalysis

Thermo Fisher Scientific has introduced new a range of Sterilin Primary Urine Tubes.

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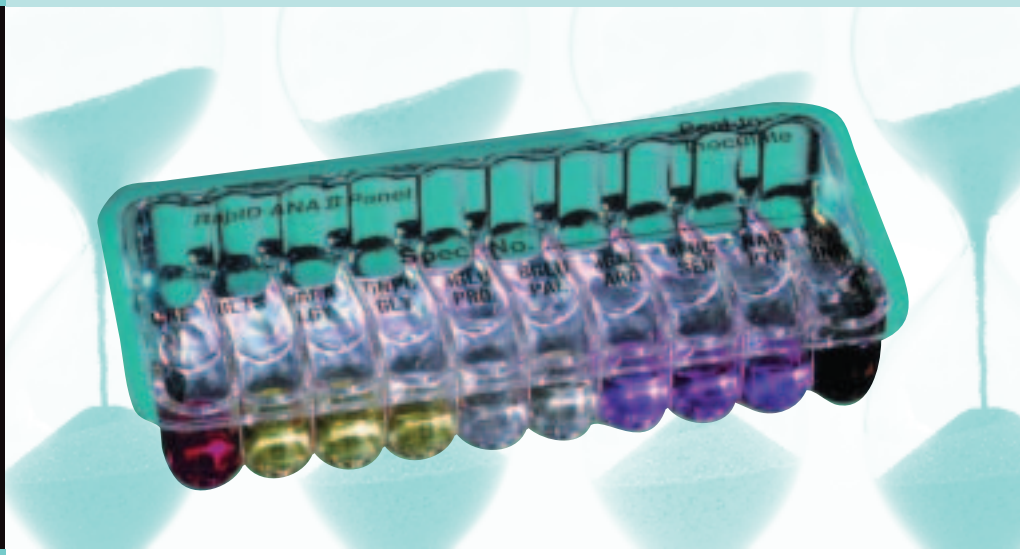

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