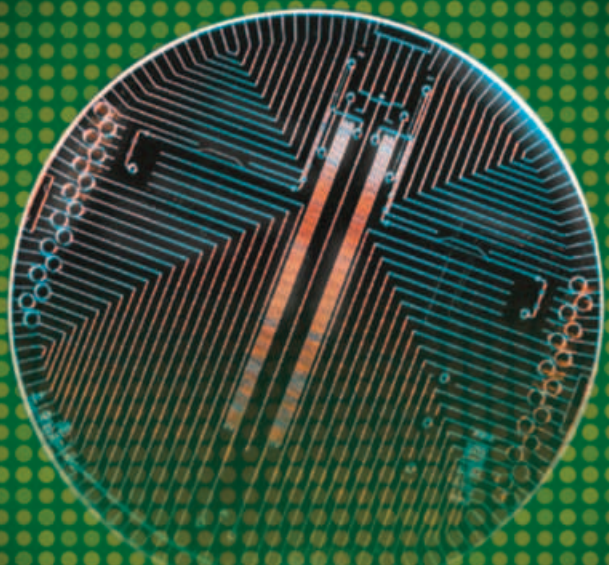
Microbiologist

The magazine of the Society for Applied Microbiology ■ September 2009 ■ Vol 10 No 3 ISSN 1479-2699



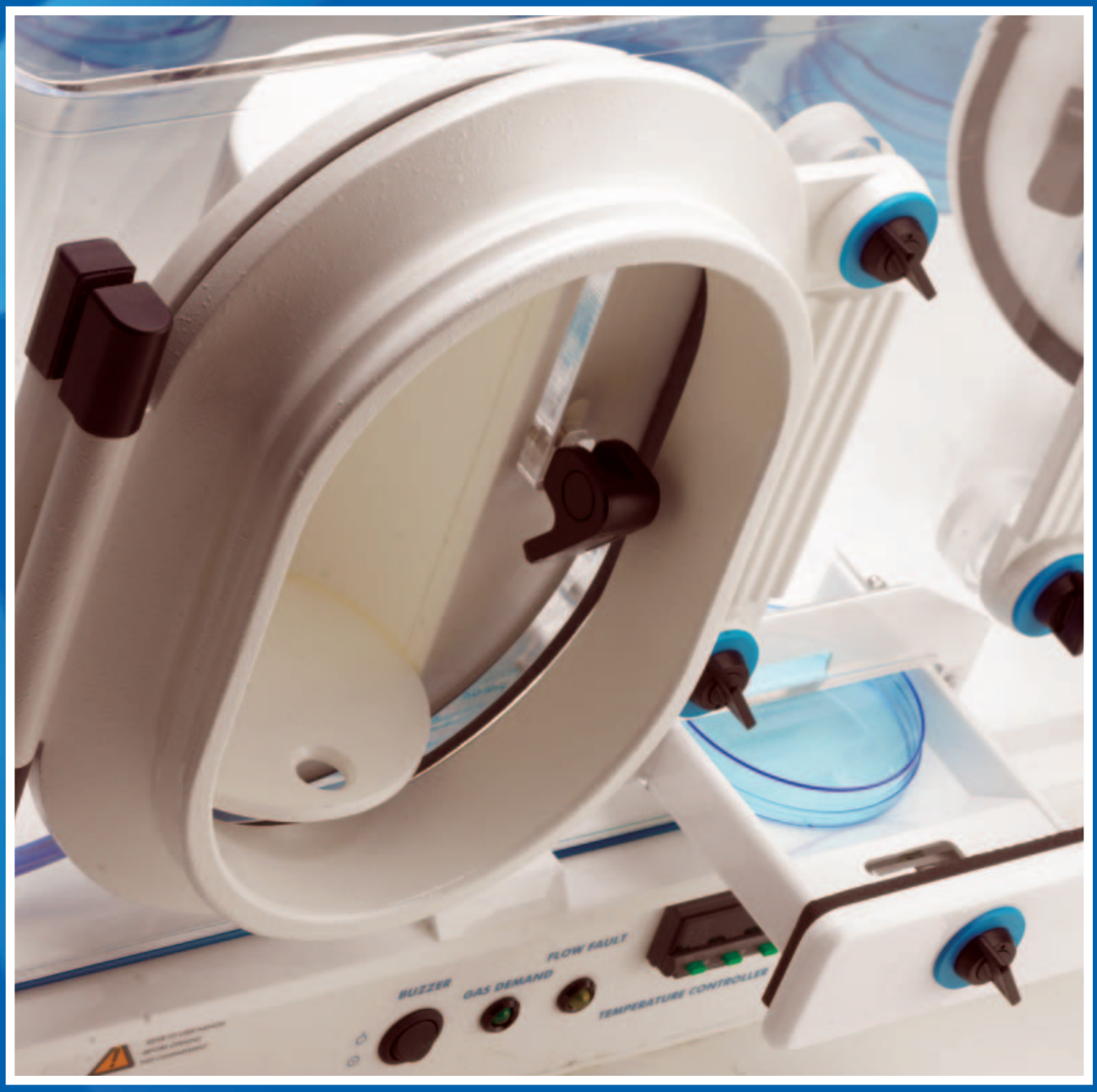
Lab-on-a-chip

Bacterial sociology in a biofilm world

INSIDE

excellence in microbiology

don whitley scientific



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Microbiologist the magazine of the Society for Applied Microbiology

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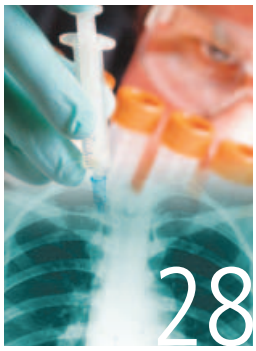
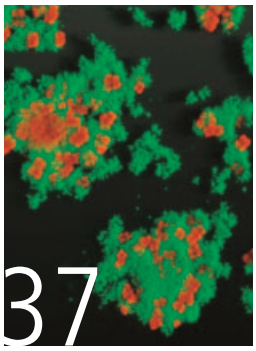


Winter meeting 2010



Bacterial sociology in a biofilm world

information

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editorial

Lucy Harper talks about communication and new technologies

In the last issue of *Microbiologist* I wrote about the reporting of Influenza A (H1N1) in the media and the importance of accurate communication of science. As we all know, the situation has developed somewhat since then with this outbreak reaching pandemic status on 11 June 2009 and treatment with antiviral drugs/prevention through vaccine development and availability being foremost on the minds of most people. I'm sure most of you will know somebody who has suffered from infection, though as I write this editorial I am happy to report that there has been a drop in the number of new cases of Influenza A(H1N1) in the UK*.

For this Editorial I will continue on the theme of communication — this time concentrating on communication via the internet. There has been much discussion regarding the success of the government pandemic flu website which was launched recently*. I know that on the day it was launched the website went 'down' for a time due to system overload. There has also been mention of people abusing the system in order to take illegitimate time off work — not something that is likely to be favoured at this time of economic uncertainty.

Some might say this is an illustration of the fragility of such online facilities as a means of information dissemination. However, my view is that online tools and new technologies are to be embraced. Online facilities — in particular interactive ones through Web 2.0 — enable instant communication across the globe and the implications of this are too many to mention in this Editorial. My enthusiasm for new technologies is probably evident from this issue of *Microbiologist* which has the theme: "New Technologies".

The feature articles for this issue are on a common theme — micro-arrays. A relatively new technology which, although not a brand new technique, has developed from a tool which measures gene expression, to one which is now being used to diagnose and inform treatment decisions (see page 30).

The second feature article remains on the subject of microarrays, but this time looking at one particular application of this adaptable technique (see page 34).

We discuss new technologies from the point of view of social networking with an article explaining how to join the new S*f*AM organisation page on the social networking website Facebook. This will enable users of Facebook to remain up to date with all S*f*AM activity and news (page 15). We also explain to those who like to tweet, how to follow "sfamtweets" — the new S*f*AM presence on the microblogging website, Twitter (page 14). Here we will be updating twitter users with real time news from S*f*AM as well as relevant news from across the twitter community.

Do you have particular views on new technologies, the internet, web 2.0 or social networking, or perhaps you have views on our reliance on the internet for global communication? Perhaps you use social networking sites regularly and don't know how you communicated without them. Perhaps you loathe even the mention of Facebook and you think Twitter is for twits. Either way, I'd be delighted to hear from you so do get in touch and let us know your views.

*Accurate at time of going to press.

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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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 digitised back files for *JAM* and *LAM* dating back to 1938
- A topical quarterly magazine, *Microbiologist*
- Substantially reduced rates for attendance at SfAM meetings and conferences
- Networking with worldwide professionals in over 80 countries
- Access to private members area of the SfAM website
- Monthly email bulletins with the latest news from SfAM
- Invitation to the annual *Environmental Microbiology* lecture
- Fostering cross disciplinary research
- A 25% discount on the extensive Wiley-Blackwell collection of titles

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

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

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

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

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

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

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

membership options

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

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

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

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

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

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

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

JOIN US!

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

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

www.sfam.org.uk

microbreak

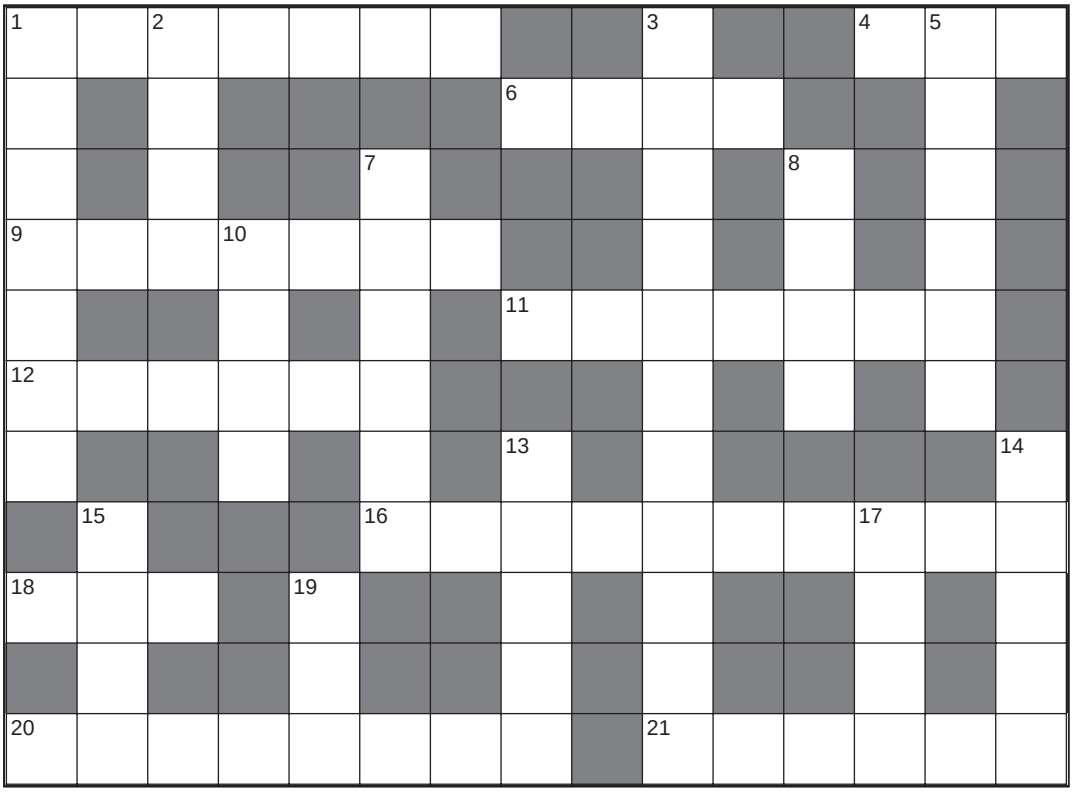
Test your microbiological knowledge by completing the crossword below and you could win an **Amazon voucher**. Get your winning entries to the Society Office by Friday 23 October 2009 to be in with a chance!

ACROSS

1. Former European Union right followed the Frenchman (7).
4. Ovoid favoured by Nagler (3).
6. Antibiotic carrier that could be compact (4).
9. Sounds like French head and tail end are suffering from clostridia (7).
11. Add up after negative response to erythema (7).
12. Enzyme sounds like you are simplicity (6).
16. Food poisoning culprit gives girl's name to fish (10).
18. Vigorous beta-lactamase (3)?
20. Quality mark to mend arrange endless time for antibiotic (8).
21. Child in front of a German reassortment produces enteric pathogen (6).

DOWN

1. Swarmer in favour of Latin you and us (7).
2. Some bugs alter media constituent (4).
3. False moans are confused in water-lover (11).
5. Garden character has point to reveal set of chromosomes (6).
7. Little Susan goes back to an upstairs room initially for *Staphylococcus* sp. (6).
8. Married lady acquires a superbug (4).
10. Right after the range is semi-solid medium (4).
13. Put silver back in beer to get bloomers (5).
14. I follow a carrier for *Lactobacillus* sp. (5).
15. Minimum inhibitory concentration with ecstasy provides testing model (4).
17. Carpet of growth from a rule in the North (4).
19. Quiet beast of burden yields virus (3).



Crossword compiled by Louise Hill-King

An Amazon voucher is waiting for the person whose entry is picked first from the Editor's in-tray! The closing date for entries is **Friday 23 October 2009**. The answers will appear in the December 2009 issue of *Microbiologist*.

Name: _____

Address: _____

_____

Simply photocopy this page and send it to: 'Microbreak Crossword', Society for Applied Microbiology, Bedford Heights, Brickhill Drive, Bedford MK41 7PH, UK.

president's column

Geoff Hanlon reviews the Society's long association with awards and prizes

Leaving our current financial problems aside it has been apparent for some time that the world is facing a number of pressing global issues including health, climate change, sustainable development and poverty. What has emerged more recently, however, is an understanding that the solutions to many of these issues are also global and do not lie solely with the developed nations. In attempting to address these problems, the participation of scientists and technologists throughout the world is now recognised as being pivotal. However, in many developing countries it is necessary first to build up local scientific capacity to enable them to address their specific local issues and ultimately to develop sustainable economies.

In 2000 the Heads of State from Governments across the world came together at the UN Millennium Development Summit and agreed to work together to achieve a more prosperous international community. From this summit a number of Millennium Development Goals were drawn up and led to multi-million pound capacity-building initiatives co-ordinated by among others the World Bank and in the UK by the Department for International Development (DFID).

There is no doubt that these large, centrally driven initiatives do have a major role to play in delivering the global development agenda. However, they are often very inflexible and can frequently miss their target. We should not lose sight of the fact that in many cases the most significant impact can be made by individuals carrying out small scale interactions on a personal level. This therefore begs the question as to whether societies like ours can embark on capacity building projects in developing countries on a smaller scale and make a real difference to individuals in those areas. Those of us who have been involved with learned societies for many years will say that our remit has always been to build capacity in our particular discipline and as we grow bigger we naturally extend this to an international scale. Learned societies such as S*f*AM are therefore ideally suited to contribute to this agenda since we are essentially clubs of scientists with extended networks and with strong links at grass roots level.

In early June this year Phil Wheat and I attended a meeting on "International Capacity Building by UK Learned Societies", held at the Royal Astronomical Society headquarters in London. The objectives of this meeting were to exchange experiences of capacity building programmes for developing countries and to explore whether there may be benefits in some co-ordinated action in this area.

Listening to contributions from various societies it became apparent that S*f*AM is already indirectly quite active in this area. For instance, we are an international society with members in over 80 different countries and unlike some societies we make no distinction between UK and overseas members. All have the same benefits and can apply for the same grants. A number of our grants are specifically directed towards developing countries such as the Endangered Culture Collection Fund and the Overseas Development Award. However, all grants including the President's Fund and the Students into Work grant are accessible to members from developing countries although applications from these regions are sparse. Under the auspices of Wiley-Blackwell, the Society's journals are available online for free (or at very low cost) to libraries in developing countries through philanthropic licensing deals such as the Health InterNetwork Access to Research Initiative (HINARI) and Access to Global Online Research in Agriculture (AGORA). I am also aware that many members undertake individual collaborative projects with fellow scientists from developing countries and that these ventures are highly successful.

Despite this, we left the meeting feeling that we, as a Society, could and should do more. A number of people including Gandhi and Churchill have been credited with saying that you measure the degree of civilisation of a society by how it treats its weakest members. There are a number of ways in which we may get involved in capacity building and below are just a few examples coming from the meeting, which I put forward without any suggestion of being prescriptive:

- Setting up collaborative research projects.
- Donation of equipment, journals or textbooks.
- Laboratory training in the UK for overseas young scientists.
- Lecturer/student exchange.
- Organising meetings/training workshops in developing countries.
- Assisting schools/universities with accreditation or curriculum design.
- Expert advice on local problems.
- Assistance with grant applications or paper writing.
- Help with forming "sister" learned societies.

I would like to set up an international sub-committee to explore ways in which S*f*AM might engage more in capacity building in developing countries. The success of these sorts of ventures

is dependent on the enthusiasm of individual members and I am conscious that any initiatives should be driven by the people on the ground in the developing areas and not formulated by those in the developed countries. The suggestion here is not to embark on a "grand plan" but simply that the Society might be able to assist members engaged in the process at grass roots level. One way in which the Society can do this is to make some limited funds available to facilitate these projects. At present we have a number of grants for which there is very little take-up. The grants sub-committee is currently undertaking a review of our grants and some of these could be re-structured to assist in the ventures outlined above. Such an international strategy can only enhance the Society's profile and reputation whilst at the same time meeting part of our objectives as a charitable organisation.

I would be very keen to hear your views on this subject particularly if you are a member from a developing country. Is it the sort of thing we should be doing? What level of activity is already being undertaken by individual members? Have you been involved in this sort of activity in the past — what were your experiences? Do you think S*f*AM would be able to assist you in what you are doing? What ideas do you have for potential capacity building projects? Please feel free to email me at g.w.hanlon@brighton.ac.uk or contact the S*f*AM office and we will pass on your views.

Professor Geoff Hanlon
President of the Society

ceo's column

Philip Wheat reports on the latest developments within the Society

Firstly I would like to thank all members who took the time to complete the S*f*AM membership questionnaire which was recently circulated. We have been overwhelmed by the response, with nearly 40% of members taking the trouble to complete the questionnaire. We are currently collating and analysing the data and once we have, I am sure it will prove extremely useful in helping to enhance the provision of services we offer to our membership.

In this issue of *Microbiologist* you will find the report on another successful Spring meeting. As with previous Spring meetings, the average scores from delegate evaluation feedback forms indicated that most aspects of the event were scored very highly. The one aspect which was poorly scored was the quality, quantity and provision of the lunch. At the event I also found this aspect of the meeting totally unacceptable. Our views were strongly expressed to representatives of the catering provision during the meeting and we have subsequently expressed our displeasure to the facility providers. I would like to make a personal apology to all delegates who attended the Spring meeting for the poor quality of the lunch which was provided by the caterer. In particular it is disappointing that the quality had significantly deteriorated since the 2008 meeting held at the same venue. I can give an assurance that we will not be using the venue for any future society-organised meetings. Also, I can confirm that we have already identified another venue at which to hold the 2010 spring meeting and full details will be announced shortly.

I have been fortunate enough to attend two meetings (ASM and IFT) in the US this year. I greatly enjoyed meeting new and existing members. You may also have met the Editor, Lucy Harper who assisted with exhibition at a third US conference (IAFP). Record numbers were enrolled at all three meetings. September and October are also busy months for exhibitions and the Society will be exhibiting at the following: Veterinary Laboratories Agency Annual Meeting, Royal Holloway, University of London, 2-4 September; European Federation of Biotechnology, Barcelona 14-16 September; Institute of Biomedical Science Meeting, Birmingham 28-30 September, and finally, the International Association of Food Protection European meeting, Berlin 7-9 October. If you are attending any of these meetings please do call by the Society stand.

Philp Wheat
Chief Executive Officer

Don Whitley Awarded Honorary D.Sc

On a beautiful summer's day on 15 July 2009, the Chancellor of the University of Bradford, Imran Khan of cricketing fame, conferred the Honorary Degree of Doctor of Science on **Don Whitley**. It was a colourful occasion in which the academic staff, fully robed and to suitable organ music, processed into the hall in which many undergraduates were already assembled to be admitted to their degrees.

Don was presented to the Chancellor by Professor Michael Gardner, who gave a full account of Don's great achievements. He described his early interest in medical science and the efforts that eventually saw him become a technician at The Women's Hospital in Leeds.

Soon the world of commerce beckoned and he joined Oxoid of which he became Sales Director. Later he founded a company to supply materials to laboratories and, after buying out his business partner in 1976, he founded Don Whitley Scientific Limited. Three generations of the family have been involved in the business. As the company developed and expanded Don became Chair of the University's Advisory Board in Biomedical Sciences and sponsored students at the University of Bradford in their industrial placements.

Don was also responsible for many benefactions, including several to this Society. Professor Gardner described how Don fostered a productive interaction between industry and academia. Paul Walton, Don's eldest son, MD of Don Whitley Scientific, commented, "*There can be few microbiologists in the world who have not used something in which Don has not had a hand.*"

Max Sussman

membership matters

S*f*AM ex-committee member recognised in Queen's Birthday Honours

S*f*AM ex-committee member, **Professor Diane Newell** has been awarded an OBE at the Queen's Birthday Honours for services to science. Whilst serving on Main Committee, Professor Newell was instrumental in developing and implementing Med-Vet-Net, the EU Framework Programme 6 project investigating zoonotic disease across Europe. Professor Newell was the Project Manager of Med-Vet-Net from 2004 until 2008 and S*f*AM as one of the partner institutes of Med-Vet-Net, would like to extend congratulations to her for this achievement.

S*f*AM **Photo Competition**

Have you taken an outstanding photograph of your beloved bugs? Do you know someone who has and you'd like to see their work in print? Perhaps you've taken a photograph while attending a S*f*AM conference which you think is worthy of reproduction?

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.

Brazil

M. Hanna Vance-Harrop

Canada

E. Beckett; M. Skulnick; S. Zenteno

China

C. Wu

Greece

N. Soultos

India

S. Bhal; R. Gaur; S. Goal; D. Gupta; D. Kaushik; S. Ramachandran; G.P. Rao

Iran

S. Shekarforoush

Ireland

S. Broderick; S. Horgan; N. O'leary; P. Rojas

Japan

N. Murayama

Korea

K. Seo

Mexico

M. Marquez-Gonzalez

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P. Chimwamurombe

New Zealand

T. Blackmore

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USA

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West Indies

S. Singh

Due to popular demand, *Sf*AM are running the photography competition again this year. We are looking for twelve eye-catching images to use for our 2010 calendar which we will be giving to all our members as a Christmas gift.

To enter this competition, please send your photographs to the Editor in the form of JPEG files which must be a minimum size of 7 x 7cm at 300dpi (800 x 800 pixels). Alternatively, you can send the original photographs in hard copy to the Society Office and we will return them to you once they have been scanned.

Photographs will appear in one of two categories:

1. Scientific — e.g., a colourful image using bacteria
2. Non-scientific but with a *Sf*AM theme e.g., taken at a *Sf*AM event

The closing date for entries for this competition is Friday 9 October 2009

OBITUARY

Dr R M Keddie

(3 February 1928 - 24 January 2009)

Dr Ronald Keddie — Ron to fellow microbiologists — was a member of the Society for all his professional life. He was born in the coal-mining community of Cardenden, Fife, Scotland, educated at Beath High School and at the Department of Agriculture, University of Edinburgh and the East of Scotland College of Agriculture – the Department and College were constituent parts of the school of Agriculture. He specialised in agricultural bacteriology and did a final year project on *Kurthia zopfii* under the supervision of Dr. T. Gibson of the College. Ron was awarded the BSc. Degree with First Class Honours in 1949 — the University records note that he completed the practical in agriculture.

On graduation, Ron joined the staff of the College and became a member of a large team funded by the Agricultural Research Council that was investigating many aspects of silage production. His task was to identify the species of *Lactobacillus* that fermented plant sugars during the initial phase of the ensilaging process. First he had to devise a selective medium to enumerate organisms on grass and silage. Following the promptings of his PhD supervisor, Dr. Gibson, he established the critical concentration of acetate buffer and the relevant pH that allowed the growth of *Lactobacillus* sp. but not that of other organisms, particularly enterococci. He found that the growth of lactobacilli was enhanced by the addition of Tween 80 and manganese to an already rich basal medium. Details of this medium were published (*Proc. Soc. Appl. Bact.*) in 1951. In that year also M. Rogosa *et al* gave details of an almost identical medium in *The Journal of Bacteriology* a derivative of which is still in

Welcome to new member of the SfAM team

common use. Ron was awarded a PhD degree in 1954.

In 1956 Ron was appointed to a lectureship in the Department of Microbiology at Reading University. This department, the first of its kind at a British university, had developed from a small unit that taught bacteriology to students in the Faculty of Agriculture. Ron was promoted to Senior Lecturer in 1964 and retired in 1985.

Ron was highly involved in the development of the BSc. degree course in microbiology and his serious and demanding approach to teaching drew respect from colleagues and students alike. He also had a lighter side, well exemplified by his utter disbelief and long-lasting amusement in retelling an occasion when a student made a streak plate from a culture of a *Lactobacillus* sp. on the inside of the Petri dish lid yet achieved reasonably acceptable single colonies!

At Reading, his research interest was centred on little known genera — *Caryophanon* and *Kurthia* (he defined a new species, *K. Gibsonii*) and particularly the poorly characterised coryneform bacteria. He linked newer methods for the characterisation of micro-organisms — *viz* cell wall analysis and numerical taxonomy — with traditional ones such as critical studies of nutritional requirements and age-induced changes in cell morphology. The latter drew on his skills in microscopy and photomicrography. Whatever method was adopted, whether ancient or modern, its application was rigorously controlled. Although not inclined to rush into print, the stature of his achievements, often in collaboration with his graduate students, can be gauged by reflecting on the prestige of the publications to which he was invited to contribute.

Ron authored or co-authored the definitive descriptions of five bacterial genera in the prestigious, internationally recognised publications — *Bergy's Manual of Systematic Bacteriology* and *The Prokaryotes*. He also co-authored the description of a new coryneform genus, *Aureobacterium*. More recently, a coryneform-like bacterium isolated from veterinary material, was named *Sanguibacterium keddieii* in his honour.

Apart from teaching and research, Ron spent much of his time overseeing the technical services of the Department at Reading and in the detailed planning of its expansion at the London Road campus and eventual transfer to Whiteknights.

On retirement, Ron and his wife, Isobel, returned to Scotland – and by this time their two daughters, Anne and Lynn were pursuing demanding careers in England. There they planned in detail and supervised with a critical eye the building of their new house at Fortrose on the Black Isle. On completion, they spent many happy years creating a beautiful home and garden.

In the eulogy given at Ron's funeral service on 30 January, 2009 the orator, a long standing friend and former Professor of Biochemistry, noted that his old friend "had a continuous, unwavering quest for meticulous accuracy in all aspects of his life". Those who knew Ron as a friend or colleague are unlikely to challenge this opinion.

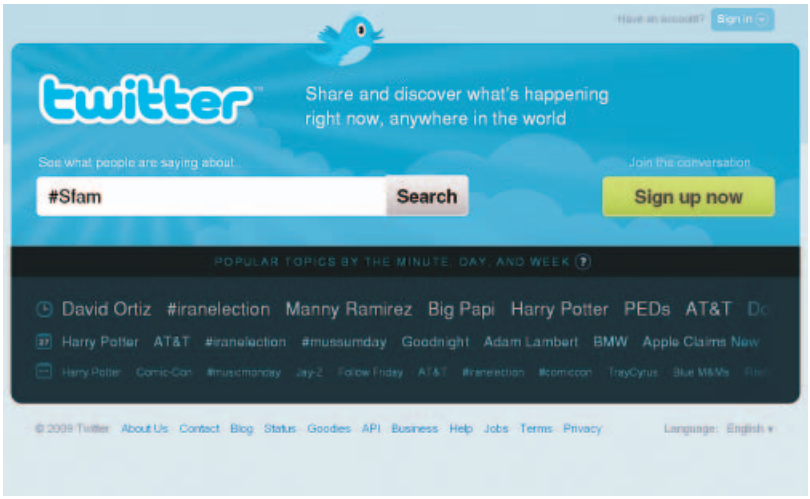
Ron G. Board, John Grainger and **Dorothy Jones**

At the beginning of June, our new Communications Officer, **Clare Doggett** joined the S*f*AM team. Clare will be working on all aspects of communications for the Society including assisting Lucy Harper with compilation and editing of the *Microbiologist*. Here she introduces herself to those of you who didn't get the chance to meet her at the recent summer conference.

I came to S*f*AM from the Royal Society of Chemistry where I had been working as a graduate trainee in communications and member services. My training with the RSC was varied and I gained a lot of experience in different areas from writing for the members' magazine through to helping to organise a conference overseas as part of the International Development team. My background is in Natural Sciences which I studied at the University of Reading. Through my time at university I studied modules in science communication as well as microbiology and virology. Even though I've only been with S*f*AM for a relatively short time, I am already thoroughly enjoying my time working at such a friendly organisation, and it has been a real delight to meet so many interesting people at the summer conference. I am very much looking forward to learning more about the Society and meeting many more members.

Clare Doggett
Communications Officer

Tweeting applied microbiology



*Sf*AM now has a presence on the popular social networking website, **Twitter**. This is a website which allows users to send short messages with a summary of recent events in real time. It is described as a micro-blogging website where people provide a short description (maximum 140 characters) of what they're doing as they're doing it — but this is just one aspect of the site. Twitter is also used by many organisations to direct people to relevant websites or their own websites, or to update people with the latest news or goings-on at their events.

For those of you who are not familiar with Twitter, this is a quick guide to signing up, creating a profile and following all those people or organisations you'd like to learn more about.

Firstly, I'll explain what Twitter is: it's a website within which you can create a profile and 'follow' anybody else with a profile. This profile is relatively simple and consists of your name, your location, a photograph (if you'd like to add one — this isn't compulsory) and a short biographical description which can be 160 characters long at most. These descriptions tend to be a brief explanation of who you are, where you're working (if applicable) and a link to a URL of your own website or that of your organisation. To create a profile, simply visit http://twitter.com, click on 'sign up now' and follow the on-screen instructions.

Once you've created a profile, you can start to follow the organisations and people of your choice. To find people, use the 'find people' link which allows you to search for others with a Twitter account by name. A good tip here is to follow somebody who has similar interests to you and see who they're following. You may find out that your favourite organisation is on Twitter and you can start to follow them. They, in turn, may be following other relevant people or organisations.

Once you have found a number of people you would like to follow, then you'll start to generate 'followers'. These are people who have searched for you, or noticed that you're following them, or found you indirectly and have decided they'd like to follow you. But don't worry if you find that you're being followed by someone who you don't wish to follow you, you can block them from your profile which means that they won't be able to follow you any more and won't receive any of your tweets.

Now you're ready to start tweeting. A tweet is the name for an update on Twitter and is limited to 140 characters of information about what you're doing, where you're going, the topic of a relevant blog post, a meeting you're attending or anything you'd like to exclaim to the Twitter community.

You can also search Twitter for relevant keywords. If you enter a word or search term into the 'search' box on your Twitter homepage, then all the tweets containing that word will appear. From this you can find others who are on Twitter with similar interests to your own and create a Twitter community based around that topic.

If you're organising an event, it's a good idea to use a 'hashtag' when referring to that event. So, you tell everyone attending the event that you're going to be 'tweeting' about it, and you give the event a short name which is preceded by a hash symbol. For example, *Sf*AM's summer conference this year was tweeted as: #sfamsc. During the event, if you had searched for #sfamsc you would have found all the tweets about the summer conference, no matter who they were from (tweets containing the hash tag can be from anyone, they don't have to be from the conference organisers). This is a good measure of the level of Twitter activity associated with your event as, if enough people are tweeting about it, your events hashtag will appear in the list of 'trending topics' on the right hand side of your homepage.

Finally, you can add links to a tweet, which will direct interested followers to relevant websites, blogs or any other online information. This is simple and merely involves pasting the URL into a tweet. You may wish to limit the number of character spaces taken up by the URL, in which case you can shorten it using one of the many online tools that are available to do this, such as tiny URL (http://www.tiny.cc/).

So, now you're ready to start using Twitter, you can follow *Sf*AM by signing up and following 'sfamtweets' — we look forward to seeing you there.

Lucy Harper
Communications Manager

Facebook fans

For those of you familiar with the social networking site, Facebook (see *Microbiologist*, vol. 8 No.4, December 2007, p4), you may be interested to hear that S*f*AM is expanding our Facebook presence. Read on to find out more

The S*f*AM Facebook presence has evolved. Group pages on Facebook are being upgraded to organisation pages by many organisations, S*f*AM included. These organisation pages are similar to personal profiles and allow a much greater level of interaction than the traditional group page. Rather than group members an organisation page has 'fans', similar to having friends on a personal profile page. Fans can post on the wall, add pictures to albums, comment on pictures and status updates and tag themselves (associate their name with a picture). Also when the page or status is updated it will appear in every fan's regular news feed keeping them up-to-date with any developments as they happen.

Anyone who has a Facebook account can become a fan of S*f*AM: just by searching for 'S*f*AM' you can find the Society's page and then all you have to do is click on the 'become a fan' button at the top. We would like to encourage people to interact with the page by posting on the wall, tagging photos and even adding photos or videos. You can also start a discussion and share your thoughts and opinions with fellow 'fans.' The page will regularly be updated with events and meetings together with links to webpages with more information.

Whilst the Society for Applied Microbiology group does still exist, we hope to gradually phase it out and eventually replace it with the S*f*AM fans page. We would, therefore, encourage all the current S*f*AM facebook group members to become fans instead so S*f*AM can continue to stay in touch and keep you updated.

Clare Doggett
Communications Officer

Book review

Infectious Disease: Pathogenesis, Prevention, and Case Studies

By N. Shetty, J. W. Tang and J. Andrews
Chichester: Wiley-Blackwell 2009. pp 674 + xiii
ISBN 978-1-4051-3543-6
Reviewed by Max Sussman

Encouraged by lurid newspaper coverage, the current significance of infectious, or rather communicable, diseases is now in the minds and fears of the general public. This textbook, directed at intermediate to senior university students is likely to attract a large readership on account of its large format and generous presentation. Though a wide range of students may find this book of value, it is in reality really addressed primarily to medical students.

The subject matter is presented in five parts, beginning with general principles and the approach is anatomical systems-based. The third part deals with infections in special groups, such as those related to pregnancy and immunocompromised hosts. The difficulties presented by this kind of arrangement come into sharp focus in Chapter 15, which is entitled, *"The fever and rash conundrum, rashes of childhood"*. It is not made clear to which of the many possible conundrums the authors are referring.

The global significance of communicable diseases is brought into sharp focus in part four. This considers tuberculosis, malaria, HIV/AIDS, viral hepatitis, influenza and the fascinating puzzle of infection in the returning traveller.

Finally, part five considers emerging and resurgent infections. While three of the chapters — all dealing with viral causes — are not unexpected, the chapter on diphtheria is something of a surprise in this part of the text.

The writing is mainly in prose paragraphs that do homage to the style of short notes; in this mode, 'good writing' is the victim. An excellent counterbalance are the boxes detailing case histories, which bring the science into a realistic focus.

It is made clear by the authors that they intend the illustrations to be central to their project. It is, therefore, an immense pity that they are often so poor. Thus, while Figure 1.3 is excellent, Figure 1.4 is very poor and similar criticism can be made of many photomicrographs. Clinical illustrations always present special problems of colour balance, because they determine the expectations of the inexperienced clinical student observer. What is the point of reproducing Figures 7.17(c) and 15.2(a) in which the overall purplish tinge obscures the characteristic measles rash, which is much better seen in Figure 15.2(b)? Otherwise the illustrations, particularly the line drawings, are very good.

This is a good book that students will appreciate and use. The criticisms are made to encourage the printer and publisher — with whom the main onus lies — to do better in a second edition.

journal**Watch**

News about the Society's journals

Journal of Applied Microbiology

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

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

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

Jofre, J. (2009) Is the replication of somatic coliphages in water environments significant? **Vol. 106**, No. 4, April 2009

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

Plumed-Ferrer, C., and von Wright, A. (2009). Fermented pig liquid feed: nutritional, safety and regulatory aspects. **Vol. 106**, No. 2, February 2009

Letters in Applied Microbiology

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

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

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

Martinez, R.C.R., Franceschini, S.A., Patta, M.C., Quintana, S.M., Candido, R.C., Ferreira, J.C., De Martinis, E.C.P., and Reid, G. (2009). Improved treatment of vulvovaginal candidiasis with fluconazole plus probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14. **Vol. 48**, No. 3, March 2009

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

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

Environmental Microbiology

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

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

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

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

Ochman, Howard (2009) Radical views of the Tree of Life. **Vol. 11**, No. 4, April 2009

Hugenholtz, Philip, and Kyrpides, Nikos C. (2009). A changing of the guard. **Vol. 11**, No. 3, March 2009

Microbial Biotechnology

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

Ueda, Akihiro, Attila, Can, Whiteley, Marvin, and Wood, Thomas K. (2009). Uracil influences quorum sensing and

biofilm formation in *Pseudomonas aeruginosa* and fluorouracil is an antagonist. **Vol. 2**, No. 1, January 2009

Daniels, Craig, and Ramos, Juan-Luis (2009). A broad range of themes in microbial biotechnology. **Vol. 2**, No. 1, January 2009

Wackett, Lawrence P. (2009). Microbial biotechnology for producing high volume chemicals. **Vol. 2**, No. 1, January 2009

Brautaset, Trygve, Lale, Rahmi, and Valla, Svein. (2009). Positively regulated bacterial expression systems **Vol. 2**, No. 1, January 2009

Editorial. The Editors, **Vol. 2**, No. 1, January 2009

Environmental Microbiology Reports

Highlights of the third issue of Environmental Microbiology Reports

Krell, Tino, Busch, Andreas, Lacal, Jesús, Silva-Jiménez, Hortencia, and Ramos, Juan-Luis (2009). The enigma of cytosolic two-component systems: a hypothesis. **Vol. 1**, No. 3, June 2009

Pignatelli, Miguel, Moya, Andrés, and Tamames, Javier (2009). EnvDB, a database for describing the environmental distribution of prokaryotic taxa. **Vol. 1**, No. 3, June 2009

Hoton, Florence M., Fornelos, Nadine, N'Guessan, Elise, Hu, Xiaomin, Swiecicka, Izabela, Dierick, Katelijne, Jääskeläinen, Elina, Salkinoja-Salonen, Mirja, and Mahillon, Jacques (2009). Family portrait of *Bacillus cereus* and *Bacillus weihenstephanensis* cereulide-producing strains. **Vol. 1**, No. 3, June 2009

Sam Holford
Wiley-Blackwell

BIOSCIENCES FEDERATION

bio **focus**

Richard Dyer discusses the progress towards integration of the IOB and BSF

The Biosciences Federation is a single authority representing the UK's biological expertise, providing independent opinion to inform public policy and promoting the advancement of the biosciences.
For further information visit:
http://www.bsf.ac.uk/default.htm

There is excellent progress towards the integration of the IoB and BSF. The formation of the Society of Biology has been approved by Privy Council, and the Society will take on the integrated roles of the IoB and BSF from 1 October 2009. Before this date the assets of the BSF will be transferred, and shortly afterwards the BSF will cease any active existence. All members of the BSF and IoB will be members of the Society of Biology.

This important realignment, that I am confident will benefit biology enormously, has only occurred because of the strength and influence that the BSF acquired in recent years. This position, of course, derived entirely from the strength of the Member Organisations and these Member Organisations will have a central role to play in building the Society of Biology.

The President, Honorary Treasurer and Honorary Secretary positions are appointments of Council: Prof Dame Nancy Rothwell will be the first President, Dr William Marshall has been appointed as the Honorary Treasurer and Prof David Coates as Honorary Secretary.

The current Interim Council (IC) will form the basis of the new Council, with a managed transition of the current members to the new profile of elected and appointed members from the two Colleges (Organisational and Individual). Council members will be elected by the Society of Biology membership.

The CEO of the Society of Biology has been appointed and should be announced very soon. Competition for the post was very strong: all interviewed were excited by the vision that has to be delivered.

Personally, my work with the BSF has now come to an end. I leave, at the end of this week (31 July 2009), with some sadness because the BSF has introduced me to many new organisations and interesting people - which I have very much enjoyed. For me it has been an enjoyable and stimulating forty three months. Dr Emma Southern is now Chief Operations Officer (COO) for the BSF. Many of you will have discovered already that she is more efficient than I am: our organisation will be in excellent hands for the next couple of months.

It is important that you, our members, contribute your views, ideas and questions about the Society of Biology. You can find out more at the temporary website: www.newbio.info. Please send any comments and thoughts by email or by adding to the blog.

Richard Dyer
Chief Executive
Biosciences Federation

The voice of applied microbiology

media**watch**

microbiology and the media

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

SfAM continues to support the Sense About Science "Standing up for science" media workshops. A chance for young scientists to find out how to promote their research through the media and to understand that journalists want to report good science as much as scientists want them to. Here, one of our delegates, **Danielle McEwan** tells us about this eye-opening experience

When I was given the opportunity by the Society for Applied Microbiology (SfAM) to attend the "Standing up for science" media workshop at The British Institute of Radiology in London, I was extremely excited. The workshop was aimed at early career scientists, PhD students and postgraduates. As a recent graduate, I did not know much about science in the media and this was the perfect opportunity for me to learn more.

The workshop was organised by *Sense About Science*; a charitable trust founded in 2002 to promote good science and evidence for the public. *Sense About Science* responds to the misinterpretations of science on important issues and has established the *Voice of Young Science* (VoYS) programme. This programme helps early career researchers who want to stand up for science in the public, finding evidence behind product claims and correcting misinformation in the media. These are important issues as bad science in the public domain affects the reputation of all scientists and can undermine scientists' work. Products should have appropriate evidence to support manufacturer's claims and VoYS encourage people to challenge these claims, if necessary, to ensure the correct message is being communicated to the public.

The day began with registration and the chance to introduce ourselves to other scientists. The first part of the workshop took place shortly

meets the Voice of Young Science

afterwards where the changing image and role of science and scientists in the public domain were discussed. There were three panellists, Dr Robin Lovell-Badge, Head of the Division of Developmental Genetics at the Medical Research Council's National Institute for Medical Research; Catherine Collins, Chief Dietitian at St. George's Hospital and Professor Mark Enright, Professor of Molecular Epidemiology in the Division of Epidemiology, Imperial College London, who all spoke about their good and bad experiences with the media. I found it useful to learn that when talking to the media we should beware of flippant remarks that the journalist or reporter may manipulate to sound different from the way it was intended. It is useful to request a copy of the transcript that the reporter has produced (although this is often not feasible), and important that we talk with confidence — we are the experts after all. There was plenty of time to ask questions of the panellists about their experiences and this, I found, was an invaluable opportunity, allowing us to get involved in the discussion.

After breaking for lunch, the next session of the workshop began with 'What are journalists looking for?' I thought this was a great idea as it allowed us to learn the journalists' point of view, what they are looking for and how we can work together to communicate accurate messages to the public. The panellists were Jason Palmer, BBC online; Kate Wighton, *The Sun* and Richard Van Noorden, *Nature*. Britain has a large community of science correspondents, with science stories being printed in most broadsheet and tabloid newspapers. The journalists explained how they approach stories, how they balance the need for news and entertainment with reporting science, and how they deal with accusations of polarising debates and misrepresenting the facts. Many people may just read the headline of an article and it is important that this does not portray incorrect information that could alarm the reader. It was clear that journalists also feel passionately that it is important that messages are correctly portrayed to the public and again, there was plenty of time for an interesting discussion.

The final session of the day was about how we, as early career scientists, can encourage good science and the sharing of evidence in the public domain when we are not necessarily the leaders in the field. We were offered practical guidance on how to get our voices heard in debates about science, how to respond to bad science when we see it and top tips for coming face-to-face with a journalist. The panellists for this session were Lucy Goodchild, Press Officer at Imperial College London; Harriet Teare from *Voice of Young Science* and Dr Leonor Sierra, also from *Sense About Science*. Being involved with VoYS is a great way to learn about the media in preparation for dealing with them later on in our careers. It also promotes our development into responsible scientists.

This workshop has helped to improve my confidence by encouraging involvement in the discussions at the end of each session. I found the day extremely helpful and informative and would certainly recommend it to my peers. In fact, I enjoyed it so much that I have joined the VoYS network and have agreed to encourage students at my own university to get involved. This workshop also made me realise that even as young scientists we have the right to challenge misconstrued information and to "stand up for science".

To get involved or find out more about VoYS workshops and projects, please contact Julia Wilson, VoYS Co-ordinator, at voys@ senseaboutscience.org. The next Standing up for Science media workshop will be taking place in Edinburgh on 6th November. For further information please visit the website www.senseaboutscience.org.

Danielle McEwan

our policy on the media

We will:

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

MED•VET•NET

The fifth Med-Vet-Net Annual Scientific Meeting took place in the Spanish town of San Lorenzo de El Escorial between 3 and 6 June 2009

Over 210 delegates met for four days at the Euroforum Infantes conference centre located next to the UNESCO World Heritage site of the El Escorial Monastery. Scientists from all Med-Vet-Net institutes were represented, and many external delegates joined us to share knowledge about controlling and preventing zoonotic diseases and to develop greater external collaborations worldwide. In total there were seven keynote lectures, 54 oral presentations, and over 172 posters. A representative from each of the 14 Workpackages also presented their results and achievements.

"After nearly five years of joint activities we are clearly seeing the benefits of our collaborative efforts," said Med-Vet-Net's Project Director, Professor John Threlfall. *"We were presented with highly topical presentations that reflect the current concerns in relation to the health of both humans and animals."*

This meeting was locally organised by the Med-Vet-Net partner institute in Spain, the Complutense University Madrid (UCM) and Instituto de Salud Carlos III (ISCIII).

"Thanks to all who participated and contributed, and also to our generous sponsors BioRad, Fort Dodge, Merial, Anaporc, Biomérieux Industry and the Spanish Ministry of Environmental, Rural and Marine Affairs, for their support of this conference," added Prof. Threlfall.

Med-Vet-Net once again, attracted a number of high-calibre, international keynote speakers who presented at the conference.

A number of SfAM members were funded by Med-Vet-Net to attend the meeting: Peter Silley, Steve Davies, Sally Cutler, Mark Fielder and Christine Dodd. In addition, the Med-Vet-Net Communications Unit were involved in the administration and communication aspects of the meeting.

The SfAM delegates kindly offered to provide a summary of each of the keynote sessions, which are presented here.

Teresa Belcher

med-vet-net

Med-Vet-Net is a European Network of Excellence that aims to improve research on the prevention and control of zoonoses by integrating veterinary, medical and food science research. Comprising 15 European partners and over 300 scientists, Med-Vet-Net will enable these scientists to share and enhance their knowledge and skills, and develop collaborative research projects.

1.Pathogenic *Escherichia coli*: contribution of the pathogen, host and microbiota — Brett Finlay

Brett introduced the idea of 'the Unholy Trinity' of infectious disease: the pathogen and its mechanisms for causing disease have been the first priority and is the aspect that has received most attention; the host and its immune response to the pathogen has been the second area of consideration and the newest area is the role of the microbiota of the normal body flora. Brett used the example of *E. coli* and focussed on Enteropathogenic and Enterohaemorrhagic (EPEC and EHEC respectively) *E. coli*. Their common mechanism of disease is the production of pedestals and attaching and effacing lesions. A key stage once the pathogen binds to the host erythrocyte is the secretion of a transmembrane receptor protein, *tir* (translocated intimin receptor) which inserts into the host cell membrane and acts as a binding site for intimin, a protein located on the surface of the pathogen. The pathogen goes on to subvert the host cell's biochemistry causing the accumulation of actin to produce the pedestal on which the bacterial cell sits. Brett's research into host response uses a mouse model infected with *Citrobacter rodentium* (the *E. coli* O157 of mice). This has suggested that the response of the host is critical in mediating disease, as an adaptive immune response is seen 14 days after infection and the organism is excluded. The role of the intestinal

▼ *Delegates at the Euroforum Infantes conference centre during the tea/coffee break*

microbiota in diarrhoeal disease is also studied relatively little. Using the mouse model, significant changes occur in the composition of the intestine three days after infection but very few *Citrobacter* are evident and *E. coli* becomes a major component. Brett's conclusion is that Citrobacter may be setting up an hospitable environment to allow *E. coli* to grow, however, this does eventually revert to a normal flora after 28 days. One practical application of the work is a vaccine against *E. coli* O157 for cattle now used in Canada which is based on Tir. This demonstrates how a fundamental understanding of mechanism of pathogenicity can be applied at the agricultural level to prevent human disease.

Christine Dodd

2. Ecogenetical dynamics of bacterial pathogens — Fernando Baquero

Fernando began his talk by explaining that mutants provide microorganisms with the ability to cope with new conditions presented by variable environments and as such they can aide dispersal. An unfit biological entity in a changing environment will struggle to thrive and survive, whilst a higher density of mutations assures fitness. This could be termed as the tolerance gradient and may allow an organism to cope with new conditions. This then leads to what was termed phenotypic diversification and the development of potentially new strains. It was suggested that organisms have a core of fairly stable genes in what was termed the basic reproductive environment. However, as the reproductive environment changes and perhaps becomes less favourable, then the less stable peripheral genes are likely to show mutations, giving rise to phenotypic variation or diversification. The success of this mutation in terms of pathogen 'fitness', is determined by the continued nature of the environment. If the environment/ecology reverts or alters further then the mutation might actually have a negative effect as it competes against the tolerance gradient. Fernando then went on to explain that variability can occur through recombination and transformation and concluded with the surprising concept that movement of chromosomal genes between bacteria appears to be coincidental!

3. Epidemiology and risk assessment: an unsettled union? — Peter Teunis

Qualitative risk assessment can predict risks from observations, but cannot predict illness. Peter explained that epidemiology is a top down approach, whereas risk assessment works from a bottom-up perspective. Risk assessment is often used in the food industry and is based upon conditional dependencies such as infection probability and dose of infective agent. So this method is good at predicting infection but not illness. Epidemiological methods rely more on case observation and are good at looking at numbers infected, but poor at estimating infection so this approach essentially measures prevalence of illness. To reconcile these two sets of outputs we need to reconsider the definition of infections. Peter explained that epidemiology and risk assessment do dwell in different realms and they can be reconciled. With the use of novel and very powerful statistical methods we can predict seroconversion rates and even start to estimate levels of asymptomatic cases in the population, allowing risk assessment and epidemiology to become a more settled union.

Mark Fielder

4. The emergence and spread of the Bluetongue virus (BTV) across Europe: the impact of climate change, insect vectors and vaccination — Peter Mertens

Peter began his talk with an apology as only one case of human infection with Bluetongue has been documented in the last 30 years, so technically it is not zoonotic. Bluetongue is the second most important disease of livestock after foot and mouth. Eradication is problematic as the infection localises in the microvascular

endothelium, probably the best place to be to ensure successful infection of new arthropod vectors.

The strain BTV-8 has recently swept through Europe, with devastating effects in The Netherlands during 2007, and is now in the UK. Greatest severity is seen among sheep; however, it can also infect cattle resulting in vertical transmission with a third of calves being viraemic. This *in-utero* infection could facilitate a means for over-winter survival when biting midges are no longer prevalent.

Peter then asked the question: why globally do we fail to control Bluetongue? The answer

▲ *Over 172 posters were submitted for viewing at the poster sessions*

resides in the heterogeneity among infecting viruses. There are 25 different serotypes of which nine are seen in Europe. The problem is the lack of cross-protection between these as vaccination against one serotype offers no protection against the others.

Remarkably, only 1-2% of midges feeding upon a viraemic host become infected. However, once infected they show highly efficient transmission to susceptible hosts with a single bite facilitating successful infection. Another major concern is the potential for global warming to facilitate spread of Bluetongue. It is probable that global warming has permitted *Culocoides imicola* to extend its range from Africa through to Spain and Turkey, then northwards into Italy and Greece. In these regions overlap with indigenous species, also competent for BTV, has enabled northern spread. Furthermore, BTV polymerase is non-functional below 10°C, bringing viral replication to a halt. Progressively milder winters extend the replicative season for this virus and it is likely that much of Europe is now compatible with BTV.

In terms of prevention and treatment, vaccines are useful, but only against an individual serotype. Live vaccines have been associated with adverse effects and sub-unit vaccines are not yet available. In the UK there has been significant use of killed vaccine (90% coverage in some areas). However, imported animals from Europe have been introduced and moved to different locations around the country, thus we are now awaiting the outcome of a national vaccine challenge study.

Sally Cutler

5. Biodiversity and evolution of pathogenic Listeria: a genomics view — Carmen Buchrieser

Carmen explained how the European consortium of 10 groups had been one of the first to sequence the *Listeria* genome and then went on to describe the six current (soon to be seven) species of *Listeria*. The current list includes *Listeria monocytogenes*, *L. ivanovii*, *L. innocua*, *L. seeligeri*, *L. welshimeri* and *L. grayi*. Of these only *L. monocytogenes* and *L. ivanovii* are thought to be pathogenic. Transmission of infection is mainly via dairy products, meat, vegetables and fish. As a rule *Listeria* is an opportunistic pathogen of the elderly, newborns, immunocompromised and pregnant women, with mortality rates as high as 30%. Carmen went on to explain the intracellular life cycle of *Listeria* and stated that it has the capability to cross three barriers within the body, namely intestinal, blood-brain and placental. *L. monocytogenes* serovar 4b is responsible for all major food-borne outbreaks of listeriosis and the majority of sporadic cases. The main focus of the talk was to determine whether this phenomenon could be explained genomically. It appears that listeria have an extensive regulatory repertoire, large numbers of transporters and an expansive range of surface proteins accounting for 7.3%, 11.6% and 5.1% of the coding cycle of *L. monocytogenes* respectively. Further work demonstrated that the genome region in listeria is much conserved. Carmen also demonstrated how several virulence genes are present in both *L. monocytogenes* and *L. ivanovii* and how *L. innocua* and *L. welshimeri* have many gene deletions in common, suggesting a common ancestor. Next using DNA arrays, Carmen went on to show that there have been three lineages of

L. monocytogenes that have different gene content but all contain the known virulence genes. Additionally, three unique marker genes for the 4b serovar were identified. She also discussed the phylogenic tree of *Listeria* suggesting that the different species may all have originated from *L. grayi* and the importance of truncated internalins. Carmen suggested that 35% of *L. monocytogenes* isolated from food express a truncated internalin, due to a point mutation, and it is only the full length internalins that are able to promote entry into cells. Finally Carmen demonstrated that the listeria virulence locus is 9kb in length and listeria appear to easily lose pathogenicity, if not required, but can switch it back on when necessary. One could argue that *L. monocytogenes* is a human pathogen aiming to get back to its environmental roots.

6. Evolution & dissemination of glycopeptide resistance operons — Patrice Courvalin

Patrice began by explaining how, to become multi-resistant, bacteria have combined a large number of defence mechanisms at both a genetic and biochemical level. He also explained that in enterococci, glycopeptide resistance can only be achieved by a combination target modification associated with target elimination. Patrice explained how glycopeptides inhibit cell wall synthesis and how resistance has developed due to the *van* A and *van* B genes. The *van* A gene encodes for both vancomycin and teicoplanin resistance, whereas *van* B gene only encodes for vancomycin resistance. However the biological cost of resistance for enterococci is extremely high and consequently to lower this cost, these genes are only expressed when needed (i.e. in the presence of a glycopeptide). As such the *van* S (sensor) and *van* R (regulator) genes work in combination to keep the organism biologically sound. Patrice and his colleagues did a lot of their work on mutant enterococci, one of which had lost its *van* S gene and therefore its resistance mechanisms are always switched on, even in the absence of vancomycin. Occasionally these strains can become glycopeptide-dependant and only grow in the presence of vancomycin.

Patrice described how resistance dissemination in enterococci and from *Enterococcus* into methicillin-resistant *Staphylococcus aureus* (MRSA), is achieved by a two-step mechanism that combines suicidal conjugation with replicative transposition, resulting in efficient transfer, stabilisation and expression of incoming resistance genes into the new host. He explained that in the US, 40% of enterococci are resistant to vancomycin and the first proven vancomycin resistant MRSA (VMRSA) was found in a renal patient in Michigan in the year 2000, due to the transfer of a *van* A gene from an *Enterococcus* into a MRSA. The number of VMRSAs isolated from patients is currently 10 and with the majority isolated in the Michigan area. Surprisingly the MICs of the Michigan strains to vancomycin are much higher than those of the New York and Pennsylvania strains. Patrice went on to explain that this was genetically driven and in the Michigan strains the *van* A gene has conjugated to the plasmid within the new MRSA host and this has led to an exponential dissemination of the resistance gene. These VMRSA also have a biological price to pay and are often slow growing which can lead to false-susceptible results on automated systems which are usually read at eight hours. Surprisingly, although these VMRSA are individually resistant to both vancomycin and oxacillin, work *in-vitro* suggests that a combination of these two agents does inhibit the organism. Further work is required but it would be a brave person that would risk such a combination on a patient.

Steve Davies

7. Epidemiological tools for surveillance and control of emerging diseases — J M Sanchez-Vizcaino

Professor Sachez-Vizcaino began his talk with a reminder that animal health issues are changing and that early detection is pivotal in our preparedness for change. We have seen an increase in new or re-emerging infections and must ask why. Important factors are likely to include climate change, travel, socio-political upheavals and globalisation of both humans and livestock (legal and illegal). Increasing demand for protein has resulted in creation of new production areas, for example in Malaysia, shortly followed by Nipah viral infections. A huge market based upon illegal animal movements has arisen accounting for a 4-6 million dollar business. Most detection tools are too slow requiring three to four months to show emerging or re-emerging disease threats. Risk assessment, particularly using social network analysis and digital simulations, can be used to analyse where diseases come from and where they might go. These models can be used to simulate the spread of diseases of both humans and livestock, thus in keeping with the "one biology one medicine" philosophy. Furthermore, they show the overlapping interactions of wild and domestic animals and human populations facilitating transmission to potentially new host species. Through these simulations we can obtain training and explore the impact of various control strategies.

Sally Cutler

information

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Spring Meeting 2009 Report

3rd broadening microbiology horizons in biomedical science

Lakeside Conference Centre, Aston University, Birmingham, UK, Wednesday 22 April 2009

The 2009 Spring meeting provided a wonderful mix of important and interesting topics devised to interest and update delegates. In the morning, we travelled from hygiene to drug resistance via bioterrorism with an update on emerging respiratory viruses — and it was a fascinating journey!

Professor Sally Bloomfield was the recipient of the SfAM/Procter & Gamble Applied Health Care Microbiology Award. Her presentation on the fall and rise of hygiene was a timely reminder of the fluctuating importance of topics associated with microbiology. After the US Surgeon General declared in 1970 that we could "*close the book on infectious disease*", hygiene slid down the agenda and acquired a faintly outdated image — until recently. With the rise in food borne disease, almost all preventable (40% are linked to infection in the home, according to WHO), hygiene in the home regained importance. Hygiene was deemed the first line of defence for influenza, being clearly associated with the *'catch it, bin it, kill it'* campaign. A need for better infection control at home would also be a consequence of earlier discharge from hospital. Sally gave an excellent overview of these current concerns, emphasising the importance of rinsing as well as cleaning and disinfection; the ability of microorganisms to survive on surfaces; and the shared role for us all in reducing risk and contamination to a level not harmful to health. The subject has of course assumed increasing importance with the current 'swine flu' concerns.

We were also reminded about other emerging respiratory viruses by Dr Kate Templeton. Her enthusiasm for the viruses that she deemed 'unknown, newly discovered, unloved and underdiagnosed' was, dare I say it, infectious! Her review of new viruses such as human metapneumovirus and bocavirus was most timely, and she raised issues associated with their clinical significance, the possible importance of mixed viral infections, and problems of diagnosis (specificity, significance, cost) and quantification.

Professor Peter Hawkey provided a sophisticated update on extended-spectrum beta lactamases (ESBLs), reminding us at the start that the acronym referred to the gene, not the germ. We were led through genetics, history and global epidemiology, ending up in plant roots and the soil, where *Kluyvera* could mark the origin of resistance for some hospital and community strains. Prof Hawkey also described the potential problems associated with environmental pressure, where quantities of quaternary ammonium compounds from industrial processes and domestic effluent (shampoos and other bathroom and domestic products), coupled with continual contact between antibiotics/animals/people/faeces/xenobiotics/water could facilitate further transmission of resistance

The key message from Professor Les Baillie was that bioterrorism should be more openly discussed and appropriate information should be widely available to the general public. This should encompass risk communication,

▼ *Sally Bloomfield receiving the SfAM/Procter & Gamble Applied Health Care Microbiology Award*

information

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You can also find details of next year's meetings on page 29 of this issue of *Microbiologist*

improved preparedness, and general but factual text, to combat the spread of fear and panic. He proposed that terrorists, both real and potential, already had access to information (and pathogens), thus a public information site would not be helping them *per se*, but would provide a useful source of rational information for the public. He is already working on the website, which has received support funding from S*f*AM and others. His presentation also provided a retrospective analysis of bioterrorism, focusing on the source of the agents used.

Joanna Verran
Manchester Metropolitan University

The afternoon session commenced with Professor Peter Lambert offering a comprehensive overview of central venous catheter related infections. These multilumen devices are commonly used and are the main cause of bacteraemia and sepsis in hospitalised patients. The main organisms responsible are linked with the skin of the patient and the hands of the healthcare worker with a smaller proportion coming from distant infection via the patient's blood. While fungal infections are less common, they tend to be more dangerous. Biofilm formation is also a major problem as these are almost impossible to treat and eradicate as the conventionally used antibiotics perform very poorly against biofilms. He also outlined the approaches that are available to prevent infections, such as the Biopatch (an American development), flushing of devices, natural products such as red algae and bacteriophage coated catheters.

Dr Tony Worthington followed with an explanation of the germination theory as it relates to *Clostridium difficile*. The concept of 'Germinate to Exterminate' was developed after it was recognised that once the spore has germinated in the small intestine, it is much more sensitive to antimicrobials. Sporicides are effective in the healthcare setting but have health and safety implications: they require wards to be vacated and can be unpleasant to use. If the spores can be treated with a germinant, such as taurocholate or certain amino acids in thioglycolate medium, then destruction of the resulting vegetative cells, especially in air, is possible.

The changing epidemiology of viral hepatitis was outlined by Laura Ryall. There have been significant changes in recent years and as well as the traditional Hepatitis A–G viruses, other causes of viral hepatitis such as cytomegalovirus and yellow fever virus are also investigated. Orphan viruses (those that have no associated, defined clinical syndrome) have also received attention as there are a number of animal infections associated with this virus and it could jump the species barrier. Laura then went on to discuss current developments and prevalence of the hepatitis viruses in the UK population as a whole as well as in high risk groups (the ageing population, transplant patients and the injecting drug population) as well as certain ethnic populations.

The afternoon session concluded with a case study of an outbreak of Q fever in Cheltenham in 2007, presented by Dr Philippa Moore. A cluster of cases led to identification of 32 *Coxiella burnetii* infections, the probable source being an infected sheep placenta which resulted in windborne aerosols. The bacterium can survive for months in the environment and is common in rural areas. Even so, the number of cases identified in June of that year was unusual and, with a hospitalisation rate of one case in five would indicate that there were in excess of 400 cases in the community as 28 of the 32 probable cases were admitted to hospital. The outcome of the investigation was an information campaign to farmers and the production of new guidelines on husbandry practices and human infections.

Louise Fielding
University of Wales Institute, Cardiff

■ We would like to extend our thanks to all the speakers for their excellent presentations

Bad Bugs Book Club

During March S*f*AM ran a public engagement event for National Science and Engineering week in collaboration with the Manchester Beacon of Public Engagement. We showed the film *'Outbreak'* to an audience of the general public with a wide range of backgrounds. Before and after the film there was a facilitated discussion around the public perception of infectious disease (see *Microbiologist*, June 2009, Vol. 10 No.2, page 31).

As a follow-up to this event, during this year's S*f*AM summer conference in Manchester, and to be aligned with the Manchester International Festival which was taking place at the time, a small collection of the delegates slipped off to a local eatery and spent the evening in contemplation of all things cultural. We were gathering for the first meeting of the Bad Bugs Book Club (try saying that quickly!), founded by Professor Joanna Verran of Manchester Metropolitan University.

The participants were from a variety of backgrounds — some scientific, some not — but the prerequisite of attendance was that all participants had read the book *'Hot Zone'* by Richard Preston and/or seen the film *'Outbreak'*. The mixed background encouraged discussion of the scientific merit of both the film and book, with the non-scientists consulting the scientists about scientific accuracy.

For those who've yet to read it, the *Hot Zone* describes the author's search for the story of the emergence of Ebola virus, as well as a recount of the story itself. The novel progresses through the authors interviews with key players. It includes several graphic descriptions of symptoms, and some detailed scientific narratives. The film *'Outbreak'* was an interpretation of this book and depicts the emergence of Ebola in scenarios comparable to the book, although occasionally using rather more artistic license in some of the scientific aspects.

As an aside, readers might be interested to learn that *'Outbreak'* was not the original film interpretation of the book. This was intended to be based on Preston's column in the New York Times entitled 'Crisis in the hot zone' with Robert Redford and Jody Foster anticipated as the lead characters. However, the film was never made and a return to its production lost momentum after the release of *'Outbreak'*.

The book club participants discussed the text from a number of perspectives over some food and drink. Some members of the group thought the book a little heavy right from the start. Some enjoyed the way in which the book began — leading the reader page-after-page through the various hazard classifications of infectious microorganisms — certainly the film remained true to the book in that context. The symptoms of patients suffering an Ebola-type viral infection were considered well represented in both the film and the book — although the book could describe more effectively the horrors experienced.

Discussion sometimes digressed from the contents of the book or film, but most importantly it always centred on the topic of infectious diseases and how they're portrayed through the media. The meeting gave the non-scientists in the group an insight into the life of a scientist and the extent to which the book and film used artistic licence to grab the attention of the audience.

This was just the first of a series of similar events run in collaboration with the Manchester Beacon of Public Engagement. To find out about future events, to suggest books, or to join the book club, visit www.sci-eng.mmu.ac.uk/intheloop or contact Professor Joanna Verran at j.verran@mmu.ac.uk. Overall, an enjoyable and enlightening evening was had by all – the event marked a successful start to what promises to be an interesting series of book club meetings. The next book will be *'Year of Wonders'* by Geraldine Brooks, which describes the plague outbreak in the Derbyshire village of Eyam. The meeting will be part of the Manchester Science Festival in October.

Lucy Harper
Communications Manager

SfAM *Environmental Microbiology* Lecture

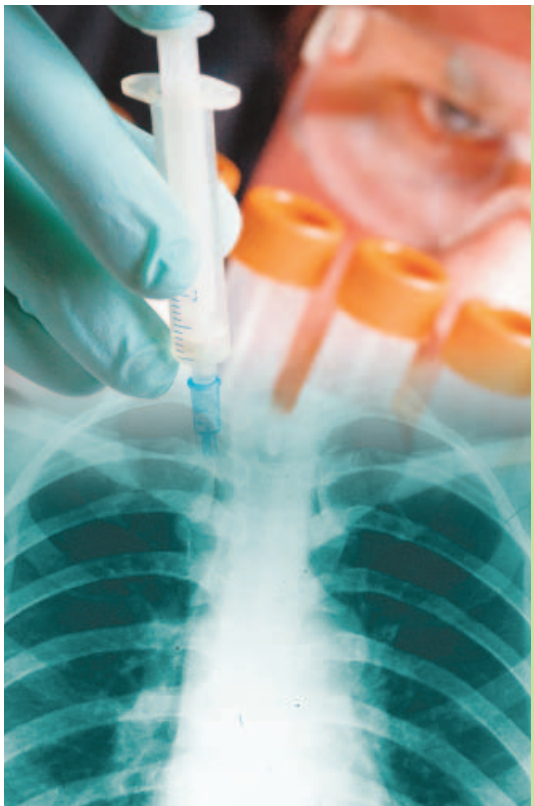
Deciphering microbial community dynamics, from genomes to biomes

presented by **Professor Edward DeLong** of Massachusetts Institute of Technology (MIT), USA

Royal Society of Medicine, London, UK • **Monday 12 October 2009**

The *Environmental Microbiology lecture* will be presented by Professor Edward DeLong of Massachusetts Institute of Techology (MIT), USA. He will present a lecture entitled *"Deciphering microbial community dynamics, from genomes to biomes"*.

New experimental strategies will be discussed. These new approaches aim to interrelate microbial genetic and peptide sequence data obtained from natural microbial communities, with their functional and ecological relevance and significance. The overarching goals of the strategies are to provide a better understanding of the complexity and dynamics of the microbial world, and its influence on large scale ecosystem processes.



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- **Advances in biocide development**
- **Tuberculosis**

Royal Society, London
Monday 11 January 2010

■ For further information please visit the Society website or contact Sally Cryer.
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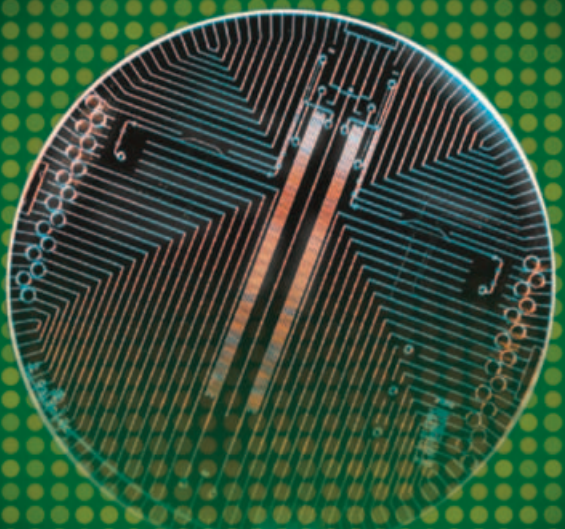


Microarrays — closing the gap between research and diagnostics tools

Dr Rod Card demystifies microarrays and explains how this powerful tool can be used to inform treatment decisions, treatment and epidemiology of infectious disease

DNA microarrays are a powerful tool for the simultaneous and specific detection of many different nucleic acids. They were first developed for the study of gene expression, as they enable the comparison of whole transcriptomes to be undertaken in a single operation. Such studies allow the identification of genes that have different expression levels under different conditions. This has provided considerable insight into the study of cancer, for example, where gene expression differences can be used to assist in the classification of particular cancers, and thereby inform treatment decisions (for a review article describing microarrays see Dufva 2009).

Other applications for DNA microarrays have subsequently been developed including comparative genome hybridisation (assessing genome content in different cells or closely related organisms) and the

identification of binding sites of DNA-binding proteins on a genome wide basis (using the ChIP-on-chip technique). DNA microarrays have also been employed for the detection of microorganisms. They have been used to screen environmental samples to obtain a microorganism profile, detect foodborne bacteria to aid food safety, and for the detection and identification of unknown pathogens in clinical samples.

Microarrays can also be employed to investigate the genome of a pathogen, providing genotypic information that can be relevant to clinical decisions. For example, there are now microarrays that allow the subtyping of human papillomavirus (Klaassen, *et al.*, 2004), as only certain strains are strongly associated with the development of cervical cancer.

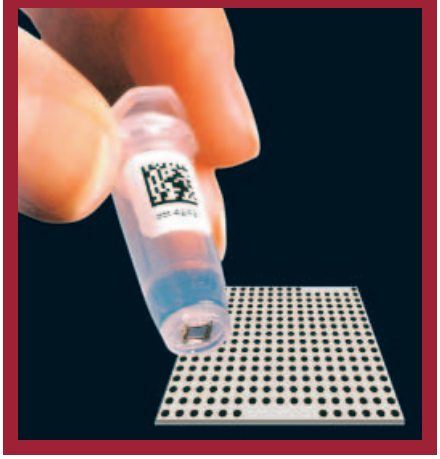
Antimicrobial resistance is another field where the multiplex capability of microarrays has proven to be very useful. Antimicrobial resistance describes the ability of microorganisms to resist the action of antimicrobial drugs (*e.g.* antibiotics) and has become a major public and veterinary health issue, as it can have a profound effect upon the treatment of infections in man and animals. Determining the antimicrobial susceptibility of bacterial isolates can help inform treatment decisions. The conventional methods for susceptibility testing require that the bacterium is first isolated in culture. The bacterium can then be exposed to a range of antimicrobials in separate assays, and susceptibility assessed by monitoring growth inhibition (Wheat, 2001). Isolated bacteria can also be screened for the presence of antimicrobial-modifying enzymes. These assays all investigate the phenotype of the organism.

Considerable effort has been put into determining the mechanisms of antimicrobial resistance and the underlying genetic components that govern it. This has resulted in the discovery and sequencing of many resistance genes, the detection of which would allow the antimicrobial resistance gene profile of an organism to be described. This information could usefully inform treatment decisions, aid surveillance studies and provide epidemiological information.

Numerous molecular assays, typically PCR, have been developed to detect specific resistance genes or gene families (reviewed in Sundsfjord, *et al.*, 2004). However, there are many genes that can confer antimicrobial resistance and to test a single bacterial isolate many PCRs would need to be performed. Even when several PCRs are multiplexed into a single reaction, the number of tests required rapidly becomes prohibitively expensive and resource intensive. A genotyping assay that allows rapid and accurate screening of many antimicrobial resistance genes in the same operation would be of considerable benefit. Microarrays allow the detection of a virtually unlimited number of genes in a single experiment and provide a powerful platform to address antimicrobial resistance genotyping needs. A number of microarrays for the detection of antimicrobial resistance genes in Gram-positive and Gram-negative bacteria have been developed, however their widespread adoption has been limited. This is likely to be due to several factors, including a belief that microarray experiments are difficult to undertake successfully and require specialised or expensive equipment and reagents.

Developments in microarray platforms and protocols have addressed many of these practical impediments to widespread use and can assist the transfer of microarrays from the research laboratory to a routine testing environment. An example of an antimicrobial resistance genotyping microarray designed with routine use in mind is the AMR-ve microarray (Identibac, Weybridge, UK) which currently targets 54 resistance genes in Gram-negative bacteria and was originally described by Batchelor, *et al.*, (2008). It was developed in a collaboration between the Veterinary Laboratories Agency (VLA) and the Health Protection Agency (HPA). This microarray consists of oligonucleotide probes printed on to the base of an ArrayTube™ (a trademark of Clondiag GmbH Jena, Germany — Figure 1).

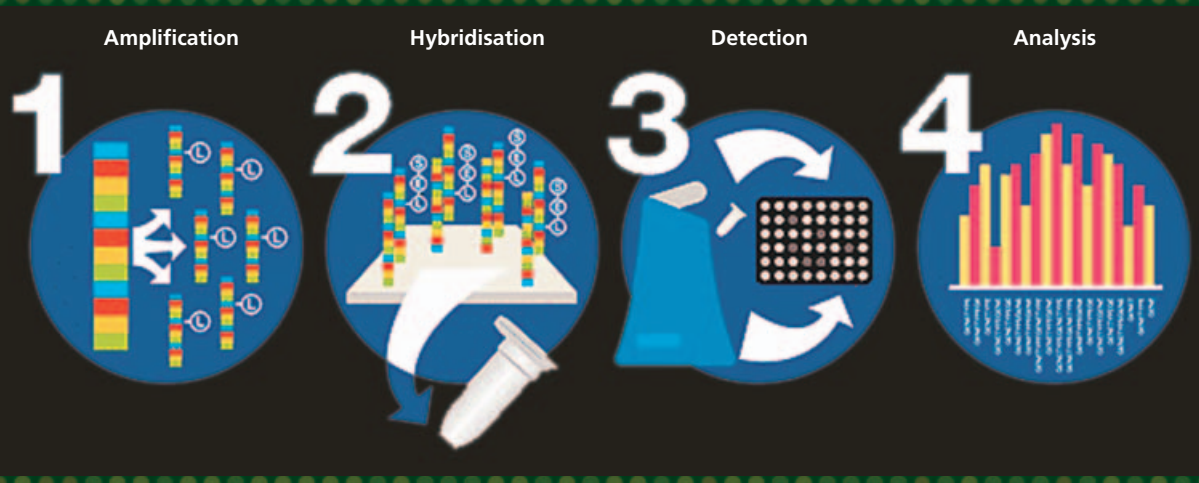
Figure 1. *An ArrayTube™*



The ArrayTube™ format was developed by Clondiag GmbH (Jena, Germany) and is used by a number of commercially available microarrays. The ArrayTube™ presents a significant advantage as it forms the reaction vessel in which all the steps of the microarray experiment take place, providing convenience and ease-of-use that is not a feature of the glass slide microarrays commonly used in research laboratories. The protocol used with an ArrayTube™ is easy to perform and little expert training is required. An outline of the procedure used by several commercially available microarrays (including the Identibac AMR-ve microarray) is given in Figure 2. The specialised equipment required to use these microarrays is limited to a thermocycler (most standard PCR machines are suitable), a thermomixer (a rotating heating block for hybridisation), and an ArrayTube™ Reader (to capture an image of the microarray). For many laboratories, it is likely that the ArrayTube™ Reader is the only item that would need to be purchased specially. All liquid handling and manipulations employ standard pipettes and equipment and reagents have been selected to be inexpensive and straightforward to use. For example, detection is achieved using an enzymatic reaction which generates a precipitate that is easily distinguished and analysed. This enzymatic system is considerably cheaper than the costly and friable fluorescent dyes employed in many other DNA microarrays. Analysis of the results is easily undertaken using software provided with the ArrayTube™ Reader and the genotype of the sample under study can be quickly determined. Results can be exported to Excel, allowing data to be tabulated and organised as desired. Analysis does not require use of complicated software packages, such as those needed to perform comparative gene expression studies, significantly reducing staff training and time costs.

ArrayTube™ microarrays have been developed to allow rapid testing and results can usually be produced within

Figure 2. *Overview of the ArrayTube™ microarray procedure*



1. *Extracted DNA is labelled with biotin (L) in a linear multiplex amplification reaction. Target for all probes can be amplified and labelled in this single multiplex reaction. The linear amplification reaction generates biotin labelled, single-stranded product. This has the advantage that the amplification products cannot serve as template for further amplification. Thus the possibility of false positive results arising from contamination of samples by amplicons generated in earlier amplifications is eliminated. This overcomes a significant concern for conventional PCR assays.*
2. *The biotin labelled, single-stranded product hybridises to the corresponding probes by complementary base-pairing. A horseradish peroxidise (HRP)-streptavidin conjugate (E) is then added and binds to biotin labelled DNA. The HRP converts the substrate SeramunGrün® (S) into a coloured precipitate that is deposited onto the microarray.*
3. *The ArrayTube™ Reader enables the capture and visualisation of the array image. The presence of a dark spot indicates a successful hybridisation.*
4. *The software supplied with the ArrayTube™ Reader is used to measure the signal intensity of each probe and determine which genes are present in the sample.*

six to seven hours, from extraction of genomic DNA to final analysis. This speed of analysis is quicker than many of the conventional methods microarrays are designed to replace or complement. For example, determining the antimicrobial resistance gene profile of a bacterium with the Identibac AMR-ve microarray can be accomplished more rapidly than many conventional methods for antimicrobial susceptibility testing.

The ability of a microarray to deliver accurate results in a dependable and consistent manner is critical to its move out of the research laboratory and into a routine testing environment. User-friendly protocols and simple analysis contribute to reliable microarray performance by reducing variation arising from the procedure. However, two additional requirements for broader acceptance of microarrays are manufacturing systems that control microarray quality and a robust validation of microarray performance.

Quality control systems should be used to demonstrate the fidelity of microarray manufacture. Accurate and precise deposition of the probes onto the microarray is critical to performance. Each production run of a microarray should be quality controlled to ensure that all probes are present, in the correct location and operate properly. Any reagents supplied with the microarray should be functionally tested on the microarray to ensure that performance meets defined standards.

Extensive testing and validation should be performed on a microarray to assess its performance. An important part of this process is the testing of a panel of samples previously characterised by a definitive benchmark test where microarray results are compared to the results obtained with the benchmark test, and the sensitivity and specificity of the microarray determined. The benchmark test can be a conventional and well established phenotypic test (*e.g.* for an antimicrobial resistance microarray this benchmark test could be conventional antimicrobial susceptibility testing). This analysis therefore compares the genotype determined by the microarray with the phenotype determined by the benchmark test, and sometimes disagreement between the two methods is seen for several reasons. For example, the phenotype could be conferred by a gene that is not detected by the microarray (either a gene with no corresponding probe or a variant gene that is not detected by the existing probes). Alternatively a gene may be present but not expressed (and thereby conferring no corresponding phenotype). These discrepancies can be investigated further by employing a different genotyping test, such as PCR or sequencing. When the data from this second genotyping test are compared to the microarray results the agreement is frequently found to be very high (see, for example, Batchelor, *et al.*, 2008). An important consideration with microarrays is that they can detect only those genes that are targeted by the probes and allow only a prediction of the phenotype to be made. However, microarrays can be expanded by the inclusion of additional probes that target newly identified genes or gene variants. When introducing new probes it is important to consider the need for additional validation, to ensure that the microarray acquires the new detection capabilities without compromising its original performance.

Despite often rigorous validation and strict quality control systems, many microarrays are designated as 'for research use only'. They cannot, therefore, be used for clinical diagnosis of samples from humans, but their utility in the veterinary field, for surveillance and epidemiology remains. For use in

clinical diagnosis in the European Union a microarray requires a CE mark, indicating that it meets all the essential requirements of the relevant European Directives, including that on *in vitro* diagnostic medical devices (98/79/EC). The assurance that a CE mark gives is widely recognised and microarrays are being developed with this in mind. An example of a CE marked microarray is Prove-it™ Sepsis (Mobidiag Ltd, Helsinki, Finland), which can identify 23 bacterial species and the mecA methicillin resistance marker in DNA extracted from positive blood culture.

The full advantage of the powerful multiplex capability of microarrays is realised when many different gene targets need to be detected in the same operation. Therefore, microarrays are best employed to address questions that involve many possible genotypes, such as the generation of an antimicrobial resistance gene profile. There are many other applications where the strengths of a microarray can be brought to bear, particularly for bacterial genotyping applications.

The application of microarrays are typified in relation to *Salmonella*. Infection with *Salmonella* species constitutes one of the most common causes of food poisoning in man and disease in animals. Two species of *Salmonella* are recognised: *Salmonella enterica* (which is divided into six subspecies) and *Salmonella bongori*. The determination of the *Salmonella* serovar is important for several reasons including epidemiology, surveillance, disease control, food safety and animal husbandry.

A microarray product that has been designed to detect all salmonellae and identify 64 common and important *Salmonella* serovars is named Premi®Test Salmonella (DSM Nutritional Products, Geleen, The Netherlands). This microarray uses the ArrayTube™ platform and has been tested with a large panel of samples (Wattiau, *et al.*, 2008). The microarray uses a novel approach that targets 13 DNA markers which are not directly associated with the genes that govern the antigenic formulae. Instead, the specific marker profile obtained with each sample tested is compared to a database of known profiles and the serovar thereby determined. Recent improvements to this microarray have increased the number of DNA markers used and the number of serovars that can be identified.

Another area where the multiplex capability of microarrays can be exploited is the pathotyping of *Escherichia coli* strains. Pathogenic *E. coli* strains present a considerable public health problem worldwide and cause disease by means of virulence factors that are encoded by a number of different genes. A microarray that can detect many of these virulence genes has been described (Anjum, *et al.*, 2007), and is able to identify virulence genes present in *E. coli* isolates of both human and animal origin that are associated with a range of pathotypes (including pathogenic extraintestinal, enterotoxigenic, and enteroinvasive *E. coli*).

A final example of bacterial genotyping microarrays is provided by those designed to detect genes or gene groups associated with antimicrobial resistance and virulence factors in *Staphylococcus aureus* (Moneke & Ehricht, 2005). Such microarrays can detect genes associated with resistance to a range of antibiotics, including methicillin and vancomycin. Given the importance of detecting and characterising methicillin-resistant *S. aureus* (MRSA), these microarrays have considerable utility.

Conclusion

Since the introduction of microarrays over fifteen years ago their powerful multiplexing capability has proven useful in a diverse range of applications. These applications include the genotyping of bacteria to generate, for example, antimicrobial resistance or virulence gene profiles. The information revealed by a single microarray experiment significantly exceeds that obtained from many PCRs and can greatly assist treatment decisions, surveillance and epidemiology.

With platforms such as the ArrayTube™, microarray techniques and data analysis have been improved and simplified. These developments have delivered microarrays with considerable user-friendliness and with relatively inexpensive set-up and running costs. This has facilitated their introduction into laboratories with minimal additional equipment or training costs. The availability of quality controlled and validated microarrays that use these simplified platforms will enable a wider range of laboratories to undertake microarray experiments with confidence. Microarrays possess an enormous potential for the rapid screening of bacteria for important genes, and they are becoming sufficiently developed to move successfully from the research laboratory into a routine testing environment.

references

- Anjum, M.F., Mafura, M., Slickers, P., Ballmer, K., Kuhnert, P., Woodward, M.J. and Ehricht, R. (2007) Pathotyping *Escherichia coli* by using Miniaturised DNA Microarrays. *Appl Environ Microbiol* **73** 5692-5697.
- Batchelor, M., Hopkins, K.L., Liebana, E., Slickers, P., Ehricht, R., Mafura, M., Aarestrup, F., Mevius, D., Clifton-Hadley, F., Woodward, M.J., Davies, R.H., Threfall, E.J. and Anjum, M.F. (2008) Development of a miniaturised microarray-based assay for the rapid identification of antimicrobial resistance genes in Gram-negative bacteria. *Int J Antimicro Ag* **31**, 440-451.
- Dufva, M. (2009). Introduction to microarray technology. *Methods Mol Biol*. **529** 1-22.
- Klaassen, C.H., Prinsen, C.F., de Valk, H.A., Horrevorts, A.M., Jeunink, M.A. and Thunnissen, F.B. (2004) DNA microarray format for detection and subtyping of human papillomavirus. *J Clin Microbiol*. **42** 2152-60.
- Moneke, S. and Ehricht, R. (2005) Rapid genotyping of methicillin-resistant Staphylococcus aureus (MRSA) isolates using miniaturised oligonucleotide arrays. *Eur J Clin Microbiol Infect Dis* **11** 825 – 833.
- Sundsfjord, A., Simonsen, G.S., Haldorsen, B.C., Haaheim, H., Hjelmevoll, S.O., Littauer, P. and Dahl, K.H. (2004) Genetic methods for detection of antimicrobial resistance. APMIS. **112** 815-837.
- Wattiau, P., Van Hessche, M., Schlicker, C., Vander, V. H. and Imberechts, H. (2008) Comparison of classical serotyping and PremiTest assay for routine identification of common *Salmonella enterica* serovars. *J Clin Microbiol*. **46** 4037-4040
- Wheat, P.F. (2001) History and development of antimicrobial susceptibility testing methodology. *J Antimicrob Chemother*. **48** Suppl 1:1-4

acknowledgement

- ArrayTube™ is a trademark of Clondiag GmbH (Jena, Germany).

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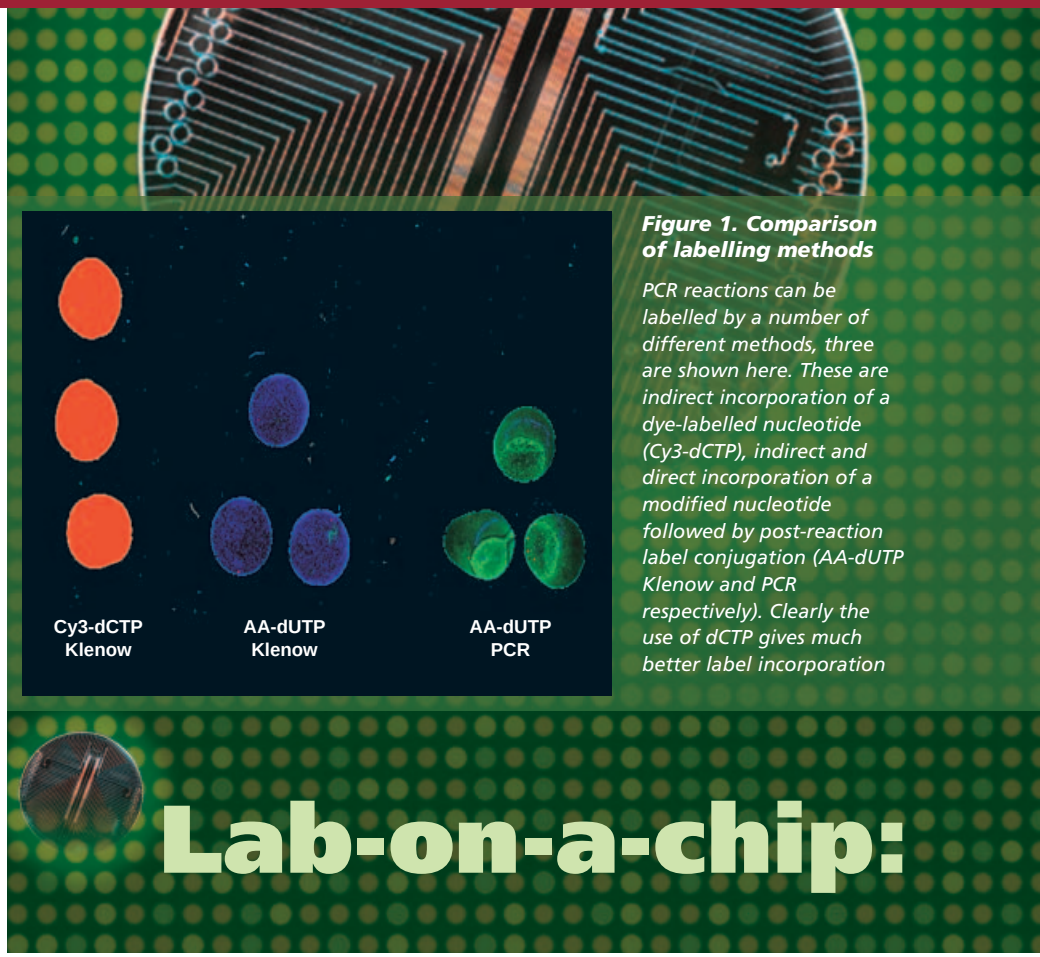

Lab-on-a-chip:

Figure 1. Comparison of labelling methods

PCR reactions can be labelled by a number of different methods, three are shown here. These are indirect incorporation of a dye-labelled nucleotide (Cy3-dCTP), indirect and direct incorporation of a modified nucleotide followed by post-reaction label conjugation (AA-dUTP Klenow and PCR respectively). Clearly the use of dCTP gives much better label incorporation

The prospect of a lab-on-a-chip is one that is incredibly exciting for microbiology. Wouldn't it be a fantastic tool to have all of the diagnostic assays that you need to run in one format without having to make decisions about the assays that need to be performed? The idea conjured up by the name is of a small size assay system which is capable of running a number of tests in parallel for biochemistry, molecular biology, immunoassay *etc*., but how real is this actual concept? At the Health Protection Agency's Porton Down laboratories we have invested considerable time and effort into exploring the potential offered by DNA microarray technology for the molecular diagnosis of infectious diseases.

Our rationale was to develop an assay system where, irrespective of the sample type and the pathogen present, we could use a consistent "front-end" approach to extract and amplify the pathogen nucleic acids irrespective of whether it had an RNA or DNA genome. It was also important that we should have an assay capable of being run to examine samples where other specific assays had yielded negative results; thus we would have no *a priori* knowledge of the pathogen present. DNA microarrays were chosen because they offered great potential to meet the above two criteria, although not without their own inherent disadvantages. The main problems were that they were not rapid assays, requiring a minimum of 16h incubation as well as the fact that as they had never been used for these type of clinical assays, we felt that the reproducibility was likely to be very low. Additionally, the only reported use for DNA microarrays at this time (2001) was to analyse changes in gene expression. The rationale behind the use of DNA microarrays for diagnostic purposes was that a single channel assay would be run; expression microarrays utilise two channels, one of which is based upon the extracted mRNA the other on cDNA. Comparison of the former against the latter gives the measure of gene expression relative to the number of gene copies within the genome. For diagnostic use, nucleic acids extracted from a sample would be amplified (this also allows conversion of RNA genomes into cDNA for hybridisation), labelled and hybridised, with the readout being a plus/minus hybridisation rather than a relative value. In this hypothetical system, it is of course possible to use the second channel as a "control", however, since the system was envisaged as a diagnostic where no preconceived decision was required, what would constitute a suitable control?

In order to develop the microarray assay, a number of key decisions needed to be made. Firstly, how would the microarray physically be constructed? Amino-silane coated glass slides (and subsequently Epoxy-coated slides) were chosen for construction of the initial arrays which would be spotted using a direct contact printer. Microarrays could be fabricated using cDNA clones, PCR fragments or long oligonucleotide probes (it should be noted that nomenclature for these assays can be somewhat different to conventional hybridisation assays; here the term probe applies to the nucleic acid fragment bound to the solid matrix). Oligonucleotides were chosen as the other two alternatives were felt to offer less control over the probe design and were more expensive to produce (Tomiuk & Hofmann, 2001). Initially the technology was evaluated using a relatively small number of different probes, all of which were based on detection of variable regions within the 16S rRNA genes of six key bacterial species (*Bacillus*, *Escherichia*, *Streptococcus*, *Mycobacterium*, *Pseudomonas* and *Neisseria*). Two 50-mer oligonucleotide probes were designed for each of the six species; these were spotted in triplicate into 16 replicate sub-arrays on the amino-silane glass slide matrices. Another key decision to be made surrounded the choice of hybridisation conditions, under which the assay would be run. The choice of 50-mer oligonucleotide probes allowed absolute control, during the design phase, of parameters such as the probe melting temperature (T_m) and secondary structure. Numerous protocols were available for the hybridisation of expression arrays; these had little comparability with each other and conditions were therefore chosen which offered medium stringency conditions for the hybridisation and wash steps of the assay. A concise review was published by Hegde *et al*. in 2000 and covered many of the available decisions and options that were then currently available.

Test hybridisation assays were performed using generic primers to PCR amplify an approximately 800bp fragment (including both of the designed probe sequences in each case) from the 5' end of the 16S rRNA gene. The resulting PCR products also needed to be labelled post-amplification in order to visualise the hybridisation reaction. Once again there were numerous

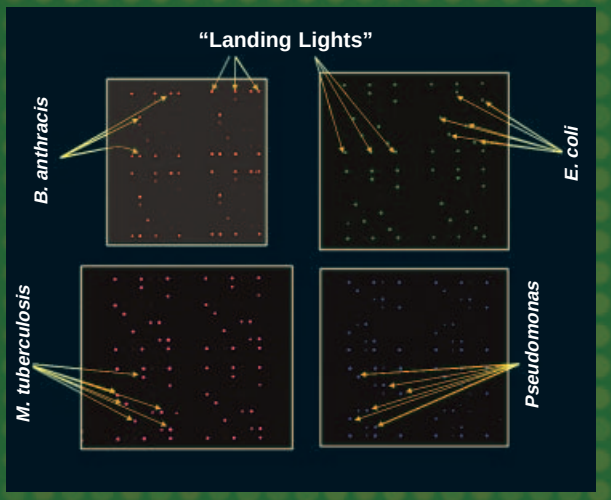

Figure 2. Panel of 4 microarray assays

Identical arrays are shown here, but incubated with DNA from four different bacterial pathogens. The spot location is indicative of the pathogen identity as the images are false-coloured for clarity. Landing light spots are indicated, these are used to locate the grids used in the analysis software

the trials and tribulations of diagnostic DNA microarray technology

methods available to label these fragments — many of which have a long provenance for use in Southern & Northern blotting techniques. Initially, we used amino-allyl dUTP in the PCR mix in order to incorporate a nucleotide which could then easily be labelled post-amplification; the fluorescent dye, Cyanine 3 (Cy3) was used for these labelling reactions. Incorporation of amino-allyl dUTP is relatively inefficient using *Taq* polymerases, as evidenced by the poor levels of dye incorporation when fragment and dye concentrations were determined prior to hybridisation. An alternative method was also investigated which used the Klenow fragment of DNA polymerase I for incorporation of these modified nucleotides employing a random priming reaction which used random nonomers to prime the second strand synthesis. This method resulted in better dye incorporation, but post-amplification coupling of dye to modified nucleotides never yielded particularly highly labelled fragments. The best method, in our hands, for ensuring that fragments were labelled with high dye incorporation ratios, was to incorporate Cy3 or Cy5 labelled dCTP directly at the PCR amplification stage. An example of the differences in dye incorporation can be seen in the figure which was produced by drying a small spot of the reaction (after removing any incorporated dye) on to a slide and scanning in the usual way.

This method of dye incorporation finally yielded our first signals with the microarray assay; every hybridisation up to this stage had resulted in a blank picture. Actually we felt that we had done quite well, as in the space of four months, we had learnt to use the microarray spotter (these are not for the faint hearted!), spotted numerous arrays, been through three different incarnations of labelling reactions and finally got a result. When we talked about our trials and tribulations with the wider microarray community, this was felt to be rather fast progress as one colleague told us, "it took us 18 months to get to the same stage". So we had the bones of an assay and it was actually giving really good, clean hybridisation patterns, albeit with probes for only six different bacterial pathogens. The next step was to try and move away from using specific PCR amplification of genes, to a generic amplification method. We knew that this would result in a decreased signal and probably higher background, however we decided that this step was critical for the development of this assay. Throwing caution to the wind, we also decided to increase the number of probes within the assay on a dramatic scale at the same time. Firstly the amplification reaction; this was a difficult decision to make (whether an amplification step should be used) as ideally one would not wish to skew results by introducing an amplification step. Neil Boonham and colleagues at the Defra Central Science Laboratories managed to achieve this with their plant pathogen array (Boonham *et al.*, 2003), however, the concentration of pathogen nucleic acids present in clinical samples is often extremely low and thus we felt that in order to achieve our aim of a true multiplexed microarray assay, that some amplification was needed. This would also allow RNA genomes to be reverse-transcribed (into cDNA), amplified and labelled in the same way as DNA.

A method based upon random amplification was chosen to avoid having to make prior decisions regarding primers sets to be used. A number of published methods were available in the literature and on microarray group websites. The method that we used was modified from that described by David Wang in his seminal paper from 2002, which was itself derived from an earlier described method (Bohlander *et al.*, 1992). This method is based upon a three-step amplification, using random short oligonucleotides (nonomers) tagged with site-specific tails in the first round, a specific PCR primer which is complementary to the site-specific tail in the second and incorporating the dye in the third. Wang and colleagues modified this method by using a reverse transcriptase in the first step instead of Klenow. We further modified both published methods by using a mixture of Klenow and a reverse transcriptase in the first step, thus allowing the amplification of both DNA and RNA genomes and then using the second round (PCR amplification step) to incorporate the dye.

The second aspect was to increase the number of probes to include known virulence genes, genotyping markers and probes for a wide range of viral pathogens. Coincident, was the publication of David Wang's viral probes set — 600 oligonucleotides covering approximately 140 viral pathogens. These probes were also synthesised and incorporated directly into our microarray assay which now comprised 2200 unique probes and covered around 150 different bacterial and viral pathogens.

The proof is in the pudding, as the saying goes, and we were not disappointed, even though there were obvious problems with the assay. One of the most obvious issues with the assay was that having vastly increased the number of probes on the array, we now had a number which showed significant cross-reactivity; this was despite all of the probes having been designed to have minimal secondary structure, equivalent T_m values and being checked by Basic Local Alignment Search Tool (BLAST) analysis against the GenBank database. To cut a very long story and considerable effort short, removal of some probes and development of the assay conditions allowed us to get the assay working well again.

Several different parameters of the assay have been evaluated. Sensitivity was felt to be one of the biggest potential issues and we published some of these data in 2005 (Burton *et al.*, 2005); direct PCR amplification of a specific gene allowed detection of femtogram quantities of pathogen nucleic acids, with the random amplification method, the limit of detection was in the low pictogram range and in the nanogram range with no amplification. These limits of detection correspond at best to approximately 10^2 organisms per mL of sample. Similarly, another potential issue was the time taken to perform the assay. Most interestingly, time course analysis of the hybridisation kinetics showed us that the favoured 16h overnight incubation was much longer than was needed and actually gave poorer results in terms of signal output. For conventional hybridisations, 2h was found to give optimal signal. Subsequently, moving the assay to an automated platform, where the hybridisation is not static improved the kinetics enormously. Using this technology, hybridisation results can now be obtained in approximately 20min.

In summary, what are the real prospects for the future of "lab-on-a-chip" assays? Hopefully what we have shown is that there are some really good prospects for this type of technology. Some of the original fears turned out to be ill-founded, as the assay can be made to be sensitive, rapid and highly reproducible, although the largest errors in the assay are unsurprisingly due to operator variation. The current choice of matrix (glass slides) are not suitable for any sort of robust assay due to their inherent fragility, but the assay could theoretically be transferred onto a bead-based system or similar, which would render it a lot more user-friendly. The biggest hurdle which still needs to be overcome is the problem associated with "contaminating" DNA present in clinical samples. This is, of course, amplified with the pathogen nucleic acid and really decreases the apparent assay sensitivity. Numerous groups have now reported such problems and until a work-around is devised, the utility of the assay remains rather limited. At the beginning I posed the question: how real was the concept of a small size assay capable of running numerous tests in parallel? Clearly there has been considerable progress towards this desire, in fact it can and has been partially achieved and I think that it certainly has a huge future potential.

references

- Bohlander, S.K., Espinosa, III R., Le Beau, M.M., Rowley, J.D. and Diaz, M.O. (1992) A method for the rapid sequence-independent amplification of microdissected chromosomal material. *Genomics* **13**, 1322–1324.
- Boonham, N., Walsh, K., Smith, P., Madagan, K, Graham, I. and Barker, I. (2003) Detection of potato viruses using microarray technology: towards a generic method for plant viral disease diagnosis. *J. Virol. Methods* **108**, 181-187.
- Hegde, P., Qi, R., Abernathy, K., Gay, C., Dharap, S., Gaspard, R., Hughes, J.E., Snesrud, E. et al. (2000) A concise guide to cDNA Microarray Analysis. *Biotechniques* **29**, 548–562.
- Tomiuk, S. and Hofmann, K. S (2001) Microarray probe selection strategies. *Brief. Bioinform* **2**, 329-340.
- Burton, J. E., Oshota, O. J., North, E., Hudson, M., Polyanskaya, N., Brehm, J., Lloyd, G. and Silman, N. J. (2005) Development of a multipathogen oligonucleotide microarray for detection of *Bacillus anthracis*. *Mol. & Cell. Probes* **19**, 349 – 357.

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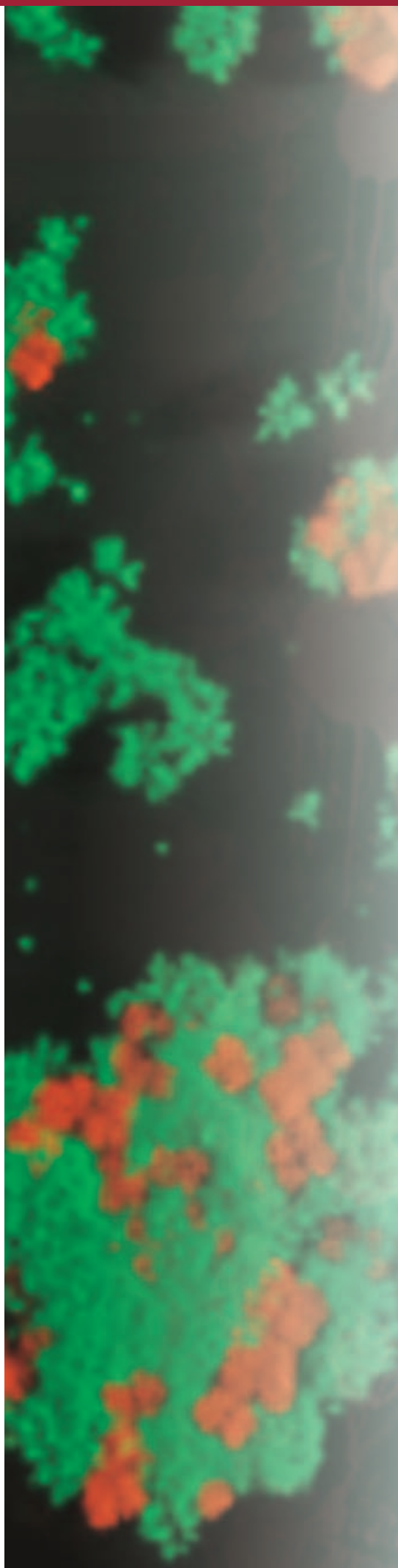


Bacterial sociology in a biofilm world

Alex Rickard, a recipient of a S*f*AM New Lecturer Research Grant, is an assistant professor at Binghamton University and is studying inter-species bacterial interactions. **Kyung Min** is a PhD student at Binghamton University and is studying coaggregation between freshwater bacteria

The words *sociology* and *biofilm* are seldom used in the same sentence, let alone together in a title. The word *sociology* is typically used as a term to describe human interaction and community composition but in general relates to social organisation, collective behavior and interaction between individuals. This word is also used to relate to developing an understanding of individuals' relationship to a group. The word *biofilm* is often used to describe a thin but robust layer of mucilage adhering to a solid surface which contains bacteria and other microorganisms that generated it. How can these two words be linked? They can be linked by the word *community*.

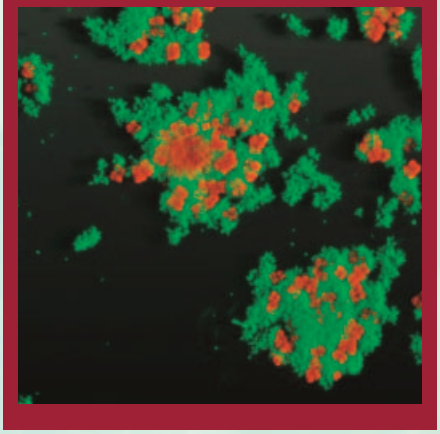
Most biofilms consist of multiple species of bacteria. For example there can be as many as 500 species of bacteria within dental plaque biofilms. Each species within this community has a unique repertoire of abilities. Species can coordinate these abilities with other species, under specific environmental conditions, to maintain a niche or even expand within a multi-species biofilm. For instance, the human oral bacterium *Veillonella atypica* is unable to ferment carbohydrates found in normal saliva and instead the species metabolises organic acids such as lactate. Lactate is a waste product of carbohydrate fermentation by oral streptococci, which also happen to be the numerically dominant species in healthy dental plaque (50-80% of the species). *V. atypica* cells recognise and specifically bind to the surface of streptococcal cells and use the *waste* lactic acid to promote its own growth. Without lactic acid, *V. atypica* cells would not grow and would be unlikely to be able to maintain a niche within dental plaque. But the story does not end there.

Early evidence also suggests that *V. atypica* may, by the act of attachment or by the production of chemical signals, upregulate *S. gordonii* α-amylase-gene-expression which is hypothesised to provide more sugar to be converted to lactic acid (Egland *et al.*, 2004). This interaction could be considered to constitute a form of inter-bacterial signaling by which *V. atypica* cells can enhance their ability to grow. Furthermore, this signaling phenomenon highlights a property that many bacteria possess – the ability to communicate with other bacterial species. Coupled with the increasing acceptance that most bacteria exist in biofilms (Costerton *et al.*, 1987), some researchers now believe that inter-species bacterial communication facilitates highly reproducible biofilm community development (Irie & Parsek, 2008; Kolenbrander *et al.*, 2006; Parsek & Greenberg, 2005).

How do bacterial species communicate with one another and become fit for *existence* in a multi-species biofilm? Simply put, there are many ways a species can communicate and potentially "socialise" — these are too numerous for this short article. However, inter-species bacterial communication can be broken down into two broad categories: physical communication strategies and chemical communication strategies. Through the use of one or both of these forms of communication, the potential for a species to maintain a niche in a biofilm community can be enhanced. It should also be noted that increasing evidence suggests that, as with any social gathering and associated forms of communication, there can be cheater species which manipulate communication mechanisms to integrate and potentially dominate within a community (Hochberg *et al.*, 2008). Thus, it is the aim of this short opinion/review to give a brief overview of inter-species physical and chemical communication, including a description of how species may ingratiate themselves or cheat their way into biofilm communities through masquerading or manipulating communication mechanisms. Oddly enough, many of these cheaters are also thought of as problematic or pathogenic species.

Figure 1.

Confocal Scanning Laser Micrograph showing the freshwater bacteria Sphingomonas natatoria 2.1 (green cells) and Micrococcus luteus 2.13 (red cells) coaggregating together and settling onto a glass-microscope slide. S. natatoria 2.1 forms small clusters of rosettes and M. luteus 2.13 forms groups of tetrads. When mixed together, the two coaggregate to form macroscopic flocs that are easily seen by eye in a test-tube.



Physical communication

Do biofilm communities consist of bacterial species that randomly clump and grow together or is there evidence that specific species exhibit a *preference* to clump with other specific species? As with most living systems, organisation can enhance functionality. Functionality improves success and success leads to self perpetuation and an improved chance of survival. Thus, if specific species could recognise and positively interact with one another, it is conceivable that their co-localisation will improve the pair's survival and maybe a form of mutualism or even altruism will develop. Indeed, since early studies of oral bacteria by Gibbons and Nygaard (1970), it has become increasingly clear that there is potential for different species to specifically recognise and adhere to one-another. This process is called coaggregation. Using visual aggregation assays, it has now also become evident that coaggregation is not just a phenomenon that occurs between oral bacteria. Coaggregation is a phenomenon that occurs between bacteria in many biofilm communities, including those from freshwater, gut, urogenital tract and wastewater ecosystems (Rickard *et al.*, 2003).

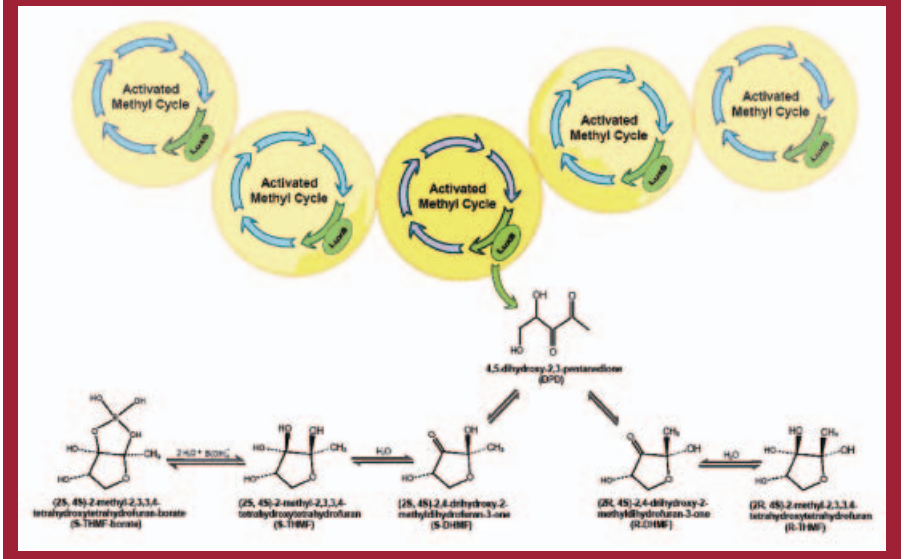
In order for coaggregation to occur, lectin-like adhesins must be expressed on the cell

surface of one species and complementary polysaccharide receptors must be expressed on the partner species' cell-surface (Kolenbrander *et al*., 2006). While this seems simple enough and studies have shown that coaggregation between oral bacteria is due to constitutively expressed adhesin and receptors, coaggregation between freshwater bacteria is growth-phase dependent (Rickard *et al*., 2003). Furthermore, expression of coaggregation by freshwater bacteria can be asynchronous, with one species only expressing adhesins when in a physiological state that is akin to stationary phase, and the other species only expressing receptors when actively growing, much like when in exponential-phase. But when time and conditions are suitable (which raises the interesting question which will not be addressed here: Why *certain* conditions?), freshwater species coaggregate (Figure 1). The question then arises, "So what? Is there a role for coaggregation other than bringing species together?" Early evidence indicates that there are certainly at least two good reasons to coaggregate. First is the obvious reason: fluid shear. In flowing environments such as the oral cavity or in a flowing stream, surfaces can be exposed to high fluid shear. Shear can prevent cells from attaching to surfaces, adhering to surface bound bacteria, or integrating into pre-existing biofilms. Because the polymers that mediate coaggregation often facilitate strong, localised physico-chemical interactions between cells (often through interactions that are similar to those mediated by lectin-saccharide interactions), the ability to coaggregate will likely enhance the potential for a species to colonise surfaces in flowing environments (Rickard *et al*., 2003). The second reason why the ability to coaggregate may be favorable for a species, relates to the importance of *juxtaposition*. Cells of different species that are in close proximity are more likely to detect and communicate with one another through chemical cell-cell signal molecules than those cells that are distantly located.

Chemical communication

Chemical inter-bacterial communication is at present receiving much attention from microbiologists. This form of inter-bacterial communication includes the production and use of metabolites, or the production and detection of low molecular weight extra-cellular cell-cell signal molecules (Kolenbrander *et al*., 2002). A variety of forms of extra-cellular cell-cell signal molecules are being studied with great interest and many mediate communication between cells belonging to the same strain or species. These signal molecules include competence stimulating peptides and acyl-homoserine-lactones (for review see Irie & Parsek, 2008). However, when considering that biofilms are typically composed of multiple species, candidate inter-species cell-cell signal molecules should also be investigated. This area of study has been spearheaded with the recent discovery of 4,5-dihydroxy-2,3-pentanedione (DPD), also called autoinducer-2 (AI-2). AI-2 is produced by LuxS, an enzyme that is part of the activated methyl cycle in many bacterial species. Chemical analysis and synthesis of AI-2 has led to the realisation that AI-2 is able to spontaneously generate a variety of inter-convertible structural forms that are in equilibrium. Different environmental conditions likely favour different forms (Figure 2). Thus AI-2 is an umbrella term, and increasing evidence suggests that many bacterial species produce AI-2 and/or can recognise different forms of the molecule.

Figure 2.



Autoinducer-2 (AI-2) is an inter-species signal molecule. AI-2 is a family of inter-convertible molecules formed by the rearrangement of DPD. In the diagram, a metabolically active streptococcal cell (bright yellow cell) is producing AI-2 by virtue of activity of the activated methyl cycle (specifically the LuxS enzyme). The far left form of AI-2 (S-THMF-borate) is recognized by Vibrio harveyi while the far right form (R-THMF) is recognised by Salmonella Typhimuruim. The form(s) of AI-2 that streptococci recognise and use to chemically communicate is unknown.

> **"Loneliness is never more cruel than when it is felt in close propinquity with someone who has ceased to communicate".**
> *Germaine Greer, The Female Eunuch (1971).*

Of recent interest is the possibility that AI-2 mediates communication between oral bacteria. This is, in part, because most human oral AI-2 producing species are also known to coaggregate and consequently juxtapose in multi-species biofilms. Summed concentrations of AI-2, when cells are in close proximity, could drive a "chemical discourse" (Bassler, 2004). Such a hypothesis, while being hotly debated, is being investigated and appears to be a valid and intriguing avenue of oral microbiology research (Kolenbrander *et al*., 2006; McNab & Lamont, 2003; Williams *et al*., 2007). Furthermore, because different oral species seemingly produce different quantities of AI-2 and also modify their local environment, different concentrations of specific forms of AI-2 may elicit different responses from a particular organism. For example, AI-2 has been demonstrated to mediate mutualism (also described as luxuriant interdigitated growth, Figure 3) between the commensal coaggregating oral bacteria *Streptococcus oralis* 34 and *Actinomyces naeslundii* T14V, when grown together in a biofilm that is fed human saliva (Rickard *et al*., 2006). Both species produce AI-2, but when *S. oralis* 34 is replaced with an isogenic (i.e., genetically identical) *luxS* mutant that is unable to produce AI-2, mutualism is abolished. Mutualism can be restored by the addition of high picomolar to low nanomolar concentrations of chemically synthesised AI-2 (Rickard *et al*., 2006). The potential for other streptococci/oral bacteria to respond to nanomolar concentrations of chemically synthesized AI-2 has also been demonstrated in *Streptococcus intermedius* (Pecharki *et al*., 2008) and *Streptococcus mutans* (Sztajer *et*

al., 2008). Of particular interest is the work by Yoshida *et al* (2005) that elegantly demonstrated that AI-2 producing oral streptococci and AI-2 producing oral pathogens *Porphyromonas gingivalis* and *Actinobacillus (Aggregatibacter) actinomycetemcomitans* restore the biofilm-forming ability of a *luxS* mutant of *S. mutans* to that of the wild-type strain. To add an extra dimension and even more intrigue to this story, very recent work by Maeda *et al*., (2008) has indicated that *P. gingivalis* cells may moderate AI-2 production during initial colonisation of oral biofilm communities. This could possibly enhance integration of *P. gingivalis* into the community.

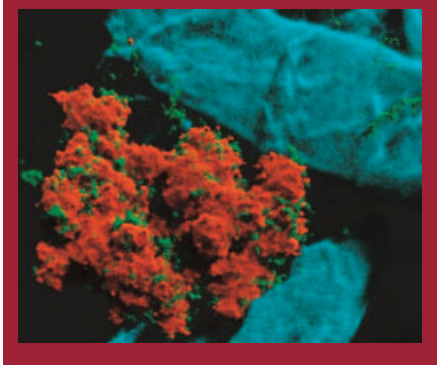
Social dynamics and an ever-developing community.

Multi-species biofilms originate from the aggregation of cells with unique properties (i.e. species). Evidence indicates that coaggregation can bring specific partner cells into close proximity and cell-cell signaling molecules mediate signaling among juxtaposed cells. In oral communities, streptococcal species predominate and this is in part due to their ability to coaggregate with one another (e.g. *S. oralis* coaggregates with *S. gordonii* which coaggregates with *S. sanguinis* and *S. mitis*). This numerical dominance may also relate to the production and detection of cell-cell signal molecules as well as being metabolically adept at growth in saliva.

Within any community there is potential for exploitation by cheaters. These include organisms that can manipulate communication mechanisms to integrate themselves into a community usually at the detriment of the other species within the invaded community. Such a possibility is now recognised to occur between many multi-cellular organisms. For example, staphylinid beetles can chemically and physically mimic activities of ants to gain access to the highly protected colonies and consume stored food and destroy ant larvae (Holldobler & Wilson, 1990; Witte *et al*., 2008). This is only wild conjecture, but a similar phenomenon may occur in oral bacterial communities, where coaggregating and signal producing pathogens attempt to integrate into biofilms that are numerically dominated by commensal *Streptococcus* species. Studies have already indicated that many oral pathogenic species such as *P. gingivalis* and *Actinobacillus (Aggregatibacter) actinomycetemcomitans* are able to coaggregate as well as produce and respond to AI-2 (James *et al*., 2006; McNab *et al*., 2003; Shao et al., 2007). If these pathogenic species can gain acceptance into a commensal oral biofilm and then grow and divide at an enhanced rate (possibly at the detriment of the streptococcal species), then the commensal community will reduce in size and the pathogenic community will dominate. Thus, it is possible that these pathogenic species integrate into commensal oral biofilms by utilising physical and/or chemical communication mechanisms and, much like the staphylinid beetle, destroy the community from within.

Figure 3.

Confocal Scanning Laser Micrograph showing luxuriant inter-digitated growth (mutualism) between the coaggregating oral bacteria Streptococcus oralis 34 (green cells) and Actinomyces naeslundii T14V (red cells) on nitrocellulose acetate filters (blue fibres) within a sorbarod biofilm system (Rickard et al., 2008). Luxuriant inter-digitated growth is mediated by the production of AI-2 by S. oralis 34.



The importance of physical and chemical communication in the development of bacterial biofilm communities outside the human oral cavity is also receiving increasing attention. The role and function of AI-2 in other communities is less clear and evidence indicates that some species neither produce nor respond to AI-2 and do not coaggregate. These bacterial species may use other physical and chemical communication mechanisms. Regardless, it is now becoming increasingly clear that bacteria are not solitary units of life and favour seemingly social biofilm gatherings as a pretext to forming complex multi-species communities.

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references

■ Bassler, B. L. (2004). Cell-to-cell communication in bacteria: a chemical discourse. *Harvey Lect* **100**, 123-142.

■ Costerton, J. W., Cheng, K. J., Geesey, G. G., Ladd, T. I., Nickel, J. C., Dasgupta, M. and Marrie, T. J. (1987). Bacterial biofilms in nature and disease. *Annual Rev Microbiol* **41**, 435-464.

■ Egland, P. G., Palmer, R. J., Jr. and Kolenbrander, P. E. (2004). Interspecies communication in *Streptococcus gordonii-Veillonella atypica* biofilms: signaling in flow conditions requires juxtaposition. *Proc Natl Acad Sci U S A* **101**, 16917-16922.

■ Gibbons, R. J. and Nygaard, M. (1970). Interbacterial aggregation of plaque bacteria. *Arch Oral Biol* **15**, 1397-1400.

■ Hochberg, M. E., Rankin, D. J. and Taborsky, M. (2008). The coevolution of cooperation and dispersal in social groups and its implications for the emergence of multicellularity. *BMC Evol Biol* **8**, 238.

■ Holldobler, B. and Wilson, E. O. (1990). *The Ants*. T Berlin/Heidelberg/New York: Springer.

■ Irie, Y. and Parsek, M. R. (2008). Quorum sensing and microbial biofilms. *Curr Top Microbiol Immunol* **322**, 67-84.

■ James, C. E., Hasegawa, Y., Park, Y., Yeung, V., Tribble, G. D., Kuboniwa, M., Demuth, D. R. and Lamont, R. J. (2006). LuxS involvement in the regulation of genes coding for hemin and iron acquisition systems in *Porphyromonas gingivalis*. *Infect Immun* **74**, 3834-3844.

■ Kolenbrander, P. E., Andersen, R. N., Blehert, D. S., Egland, P. G., Foster, J. S. and Palmer, R. J., Jr. (2002). Communication among oral bacteria. *Microbiol Mol Biol Rev* **66**, 486-505, table of contents.

■ Kolenbrander, P. E., Palmer, R. J., Jr., Rickard, A. H., Jakubovics, N. S., Chalmers, N. I. and Diaz, P. I. (2006). Bacterial interactions and successions during plaque development. *Periodontol 2000* **42**, 47-79.

■ Maeda, K., Tribble, G. D., Tucker, C. M., Anaya, C., Shizukuishi, S., Lewis, J. P., Demuth, D. R. and Lamont, R. J. (2008). A *Porphyromonas gingivalis* tyrosine phosphatase is a multifunctional regulator of virulence attributes. *Mol Microbiol* **69**, 1153-1164.

■ McNab, R., Ford, S. K., El-Sabaeny, A., Barbieri, B., Cook, G. S. and Lamont, R. J. (2003). LuxS-based signaling in *Streptococcus gordonii*: autoinducer 2 controls carbohydrate metabolism and biofilm formation with *Porphyromonas gingivalis*. *J Bacteriol* **185**, 274-284.

■ McNab, R. and Lamont, R. J. (2003). Microbial dinner-party conversations: the role of LuxS in interspecies communication. *J Med Microbiol* **52,** 541-545.

■ Parsek, M. R. and Greenberg, E. P. (2005). Sociomicrobiology: the connections between quorum sensing and biofilms. *Trends Microbiol* **13**, 27-33.

■ Pecharki, D., Petersen, F. C. and Scheie, A. A. (2008). LuxS and expression of virulence factors in *Streptococcus intermedius*. *Oral Microbiol Immunol* **23**, 79-83.

■ Rickard, A. H., Gilbert, P., High, N. J., Kolenbrander, P. E. and Handley, P. S. (2003). Bacterial coaggregation: an integral process in the development of multi-species biofilms. *Trends Microbiol* **11**, 94-100.

■ Rickard, A. H., Palmer, R. J., Jr., Blehert, D. S., Campagna, S. R., Semmelhack, M. F., Egland, P. G., Bassler, B. L. and Kolenbrander, P. E. (2006). Autoinducer 2: a concentration-dependent signal for mutualistic bacterial biofilm growth. *Mol Microbiol* **60**, 1446-1456.

■ Rickard, A. H., Campagna, S. R. and Kolenbrander, P. E. (2008). Autoinducer-2 is produced in saliva-fed flow conditions relevant to natural oral biofilms. *J Appl Microbiol* **105**, 2096-2103.

■ Shao, H., Lamont, R. J. and Demuth, D. R. (2007). Autoinducer 2 is required for biofilm growth of *Aggregatibacter (Actinobacillus) actinomycetemcomitans*. *Infect Immun* **75**, 4211-4218.

■ Sztajer, H., Lemme, A., Vilchez, R., Schulz, S., Geffers, R., Yip, C. Y., Levesque, C. M., Cvitkovitch, D. G. and Wagner-Dobler, I. (2008). Autoinducer-2-regulated genes in *Streptococcus mutans* UA159 and global metabolic effect of the luxS mutation. *J Bacteriol* **190**, 401-415.

■ Williams, P., Winzer, K., Chan, W. C. and Camara, M. (2007). Look who's talking: communication and quorum sensing in the bacterial world. *Philos Trans R Soc Lond B Biol Sci* **362**, 1119-1134.

■ Witte, V., Leingartner, A., Sabab, L., Hashim, R. and Foitzik, S. (2008). Symbiont microcosm in an ant society and the diversity of interspecific interactions. *Animal Behaviour* **76**, 1477e1486.

■ Yoshida, A., Ansai, T., Takehara, T. and Kuramitsu, H. K. (2005). LuxS-based signaling affects *Streptococcus mutans* biofilm formation. *Appl Environ Microbiol* **71**, 2372-2380.

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

Comparison of regression lines

Statnote 18

The relationship between two variables may have been studied at various times or in different laboratories giving rise to two or more independent estimates of the relationship between Y and X. In these circumstances, it may be of interest to discover whether the different regression lines are the same, and if they are, an investigator may wish to combine the data from the different studies and fit a single regression line to the whole of the data. In addition, an investigator may wish to determine the relationship between Y and X given one set of conditions compared to the relationship under a different set of conditions. Hence, regression lines can be compared not only with the intent of combining data but to reveal that they are in fact different, potentially as a consequence of the different conditions. This Statnote describes the statistical methods associated with comparing two or more regression lines.

Scenario

To illustrate the method, we return to the scenario first described in Statnote 14 (Hilton & Armstrong, 2008). Essentially, adequate skin antisepsis prior to invasive procedures is important in preventing infections. Nevertheless, skin antiseptics permeate poorly into the deeper layers of the skin and into hair follicles, which may harbour microorganisms and cause infection when the protective skin barrier is broken. One potential mechanism of delivering antiseptics deeper into the skin is to co-administer a 'carrier' compound to facilitate movement of the biocide through the various skin layers. Hence, the aim of the study was to evaluate the permeation of a commonly used biocide into the full thickness of human skin when applied alone, or in combination with a 'carrier' compound.

Full thickness human skin samples were obtained from patients undergoing breast reduction surgery. The skin permeation studies were performed using vertical diffusion cells with the stratum corneum of the skin sample uppermost. One millilitre of antiseptic solution in the presence or absence of the carrier compound was aliquoted onto the skin, and incubated for two minutes, 30 minutes, or 24 hours. The assay was performed in triplicate. Following the exposure to the antiseptic solution (+/- carrier) the skin was washed with PBS and three 7mm punch biopsies taken from each sample. The biopsies were cut with a microtome into 20μm slices from the skin surface to a depth of 600μm and 30μm slices from 600μm to 1500μm. The weight of the skin samples was determined and each sample was analysed by HPLC to determine the concentration of antiseptic present as μg antiseptic per mg of tissue.

A number of mathematical models might describe the pattern of penetration of the antiseptic into the skin. In the correlation analysis described in Statnote 14, the data suggested that a model based on passive diffusion poorly described the penetration profile observed and further models were then tested and specifically, whether the antiseptic alone would have penetrated the skin according to a *'power-law'* model similar to the inverse square law. A variable Y is distributed as a power-law function of X if the dependent variable has an exponent 'a', i.e., a function of the form $Y = CX^{a}$. If penetration of the antiseptic does follow such a law, then a log-log plot of the data should result in a linear relationship between Y and X. In Statnote 16 (Hilton & Armstrong, 2009), this model was fitted to a single set of data, i.e., without carrier. In the present Statnote, we extend this analysis to test whether the presence of the carrier 'EO' affected the degree of penetration through the skin.

Data

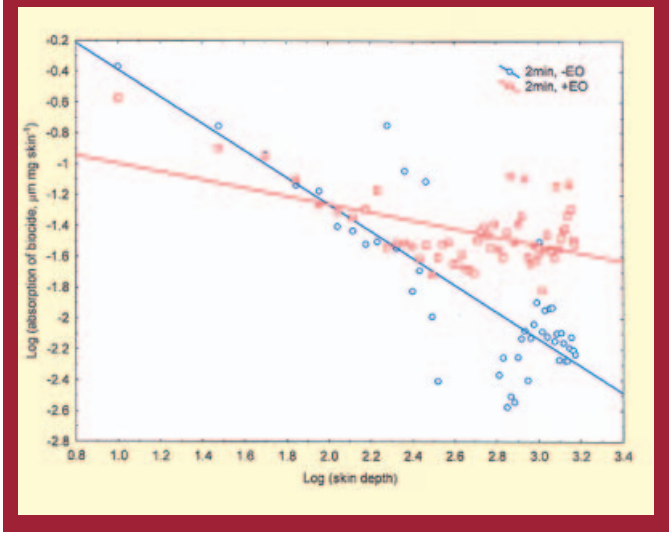
The data comprise several pairs of measurements of two variables, *viz.*, the concentration of antiseptic (Y) and skin depth (X) with (Y_1,X_1) under two conditions, *viz.*, with and without the carrier 'EO' (Y2,X2) and the data are illustrated in Figure 1.

Analysis

How are the test statistics calculated?

Regression lines may differ in three properties. First, they

Figure 1. *Relationship between the degree of penetration of the biocide with (+EO) and without (-EO) carrier and depth of skin (Pearson's correlation coefficient -EO r = -0.84, r^2 = 0.71; +EO r = 0.53, r^2 = 0.28).*



may differ in residual variance, i.e., in how good a fit the lines are to the data and therefore, one line may fit the data better than the other. Second, they may differ in slope and hence one line may exhibit a greater change in *Y* per unit of *X* than the other. Third, the lines may differ in elevation, i.e., if the two lines have similar slopes and are therefore parallel they will intersect the *Y* axis at different points. Whether the lines differ in 'goodness of fit' can be tested by comparing their residual variances using a two-tail 'F' test (see Statnote 5, Hilton & Armstrong, 2006). Whether the two lines differ in slope or elevation can be tested using an analysis based on analysis of covariance (an extension to analysis of variance in which a covariate *Y* is included in the analysis) (Snedecor & Cochran, 1980). Essentially, individual regression lines are fitted to each set of data separately and a further common line is then fitted to the data pooled from both sets of data; the analysis essentially comparing the individual fits with that of the pooled regression. Differences in the slopes and elevations of the lines can then be tested. If there is a significant difference between the slopes of the regression

Table 1. Analysis of covariance comparing the slopes and elevations of two regression lines

Variation	Sums of squares (SS)	DF	Mean square (MS)	'F'
Between slopes	2.2375	1	2.2375	39.63***
Error	5.7596	102	5.65×10^{-2}	
Between elevations	4.4217	1	4.4217	
Error	7.9971	103	7.7642×10^{-2}	

lines, then it is not necessary to test the elevations as these will vary at each value of *X*. If the lines have similar slopes, then their elevations can also be tested. If two or more regression lines are shown to have the same slopes and elevations, then they are statistically similar and the investigator may wish to combine the data in a single regression analysis.

Interpretation

The first stage in the analysis is to fit a linear regression to each set of data separately and the lines of best fit are shown in Figure 1. Examination of these lines suggests that they differ in slope. The values of '*r*' and 'r^2' indicate that both sets of data can be fitted adequately by straight lines, although the linear regression is a better fit to the data without the carrier 'EO'. A regression line is then fitted to the data pooled from both data sets and this enables the slopes and elevations of the lines to be compared. The analysis of covariance table is shown in Table 1. There is a highly significant difference between the slopes of the two regression lines ($F = 39.63$, $P < 0.001$). The comparison between heights or elevations of the two regressions is also included here but would not be relevant in this example as the lines have different slopes. Note that if the slopes of the two lines were not significantly different, i.e., the lines were parallel, then it would be important to test the difference in elevation.

Hence, the regression of log absorption of biocide declines less rapidly with log skin depth when the carrier compound 'EO' is present. Note that the *Y* axis represents a negative logarithmic scale, and hence the lower negative numbers represent a greater degree of absorption. Hence, we can conclude from this experiment that the presence of the carrier 'EO' does alter the kinetics of penetration of the antiseptic into the skin and increases the amount of antiseptic reaching the deeper levels of the skin.

Conclusion

In many circumstances, it may be of interest to discover whether two or more regression lines are the same. Regression lines may differ in three properties, *viz.*, in residual variance, in slope, and in elevation; all of which can be tested using analysis of covariance. If there are no significant differences between regression lines, an investigator may wish to combine the data from different studies and fit a single regression line to the whole of the data.

references

- Hilton, A. and Armstrong, R.A. (2006) Statnote 5: Is one set of data more variable than another? *Microbiologist* **Vol. 7** No.2, pp34-36.
- Hilton, A. and Armstrong, R.A. (2008) Statnote 14: The correlation of two variables (Pearson's 'r'). *Microbiologist* **Vol. 9** No.3, pp34-36.
- Hilton A and Armstrong R.A. (2009) Statnote 16: Fitting a regression line to data. *Microbiologist* **Vol. 10** No. 1, pp40-42.
- Snedecor, G.W. and Cochran, W.G. (1980) Statistical methods, 7th Ed. Iowa State University Press, Ames Iowa.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careers

Science Communication

Editor of *Microbiologist* and Communications Manager of S*f*AM, **Lucy Harper** explains how and why she came to be in such a role and discusses the key skills and qualities required

I began my career taking the traditional science route — I studied my undergraduate degree in Medical Biochemistry at the University of Birmingham and remained in Birmingham for many years taking a number of short-term research posts. The last of these posts generated sufficient results for publication and a PhD, and I was awarded my PhD from Aston University in 2002.

I've always loved writing and was one of the few bench scientists I knew at the time who actually preferred writing up results to generating them. During my PhD and subsequent post-doctoral positions in microbiology, I wrote a number of non-peer-review articles for various publications, including the then 'S*f*AM News' and later, *'Microbiologist'*. I loved the creativity of writing and the way I could essentially paint a picture with words. I also relished the opportunity to communicate complex scientific principles to a non-specialist audience enabling all to understand the principles behind the science I was explaining.

During what was to become my final post-doctoral position, I was given the opportunity to apply for the position of Honorary Editor of *Microbiologist* and I must admit I didn't immediately jump at the chance. It's a big responsibility editing a magazine which will reach thousands of people worldwide. I was very wary of potentially making a mistake when producing such a visible 'product' as this could reflect badly on the Society. However, despite my initial hesitancy, I decided to bite the bullet and was successful.

To say I enjoy working on the *Microbiologist* is an understatement and probably reflected in the fact that I'm still doing it five years on. I love the creativity of the job and the fact that I am able to commission articles from such a wide variety of scientists who are experts in a diverse range of areas. Tying different areas of microbiology together with a common theme is sometimes a challenge, but it's always very satisfying to see the finished product lying on someone's coffee table (and that of the S*f*AM office of course).

So, I was working as a post-doctoral researcher and editing *Microbiologist* in my spare time and, with only six months left on my contract, I was contemplating my next career move. Then I heard about a new workshop on communicating science through the media, run by the then newly-established organisation called Sense About Science. The workshop was called 'Standing up for Science' and those who wanted to attend were asked to apply for a place by sending in a CV, so I applied straight away keeping my fingers crossed (see page 18 for a write-up of the most recent of these workshops which I'm glad to say are still going strong). I was lucky enough to be afforded a place at the workshop and found the whole experience enlightening and inspiring. I was determined from then on to make a career of communicating science to the general public. I wasn't entirely sure what type of job would enable me to do this, but I knew from then on that science communication was where I wanted to be.

My experience as Editor enabled me to apply for editing or writing roles, but in addition I applied for general communications roles in an attempt to expand my communications repertoire. I left the laboratory to become the Communications Officer for Med-Vet-Net, the EU framework programme 6 network of excellence for zoonoses research (see page 20). As S*f*AM are the partner institute for dissemination within Med-Vet-Net, this position enabled me to continue in my editorial role (albeit in my spare time) and to expand my communication skills through training. Whilst in this position I received training in a wide range of communications skills from how to write a press release, to using desktop-publishing software. I learnt about the communication of science at science centres, crisis management in the corporate world, science policy and the less tangible aspects of communication such as influence and the public perception of science.

Whilst working for Med-Vet-Net it became clear that S*f*AM needed somebody in a communications role in addition to the role of the Editor of *Microbiologist*. Med-Vet-Net also required additional communications support as the network delivered its objectives, so Med-Vet-Net employed a Senior Communications Officer and I became the Communications Officer for S*f*AM — a role which would enable me to put all my new-found skills into practice.

If I were to describe the work of a Communications Manager* in one word I would use the word "diverse". Every day is different and often the most stringent, carefully thought-out plans go to waste in the time it takes to receive a phonecall.

My role at S*f*AM involves most aspects of science communication and can be split into six general categories:

- Editor — *Microbiologist*
- Website and new technologies (e.g. podcasting/Facebook/Twitter)
- Media — both media enquiries and proactive media work (press releases/press briefings)
- Policy — responding to relevant government consultations

- Public engagement/outreach
- Internal communication

I don't have the space to go into every aspect of this role: a whole article could be devoted to careers in each of these areas of science communication. But many organisations have individuals or even departments dedicated to each of these areas of communication, so it's not difficult to imagine the diversity of the skills set required of a Communications Manager.

Skills and qualities

The ability to write is a fundamental skill required of a Communications Manager. Not only should one be able to write, but the writing style must be adapted depending upon the nature of the written piece. Before beginning a piece of writing it is vital that the writer *understand* (not just know) who their audience is going to be — this could be school children, journalists or scientists and the level at which the work is pitched will be very different for each audience. Before writing, it can be useful for a writer to spend some time researching the audience's demographics, their training, and their likely level of scientific understanding. This will ensure the finished product has the correct pitch so the majority of readers will benefit from a greater understanding of the subject.

It is imperative that a Communications Manager remains up to date with the development of all kinds of new technologies. The tools people use in order to communicate are changing rapidly. Keeping up with the development of new technologies is essential to science communicators – for example, so many people rely on Twitter as a means of communication and information dissemination, that to ignore this communication tool could mean that you are ignoring a large proportion of a potential audience. It is also important to consider the effect of such developments on those in associated professions. For example, the role of science journalists is changing as a result of new technologies which enable news to be 'reported' in real time through Twitter, Facebook and blogs. So the approaches science journalists are taking to report science are also changing. As a press officer, a Communications Manager is a potential supply of information to a science journalist through press releases or briefings. If a journalist needs a certain depth of information, or information presented in a certain way that's different to the way the information has been supplied in the past, it's the Communications Manager's job to ensure current information is supplied in a usable format.

Time management and versatility are inherent in the role of a Communications Manager. For example I could be engrossed in proof-reading an article on influenza for the magazine when the phone rings and suddenly I'm thrust into the role of press officer and I have to drop everything to find an expert for a journalist who's doing a piece on antibiotic resistance. Equally, I could be assisting in the organisation of a public engagement event where I'm required to find venues for the event, produce costings and report my findings to various different subcommittees, whilst at the same time trying to get to the bottom of a technical problem with the website. So it is important that Communications Managers are comfortable with switching from one type of task to another quickly and seamlessly to get results.

Training and related organisations

Briefly, it is possible to go into science communication without any formal qualification in the subject, but it is advisable to seek training where possible. This can take the form of a MA or MSc course in science communication, many of which are run by UK universities including Imperial College London and the University of Bath. In addition there are many accredited courses which can be undertaken alongside full time employment.

Some of the career paths taken by those in science communication can include:

- Communications Officer/Manager
- Press Officer — for university, research council, or government science organisation (see *Microbiologist* vol. 10., no.2, pp12-13)
- Public Relations Officer/Manager
- Public Outreach/Engagement Officer
- Editor
- Science Writer (see *Microbiologist*, vol. 9., no. 1., pp40-41)
- Medical Writer for a medical communications agency (see *Microbiologist*, vol. 9., no. 4., pp44-45)
- Science Journalist (see *Microbiologist*, vol. 9., no. 1., pp40-41)
- Publications Manager (see *Microbiologist*, vol. 8., no. 2, pp42-43)
- Guide at hands-on science centre

There are many organisations which provide support, guidance, advice and career development for science communicators, including:

- Science Technology Engineering and Medicine Public Relations Association (STEMPRA) http://www.stempra.org.uk/
- Science Media Centre (SMC) http://www.sciencemediacentre.org/pages/index.html
- Sense About Science http://www.senseaboutscience.org.uk/
- American Association for the Advancement of Science (AAAS) http://www.aaas.org/
- British Science Association (formerly British Association) http://www.britishscienceassociation.org/web/
- Association of British Science Writers (ABSW) http://www.absw.org.uk/
- World Federation of Science Journalists (WFSJ) http://www.wfsj.org/
- Wellcome Trust http://www.wellcome.ac.uk/
- Royal Society (report) http://royalsociety.org/downloaddoc.asp?id=3052
- Beacons of Public Engagement http://www.rcuk.ac.uk/sis/beacons.htm
- Chartered Institute of Public Relations (CIPR) http://www.cipr.co.uk/

NB: This is a selection of the organisations available and should not be considered a comprehensive list.

If you're interested in science communication but you don't want to leave the laboratory, there are numerous ways in which active scientists can participate in science communication activities. Providing expertise to journalists and press officers, participating in science festivals and other public engagement activities, and visiting schools to talk about science, are just a few of the ways scientists can play a part. In fact this is fast becoming an integral aspect of a scientist's career, with research councils and funding bodies recognising the dissemination of scientists work outside of peer review as a metric for evaluation.

I find the diversity, variety and subject of my job fascinating. I'm constantly being stretched and finding new ways of tackling sometimes difficult tasks, situations and (rarely) people. I love the fact that my job requires me to meet so many interesting experts, and keep up-to-date with changing technology (this feeds my inner geek). I would recommend a career in science communication to anyone who values diversity, complexity and who relishes a new challenge every day.

*As I am now fortunate to be the Communications Manger for SfAM the article is written using this job title but the reader should note this information also applies to the Communication Officer role

Lucy Harper
Communications Manager

In the loop pecs

News from the SfAM Post-Graduate and Early-Career Scientist Committee

PECS NEWS

George Aboagye and Jess Rollason have stepped down from their committee positions and we would like to thank them for their dedicated work with PECS. We are pleased to announce that, Simon Gould (Chair) and Amara Anyogu (Secretary) have been duly elected into these positions, welcome to you both.

The PECS committee invites nominations for events officer, events team and communications officer. The posts are available from October 2009 (for one year's term) and are open to all student or early career scientist members of SfAM.

Congratulations!

Congratulations go to Jo Heaton a SfAM early career scientist who was awarded the SGM outreach award for 2009 in recognition of her dedicated work promoting microbiology to school children.

If you know a SfAM student or early career scientist who has been awarded a PhD/ prize/award then get in touch, email: v.l.mccune@newcastle.ac.uk.

Vicki McCune
PECS Communications Officer, Newcastle University

Summer conference 2009 student session — Networking

So many postgraduate and early career scientists find the prospect of networking daunting, therefore this year's summer conference students session organised by the PECS events team, was an interactive workshop on how to make the most of a networking situation. The hosts of the session were Beverley Gartside and David Harrison from Fairplace. Some of the skills taught included how to enhance your ability to network successfully, what to say and what to ask, what you can observe about others and how to feel more confident. The workshop highlighted why networking is so important for scientists at the beginning of their careers, especially as it enables them to build relationships that could lead to collaborative work or even getting a new job at the end of their PhD.

Beverley summarised what networking is by quoting Steven D'Souza; *"networking is the art of building reciprocal relationships that help individuals and the community as a whole to achieve their goals"*. One of the most interesting aspects of the session focused on "your personal brand". Successful commercial brands were discussed and then participants had to think about their sixty second story; what is it that others think about them during an initial meeting and what makes them stand out? According to Mehrabian's rule of communication only 7% of good communication skills are associated with spoken words, 38% is associated with the tone of voice and 55% with body language. If you think about a person you know who is good at networking, what do you notice about them? They usually have good posture giving the appearance of confidence, a smile and are interested in what others are saying.

The following top tips were also given for breaking in and out of groups. Breaking in: aim for groups of three as this will not be a closed conversation, look for an opening in the group and walk towards it with confidence, then mirror the body language of the group. When the alpha finishes speaking be the first to ask a question or give a compliment. Breaking away: connect the person you are speaking with to someone else, give a plausible reason to leave, i.e., getting a drink or using the facilities and always leave a positive impression.

By the end of the hour those attending where certainly more confident and prepared to test out their newly acquired networking skills at the summer conference trade show!

Katie Fisher
PECS Events Officer
University of Northampton

Students into Work Grant reports

information

am I eligible - can I apply?

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

For further information visit:
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Resistance of strains of *Pseudomonas aeruginosa* to photodynamic antimicrobial chemotherapy

During the summer before my final year, I decided to undertake a research studentship within the School of Pharmacy at Queens University, Belfast (QUB). I believed that this work would provide me with an insight into the industry and research side of pharmacy, as well as giving me some indication of what a PhD might involve. It would also help prepare me for my final year project for my MPharm degree.

The main aim of my research was to investigate the resistance of various strains of *Pseudomonas aeruginosa* to photodynamic antimicrobial chemotherapy (PACT). Resistance of bacteria to PACT is an area in which very little research had previously been carried out and experts believed that resistance was limited.

PACT is the combination of a photosensitiser, alongside light and *in situ* oxygen. In this case the photosensitisers meso-tetra (N-methyl-4-pyridyl) porphine tetra tosylate (TMP) and methylene blue (MB) were chosen. The photosensitisers accumulate both rapidly and selectively in the cell membranes of the bacteria. When light of a suitable wavelength is irradiated on the cell this causes the production of a reactive oxygen species which leads to the destruction of microbial cells but leaves the host tissue unharmed.

The original aim of the project was to choose a bacterial species which was resistant to antibiotics and to determine if combining sub-lethal concentrations of PACT with the antibiotic would lead to cell death. However, some problems were encountered over the ten weeks and the aim of the research moved in a different direction. The bacterium chosen to be the main focus of testing was *P. aeruginosa* strain DF1. This was known to be resistant to, among others, ceftazidime and meropenem. After initial testing the strain was exhibiting low percentage kill with MB and therefore a decision was made to change the photosensitiser to the stronger toluidine blue O (TBO). However, after many attempts this also was showing a low percentage kill. As this particular strain was more resistant than originally expected, the irradiation time was increased from five to ten minutes. This proved to be beneficial as a kill of 99.3% was achieved.

Due to this strain displaying greater resistance than other bacteria previously tested, the aim of the research was changed to investigate if other *P. aeruginosa* strains, grown planktonically, displayed the same degree of resistance. Further problems were also encountered with the light source used, as it had to be returned to the manufacturers. During this period, testing was carried out using a red LED light source. However this produced results which were insignificant as 100% kill was achieved both with and without the presence of light.

Over the course of the ten weeks, twelve different strains of *P. aeruginosa* were tested and they generated a wide range of results, with very little trend displayed between the different strains. On various occasions some of the strains which were proven to be resistant to antibiotics also displayed a higher degree of resistance to PACT; however, this was not observed in every case. In order to achieve an acceptable level of kill, each strain required either five or ten minutes of irradiation and different TBO concentrations. These ranged from a minimum of 25μg/ml for more susceptible bacteria to 500μg/ml for the more resistant bacteria. For each of the strains tested, the method was carried out a minimum of two times. The effect of another photosensitiser, TMP, was tested by another summer student and was found to exhibit the same variation in results as was demonstrated with TBO. A possible explanation for the variation observed between the strains could be the presence of efflux pumps in the bacterial cell. It is believed that these pump the photosensitiser out of the cell membrane and inhibit its effects.

Overall, although the results were not what we originally expected, I believe that those generated are significant to

the field being researched due to the fact that they show that bacteria, especially *P. aeruginosa*, do display a certain level of resistance to PACT. It would be both beneficial and interesting to further investigate this using the antibiotics as originally planned. This would help to determine if there is any similarity between bacterial resistance to antibiotics and resistance to PACT. Further research could also be carried out to see if isolates growing in biofilms produce different results to those grown planktonically. I hope that my work will be continued within the School of Pharmacy and that one day this field of research will prove to be clinically useful.

Finally, I would like to thank Ryan Donnelly and the School of Pharmacy, QUB for allowing me to carry out this research and giving me the opportunity to extend my knowledge in the field of microbiology.

Kathryn Sally

Identification and antibiotic treatment of bacteria isolated in sputum from patients with Cystic Fibrosis

Cystic fibrosis (CF) has been identified as one of the most common genetic disorders of the Caucasian population ultimately leading to death. It is known that CF is the result of a defect in the CF transmembrane conductance regulator gene. One of the outcomes of this mutation is that the CF patient produces thick mucous secretions in their lungs that they are unable to clear which results in frequent and chronic airway infections. Fortunately, the prognosis of CF has improved dramatically with the introduction of antibiotics.

One of the principal pathogens causing acute pulmonary exacerbations is *Pseudomonas aeruginosa*. (Tunney *et al.*, 2008). Thus, during the first few weeks of this summer placement selected *P. aeruginosa* isolates, cultured from sputum samples from CF patients before and after treatment of an acute exacerbation, were tested for their susceptibility to ceftazidime, meropenem, tobramycin and piperacillin/tazobactam. These antimicrobial agents are commonly used in the management of pulmonary infection in CF. Therefore, the aim of this initial investigation was to establish if the susceptibility of the bacteria to these antibiotics differed before and after an acute exacerbation. The susceptibility was determined using E-test strips (Bio-Stat, Stockport, UK) according to the manufacturer's instructions. The desired isolate was cultured on MHA (Mueller-Hinton Agar) at 37°C for 24 hours and then the minimum inhibitory concentrations (MICs) were read. The findings of this study were that, in general, the susceptibility of each isolate to the various antimicrobial agents did not change. For example, those *P. aeruginosa* isolates which were initially susceptible to the antibiotics also remained susceptible after an acute exacerbation.

The second aim of the placement was to identify aerobic isolates cultured from CF sputum. Genomic DNA was first extracted from the isolates and screened for *P. aeruginosa* using the polymerase chain reaction (PCR). To identify an unknown isolate as *P. aeruginosa*, one of the published gene sequences, OprL, was targeted for PCR amplification using a previously described method (Xu *et al.*, 2004). The PCR products were then visualised by gel electrophoresis. Subsequently, PCR amplification of the 16S ribosomal RNA gene was performed for those isolates which were negative for the OprL gene (LiPuma *et al.*, (1999) The PCR amplicons were then sequenced with nucleotide sequences compared with previously published sequences using a basic local alignment sequence tool (BLAST).

Surprisingly, after plating out one of the selected isolates on agar, two different colonies could clearly be viewed indicating that two potential pathogens were growing on the solid medium. One colony of each variant was selected separately, streaked out on fresh agar and incubated aerobically before PCR amplification of the OprL gene. Results revealed that the first colony was positive for *P. aeruginosa* whilst the second colony was negative. These colonies were different in appearance to those previously inspected and a Gram stain also revealed that the microorganisms cultured were small Gram-negative rods. Morphological and Gram stain appearance suggested that this isolate may have been *Haemophilus influenzae*. This bacterium possesses complicated nutritional requirements and, therefore, to optimise growth the isolate was cultured on supplemented Brucella blood agar in 5% CO_2. The growth of this suspected *H.influenzae* isolate resulted in the project taking a new direction. To confirm the identity of this isolate as *H.influenzae*, PCR was required. However, there was no established protocol for identifying *H.influenzae* using PCR amplification. Therefore, during the latter half of my placement the aim was to develop a specific PCR procedure to detect this pathogen.

Firstly, oligonucleotide primers were designed by Dr. Deirdre Gilpin, which were complementary to unique sequences next to the gene segment (gyrase B gene) to be amplified. The predicted amplicon size was 225bp which is ideal. In order to ensure specificity, the conditions for the PCR reaction were determined by trial and error. Two variables which can easily be altered are the annealing temperature and the concentration of magnesium chloride ($MgCl_2$) present in the mastermix. The $MgCl_2$ concentration was increased in increments of 0.5mM from 2.0 to 5.0mM. Low annealing temperatures of 50°C and 55°C were also investigated. A bright band was observed on the agarose gel when 2mM $MgCl_2$ was present in the mastermix and the PCR reaction was carried out at 55°C. Hence, these proved to be the optimum reaction conditions. Furthermore, to ensure that the PCR method only amplified the gyrase B gene in *H.influenzae*, other isolates (*Escherichia coli*, *P. aeruginosa* and *Staphylococcus aureus*) were screened with no bands apparent for any of these isolates.

In addition to aerobic bacteria, anaerobic bacteria such as *Prevotella* and *Actinomyces* species, have been isolated from the sputum samples of CF patients (Tunney *et al.*, 2008). Hence, several selected anaerobic isolates were cultured under strict anaerobic conditions. As previously described, the susceptibility of these isolates to various antimicrobial agents (clindamycin, meropenem, metronidazole and piperacillin/tazobactam) were determined using E-test strips.

Resistance was apparent to both clindamycin and metronidazole.

Towards the end of the placement, ten anaerobic isolates were selected at random and cultured on milk agar. It was proposed that if the isolate produced proteases, which contribute to the poor lung function in the CF patient, then these enzymes would degrade the protein present in the milk. After incubation at 37°C for one week in the anaerobic cabinet, white colonies could be observed on 10/10 plates. However, the nutrient medium for 2/10 isolates had turned completely translucent whereas the remaining 8/10 remained opaque. Therefore, those isolates, whose growth resulted in the medium undergoing the transition from opaque to translucent, were believed to produce proteases.

This placement introduced me to various microbiological techniques such as culturing bacteria under aseptic conditions and also molecular methods including PCR. I have also gained invaluable experience of working in a laboratory and how to organise my time appropriately to ensure that I work as efficiently as possible. This has allowed me to gain confidence to work on my own in unfamiliar situations and shown me how challenging and rewarding a career in research could be.

I would like to thank my supervisor, Dr. Michael Tunney for giving me the opportunity to study this project, as well as all the staff of the School of Pharmacy, in particular Dr. Deirdre Gilpin, for their assistance, guidance and patience during my summer placement. Finally I would like to thank S*f*AM for making this placement possible.

References

- Tunney *et al.*, (2008). Detection of anaerobic bacteria in high numbers in sputum from patients with cystic fibrosis. *American Journal of Respiratory and Critical Care Medicine*, **Vol 177**, pp995-1001
- Xu *et al.*, (2004). Molecular (PCR) detection of *Pseudomonas* spp. other than *P. aeruginosa* directly from the sputum of adults and children with cystic fibrosis. *British Journal of Biomedical Science*, **Vol 61**, pp147-149
- LiPuma *et al.* (1999). Development of rRNA-based PCR assays for identification of *Burkholderia cepacia* complex isolates recovered from cystic fibrosis patients. *Journal of Clinical Microbiology*, **Vol 37**, pp3167-3170

Laura Gillanders
School of Pharmacy, Queen's University Belfast

Evaluating and improving the performance of footbath product for preventing bacterial growth on the feet of farmyard animals

For eight weeks I have been working in collaboration with Provita — a company in Northern Ireland specialising in the manufacture of animal healthcare products. Nearing completion of year three of a four year MPharm course I applied for a grant in order to enable me to gain experience of laboratory research. I felt that the project would give me an insight into the work that may be involved in a PhD and industrial pharmacy and would be of great help in undertaking my final year project at university.

The focus of the project was to investigate the antibacterial properties of a footbath preparation and to compare it with other available products.

Footbath preparations are used in farmyard footbaths primarily for dairy cattle to prevent the growth of bacteria such as *Escherichia coli* on the feet of animals. Damp conditions and slurry promote this growth and footbathing is the choice action to controlling infection. If the animal is not managed correctly and incurs large amounts of bacterial growth around the hooves, it will develop painful sores and may become lame. This can predispose cattle to other diseases thus lowering production and fertility at the expense of the dairy farm business. The cost of lameness is estimated by Esslemont and Kossaibi to be in the region of £178 per affected cow.

Lameness continues to be a serious problem on many farms despite the fact that it can be relatively easily prevented through careful consideration of animal husbandry factors, such as nutrition and environmental conditions. Despite advances there has been little improvement in lameness over past years. It is clear to see why footbath products are important in agriculture not only to maintain profit and good supply but more importantly to safeguard animal welfare.

I began my investigations by establishing the minimum inhibitory concentration (MIC) for a range of antibacterial footbath products. Various concentrations of the market dilution were prepared. *E.coli* was cultured overnight and centrifuged to a pellet followed by dilution to optical density 0.3 followed by further dilution for addition to the test tubes. A sample of dung was collected and autoclaved. It was then added to the tubes in suitable portions to mimic environmental conditions. After running the experiment, test solutions were plated and incubated overnight to observe any bacterial growth. The desired outcome was to discover the lowest concentration of antibacterial required to inhibit bacterial growth.

The rate of kill was later investigated. The products were prepared to market dilution and two repeats run for each. Recordings were taken at five different time points in clean and dirty conditions. This determined the time taken for a given footbath product to achieve a bacterial load of zero.

A formulation study was also carried out by isolating constituents of a given footbath preparation and investigating their rate of kill individually. Different concentrations were investigated in order to seek the optimum formulation. My overall experience of the Students into Work scheme has been very positive. It was flexible and enjoyable and has taught me a volume of skills, both specialist skills such as operational techniques and experimental procedures and general skills such as organisation, team work and time management.

As a result, my confidence in the laboratory has greatly improved.

An added benefit to this particular project is the involvement it has presented with an industrial company. This has provided me with experience in a field of pharmacy that not many students get the chance to obtain.

I would definitely recommend a summer research post to anyone with a keen interest in microbiology or those considering a PhD or seeking an alternative to community pharmacy. I believe it best to explore all possible options before choosing a career pathway and a research project will certainly help with those decisions, not to mention make a great addition to your CV.

Finally I wish to express my gratitude toward all those involved in the project: Cecilia Brennan and Dr Teresa Allen, both representing Provita, who supervised and guided my work, Dr Brendan Gilmore and Professor David Jones of Queen's University Belfast who oversaw and directed the project and the funding body of S*f*AM who supplied the grant for the eight weeks.

Jessica Edwards

President's Fund reports

information

am I eligible - can I apply?

If you often find difficulty in funding attendance at meetings.

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.

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Characterisation of actinomycetes from Helmcken Falls volcanic cave and their antimicrobial activity against the honey bee pathogen *Paenibacillus larvae*

American foulbrood disease (AFB) is one of the most important bacterial diseases of honey bees around the world (Neuendorf *et al.*, 2004). The cost in lost income, due to destruction of bees and equipment associated with infected hives and the possibility of antibiotic residues left in the hives is estimated in millions of dollars (Clay 2000). The responsible agent is the Gram-positive, spore-forming bacterium, *Paenibacillus larvae* (Allipi *et al.*, 2002). This pathogen causes infection of the gut of honeybees larvae (*Apis mellifera* L.) leading to death in the larval stage. Death occurs when the bacterium breaks down the epithelium of the mid-gut region of the gastrointestinal tract. The bacterial spores are resistant to a variety of adverse environmental factors such as heat, cold, desiccation and chemical disinfectants and may remain viable for 35 years or more (Chantawannakul & Dancer, 2001). In Canada, McRory (2004) reported that 7% of 719 Ontario bee yards showed signs of AFB infection. Currently, oxytetracycline (OTC) is the only approved antibiotic for treatment of AFB worldwide. However, in recent years, OTC resistant strains have emerged in the United States of America, Canada and Argentina (Miyagi *et al.*, 2000).

It has been shown that actinomycetes, filamentous Gram-positive bacteria with a high G+C content, are a prolific source of new bioactive compounds (Lazzarini *et al.*, 2000). Approximately two-thirds of naturally occurring antimicrobial products are produced from actinomycetes (Okami & Hotta, 1988). Large numbers of novel actinomycetes were reported to be present in unexplored habitats worldwide including cave environments (Groth & Saiz-Jimenez, 1999; Jones, 2001; Northup & Lavoie, 2001). Several novel actinomycetes were isolated from caves

as exemplified by the description of *Beutenbergia cavernae*, a new genus of L-lysine-containing actinomycete (Groth *et al.*, 1999) and *Agromyces subbeticus* being isolated from a cave in southern Spain (Jurado *et al.*, 2005).

The significance of microbial diversity in the search and discovery of novel metabolites has been underlined in a recent article (Keller & Zengler, 2004). Consequently, the main focus of this study was to search in previously uncharacterised habitats to uncover novel actinomycetes that may produce antimicrobial agents against *P. larvae*, the causative agent of AFB. A volcanic cave habitat was chosen as a potential source of antimicrobial producing actinomycete strains because of their unique characteristics and the lack of study in such habitats that may lead to higher probability of finding such novel strains.

This report represented some preliminary work on the isolation of cave actinomycetes and screening for antimicrobial agent(s) that is a part of a larger study on microbial diversity in caves of Canada.

One hundred and nineteen strains of actinomycetes were isolated by dilution plating from sixteen soil samples collected from Helmcken Falls, a volcanic cave in Wells Gray Provincial Park, British Columbia and screened for their antimicrobial activity against *P. larvae* using a paper disc diffusion method. The antimicrobial activity was carried out in three fermentation broths namely YMG, AMHU-4, and V8 juice, at 28°C. Among these, strain E9 grown in V-8 juice medium showed significant antibacterial activity against *P. larvae*, and produced a zone of inhibition equivalent to that of a 30μg tetracycline control disc. E9 began to produce antimicrobial activity against *P. larvae* after 2 days of incubation and continued up to day 8. The antimicrobial activity peaked on day 4 of incubation with an inhibitory zone of about 10mm. During fermentation, the pH of the media varied from 7 to 9 with an optimal antimicrobial activity at pH 7. The growth pattern of E9 during antimicrobial agent(s) production was observed and presented as the average percentage of packed cell volume (%PCV). Strain E9 showed a typical bacterial growth curve during the 10-day fermentation in V8 juice medium. This information could be very useful for both optimisation studies and large-scale production of antimicrobial agent(s).

Strain E9 was identified using a combination of genotypic and phenotypic characterisations. Strain E9 morphology and chemotaxonomic markers matched with those of *Streptomyces* species. Phylogenetic analysis based on almost complete 16S rRNA gene sequence revealed that strain E9 is a member of the genus *Streptomyces* and closely related to *Staphylococcus aureus*. In addition, strain E9 showed distinct differences to other validly described *Streptomyces* species, there is a possibility that it may represent a new species. However, this requires further study, in particular DNA:DNA hybridisation to establish its taxonomic status.

This study suggests a high possibility of finding new antimicrobial agent(s) from less-studied actinomycetes in volcanic cave habitats. There is no previous record of any antimicrobial production in *S. aureus*. This is the first report on antimicrobial activity against *P. larvae* from a volcanic cave actinomycete isolated in Canada.

This article is based on a poster presentation made at the 14th International Symposium on Biology of Actinomycetes in Newcastle upon Tyne, UK in August 2007. I would like to thank S*f*AM for giving me the opportunity to attend and present this work at this meeting.

Wasu Pathom-aree

References

- Alippi, A. M., López, A. C., Aguilar, O. M. (2002). Differentiation of *Paenibacillus larvae* subsp. *larvae*, the cause of American foulbrood of honeybees, by using PCR and restriction fragment analysis of genes encoding 16S rRNA. *App and En Mic*, **Vol 68**, pp3655-3660
- Chantawannakul, P., Dancer, B. N. (2001). American foulbrood in honey bees. *Bee World*, **Vol 82**, pp168-180
- Clay, H. (2000). American Foulbrood is back. Hivelights [online]. Available from http://www.honeycouncil.ca/users/folder.asp?FolderID=1043 [cited 14 September 2005]
- Groth, I., Schumann, P., Schuetze, B., Augsten, K., Kramer, I., Stackebrandt, E. (1999). *Beutenbergia cavernae* gen. nov., sp. nov., an L-lysine-containing actinomycete isolated from a cave. *International Journal of Systematic Bacteriology*, **Vol 49**, pp1733-1740
- Groth, I., Saiz-Jimenez, C.(1999). Actinomycetes in hypogean environments. *Geomicrob Jnl*, **Vol 16**, pp1-8
- Jones, B.(2001). Microbial activity in caves: a geological perspective. *Geomicrob Jnl*, **Vol 18**, pp345-357
- Jurado, V., Groth, I., Gonzalez, J. M., Laiz, L., Saiz-Jimenez, C.(2005). *Agromyces subbeticus* sp. nov., isolated from a cave in southern Spain. *Int Jnl of Sys and Evolutionary Microbiology*, **Vol 55**, pp1897-1901
- Keller, M., Zengler, K.(2004). Tapping into microbial diversity. *Nature Reviews Microbiology*, **Vol 2**, pp141-150
- Lazzarini, A., Toppo, C.G., Marinelli, F.(2000). Rare genera of actinomycetes as potential producers of new antibiotics. *Antonie van Leeuwenhoek*, **Vol 79**, pp399-405
- McRory, D. Ontario Provincial Apiarist Annual Report, Ontario, Canada (2004)
- Miyagi, T., Peng, C., Chuang, R.Y., Mussen, E.C., Spivak, M.S., Doi, R.H.(2000). Verification of oxytetracycline-resistant American foulbrood pathogen *Paenibacillus larvae* in the United States. *Journal of Invertebrate Pathology*, **Vol 75**, pp95-96
- Neuendorf, S., Hedtke, K., Tangen, G., Genersch, E.(2004). Biochemical characterization of different genotypes of *Paenibacillus larvae* subsp. *larvae*, a honey bee bacterial pathogen. *Microbiology*, **Vol 150**, pp2381-2390
- Northup, I., Lavoie, K.(2001). Geomicrobiology of caves: A Review. *Geomicrob Jnl*, **Vol 18**, pp199-222
- Okami, Y., Hotta, K.(1988). Search and Discovery of new antibiotics. In M. Goodfellow, S.T Williams, M. Mordarski. (Eds): *Actinomycetes in Biotechnology*. Academic Press Ltd., London pp33-67

Essential oils for improved skin antisepsis: the way forward?

Advances in modern biomaterial science have lead to an increase in the diversity, availability and therefore use of a variety of medical devices, for example intravascular and peripheral catheters. Hospitalisation, especially in in-patients receiving intense management, is often accompanied by the use of such devices; however surgical insertion carries a risk of infection as the barrier properties of the skin become breached. Infections associated with intravascular catheters can vary in severity from local infections around the site of insertion, to systemic infections of the bloodstream, which may in turn lead to further complications (Worthington & Elliott, 2005). They are thus associated with significant patient morbidity and mortality, as well as increasing costs to the health service. The main bacteria responsible for causing intravascular device related infections are the skin microorganisms, in particular *Staphylococcus epidermidis*, although

Staphylococcus aureus, Gram-negative bacilli and *Candida* species are also often associated with infection. Many of these microorganisms are capable of producing biofilms that allow them to become irreversibly attached to surfaces such as catheters, as well as protecting the microorganism, reducing their susceptibility to the effects of antimicrobial agents and host defence mechanisms. Prevention of infection associated with surgical incision of the skin is governed by adequate skin antisepsis prior to breach.

Several UK hospitals use 0.5% (w/v) chlorhexidine in 70% (v/v) alcohol for skin antisepsis, however, recent recommendations in the Evaluation of Processes and Indicators in Infection Control (EPIC) guidelines list that 2% chlorhexidine gluconate in 70% isopropyl alcohol (IPA) should be used (Pratt *et al.*, 2007). Chlorhexidine is one of the most widely used antimicrobials within clinical practice, yet its efficacy is significantly reduced by pH and organic matter (McDonnell & Russell, 1999). For these reasons, along with the increasing prevalence of resistant microorganisms, it is becoming more important to investigate novel strategies for improving skin antisepsis.

Many antimicrobial agents have been tested for use as preventative measures against bacteria. These include a range of chemicals such as aqueous chlorhexidine, as well as many complementary and alternative medicines including essential oils, which are regarded as mixtures of compounds that produce a flavour or aroma, and are from natural sources such as flowers, spices and aromatic herbs. Phenolic compounds are responsible for most of the antimicrobial activity of essential oils derived from herbs and spices, but secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids found in plants also possess antimicrobial properties.

The medicinal properties of tea tree oil were first reported in the 1920s in a series of papers published by Penfold, however, Aborigines were the first people documented to have benefited from the plant, by using crushed leaves to heal wounds and making broths with soaked leaves to heal sore throats. Like many other natural remedies, the use and popularity of tea tree oil has increased over recent years and is sometimes used in low concentrations to treat insect bites and cuts. The oil is well reported to have many assets including broad-spectrum antibacterial, antifungal, antiviral, and anti-inflammatory properties and could therefore be beneficial in the treatment of a wide range of infections. There are a number of compounds within tea tree oil that possess antibacterial activity including α-pinene, β-pinene and linalool, but the principal antibacterial constituent is terpinen-4-ol. The active components of tea tree oil cause loss of membrane function and integrity. Some bacteria lose intracellular material; some lose the ability to maintain homeostasis while others are inhibited from respiring.

Studies into the clinical efficacy of tea tree oil have shown it to be effective in the treatment of acne as well as the eradication of MRSA carriage when given as a nasal ointment (Bassett *et al.*, 1990; Anderson & Fennessy, 2000). At present, it is used as an antiseptic in a range of skincare products and some cosmetics, in concentrations of between 2 and 5%, and interest is being shown into the possibility of adding it to hand-wash solutions within clinical and care settings.

Recent research at Aston University, UK, has confirmed the antimicrobial activity of a wide range of essential oils that may potentially be incorporated into a skin antisepsis regime, in particular eucalyptus oil (Karpanen *et al.*, 2006). Eucalyptus oil is also plant-derived and demonstrates antimicrobial properties when applied directly to the skin as a balm, used in mouthwashes or inhaled as a spray. Around 70% of the oil is eucalyptol (or cineole) and about 14% is α-pinene, though the amount of oil and its contents varies between eucalyptus species. Eucalyptus oil contains volatile components such as alcohols, ketones and aldehydes, and like tea tree oil, eucalyptus contains monoterpene hydrocarbons and terpenoids of which the main constituents are terpinen-4-ol, α-terpineol and 1,8-cineole. Research at Aston has demonstrated that while eucalyptus oil alone possesses excellent antimicrobial activity, it exhibits synergistic activity against biofilms of *S. epidermidis* when in combination with 2% chlorhexidine digluconate. Furthermore, the lipophillic nature of eucalyptus has been known to facilitate the permeation of chlorhexidine into the deeper layers of the skin, where many microorganisms exist as biofilm-like micro-colonies, surrounded by sebaceous secretions.

The antimicrobial properties of many other oils are also currently being investigated and include cinnamon, lemon and rosemary oils. However, there are at present limitations that need to be overcome before essential oils can be routinely used in skin antisepsis. Research is needed into the safety of oils as both tea tree and eucalyptus oils are known to be toxic if ingested and can cause irritation and occasional allergic reactions when applied to the skin, although these depend in part upon the concentration used and sensitivity of the person exposed to the oil. Also, the quality of the final product would need to be assessed as quantities of antimicrobial compounds within all essential oils may vary with season or strain of plant. When this is combined with the knowledge that essential oils are volatile and have poor solubility, common use of them in the healthcare profession will require further research to enable restrictions in clinical application to be overcome.

References

- Anderson, J. N. and Fennessy, P. A. (2000). Can Tea Tree (Melaleuca alternifolia) Oil Prevent MRSA? *Journal of Australia* **173**, pp489.
- Bassett, I. B., Pannowitz, D. L., and Barnetson, R. S. (1990). A Comprehensive Study of Tea Tree Oil Versus Benzoylperoxide in the Treatment of Acne. *Medical Journal of Australia* **153**. pp455-458.
- Karpanen, T. J., Worthington, T., Rathbone, D., Lambert, P. (2006). P6.18 Activity of Thiosemicarbazone and Carboxamidrazone Compounds and Essential Oils Against Microorganisms Associated with Intravascular Device Related Infections. *Journal of Hospital Infection* **64**, ppS37.
- McDonnell, G. & Russell, A. D. (1999). Antiseptics and Disinfectants: activity, action and resistance. *Clinical Microbiology Reviews* **12**, pp147-179.
- Pratt, R. J., Pellowe, C. M., Wilson, J. A., Loveday, H. P., Harper, P. J., Jones, S. R. L. J., McDougall, C., Wilcox, M. H. (2007). epic2: National Evidence-Based Guidelines for Preventing Healthcare-Associated Infections in NHS Hospitals in England. *Journal of Hospital Infection* **65**, ppS1-S59.
- Worthington, T. and Elliott, T. S. J. (2005). Diagnosis of Central Venous Catheter Related Infection in Adult Patients. *Journal of Infection* **51**, pp267-280.

Emma Hendry

Control of *Aspergillus flavus* in corn using natural materials

I would like to thank SfAM for awarding me a President's Fund grant to attend the 9th European Conference on Fungal Genetics held in Edinburgh, UK on 5-8 April 2008.

I presented my paper on the organisms that produce the mycotoxin, aflatoxin, especially *Aspergillus flavus*. This can infect important food and feed crops, including corn, before, during and after harvest. These fungi are normal soil-borne inhabitants in our environment, growing on both living and decaying plant matter. Mycotoxins include metabolic by-products produced by a number of different fungi.

The importance of this study comes from the prevalence of *Aspergillus flavus* producing aflatoxin in corn, wheat and barley crops. It is possible to control contamination by preventing the presence of the fungus (*Aspergillus flavus*) and removing or affecting its capability to produce the toxin. This could be done by using natural materials and may lead to the production of toxin-free food and feed and increased safety from a health point of view whilst preserving grain food wealth.

I studied the control of *Aspergillus flavus* using natural materials namely, the essential oil marjoram (*Origanum majorana* L.) and salt (sodium chloride) and their effect on fungal growth and the quantity of aflatoxin production.

Effect of different concentrations of marjoram (*Origanum majorana* L.) essential oil and salt (sodium chloride) on *Aspergillus flavus*:

One millilitre of *Aspergillus flavus* containing $5.0x10^4$cfu/ml was added to 100 grams of corn (sterilized in a 250 ml conical flask) and distributed without the addition of marjoram or salt (control sample). Also, one millilitre of the same *Aspergillus flavus* with the addition of different volumes of marjoram essential oil (0.05, 0.1, 0.2, 0.3, 0.5 and 0.7 ml) and one ml of the same *Aspergillus flavus* with the addition of different weight of salt (0.2, 0.4, 0.6, 1.0, 1.5 and 2.0 grams) were each added to 100 grams of sterilized corn (placed in a 250 ml sterilized conical flask) and mixed. Sterilised distilled water was added to raise the moisture content to 18% and the mixture was shaken to make it homogenous. These were stored at 25°C for 21 days. Sampling to determine *Aspergillus flavus* count and aflatoxin concentration was made at intervals of 7 days until 21 days.

Adding 0.05 ml of marjoram essential oil did not stop the growth of *Aspergillus flavus* during storage and up to 21 days, in inoculated corn. At the beginning of storage *Aspergillus flavus* count was $4.0x10^2$ cfu/g and gradually increased on day 21 to reach $1.5x10^3$cfu/g.

The corresponding values in the control sample were $4x10^2$ *Aspergillus flavus* count and 0 ppb aflatoxin production at the beginning of storage and $3.2x10^3$ *Aspergillus flavus* and 150 ppb aflatoxin production at the end of storage (21 days). This indicates that the addition of 0.05 ml marjoram caused a reduction of *Aspergillus flavus* count of 62.5% at 21 days of storage. Raising the level of marjoram to 0.1 ml inhibited the growth of *Aspergillus flavus* and aflatoxin production completely at 7 days until the end of storage. These results are in agreement with those of Paster *et al.*, (1995) who reported that the essential oil of marjoram has been shown to inhibit mycelial growth and aflatoxin production in *Aspergillus parasites* and *Aspergillus flavus* — both fungi which grow on stored grains. This observation was noted clearly with other higher marjoram levels.

It can be observed from the effect of different salt concentrations on *A. flavus* count during storage of inoculated corn up to 21 days that the control sample gave a gradual increase in *A. flavus* number during storage from 0 to 21 days. It increased from $6x10^2$ cfu/g to $5x10^3$cfu/g at 0 and 21 days storage period respectively. The corresponding values, in inoculated corn sample treated with 0.2 grams of salt were $6x10^2$cfu/g to $9x10^2$cfu/g at the same periods of storage. Although the salt concentration (0.2 gram) was quite low it still produced a slight effect towards reducing the rate of growth of *A. flavus* during storage. This trend increased during storage, and was more obvious when 0.4 grams of salt was added. At this concentration the number of *A. flavus* at 0, 7, 14 and 21 days storage period became $6x10^2$, $6.3x10^2$, $6.6x10^2$ and $7x10^2$cfu/g, respectively. At 0.6% salt concentration growth inhibition reached 83.3% and aflatoxin production reached 50 ppb between 0 and 21 days while the control sample reached 180 ppb during the same period. Hence this concentration is not suitable for controlling *A. flavus* growth during storage.

Raising salt concentration to 1 gram caused a complete inhibition of *A. flavus* growth and aflatoxin production during the whole experimented storage periods (7, 14, and 21 days). The same observation was noted when 1.5 and 2 grams of salt were used. Accordingly the minimum inhibitory concentration to prevent *A. flavus* growth in corn is 1 gram salt/100 gram corn and can be successfully used in the storage of corn up to 21 days. It is also suitable to prevent aflatoxin production. Moreover salt is traditionally used as a food, feed ingredient and food preservative, so the hazard would appear negligible.

It is possible to conclude that using marjoram at concentration of 0.1 ml and above per 100g corn at 18% moisture inhibited the growth of *Aspergillus flavus* completely at 7 days and up to the end of storage also prevented aflatoxin production.

Also it can be concluded that the minimum inhibitory concentration to prevent *A. flavus* growth in corn is 1 gram salt/100 gram corn. This concentration can be successfully used in the storage of corn up to 21 days. It is also suitable to prevent aflatoxin production.

References

- Aurelio Lo´pez-Malo, Stella Maris Alzamor, Enrique Palou (2005) *Aspergillus flavus* growth in the presence of chemical preservatives and naturally occurring antimicrobial compounds. *International Journal of Food Microbiology* **Vol 99**, pp119–128
- Paster, N., Menasherov, M., Ravid, U. and Juven, B. (1995) Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attaching stored grains. *Journal of Food Protection*, **Vol 58**, pp81-85
- Uraih, N., Chipley, J. R. (1976) Effects of various acids and salts on growth and aflatoxin production by *Aspergillus flavus* NRRL 3145. *Microbios*, **Vol 17** No. 67, pp 51-9

Youssef Ahmed Youssef Gibriel

corporate news

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

New, Compact Automated Pourer Stacker

New from Don Whitley Scientific is **APS One**, the ergonomically designed, automated pourer stacker that can process 800 plates (18ml) in an hour. Manufactured by AES Chemunex, the APS One is particularly compact and is only 845mm tall. This low overall height has several advantages but one major benefit is that it enables technicians to load the columns more easily.

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Campden BRI produces a number of manuals of relevance to laboratory microbiologists. In addition to the well established *Manual of Microbiological Methods* (Guideline 43), now in its fifth edition, we have recently published the sixth edition of the *Catalogue of Rapid Microbiological Methods* (Review 1). Using simple tables for ease of cross-referencing, it lists over 400 kits from around 50 kit manufacturers. It covers pathogens of interest to the agri-food chain, spoilage organisms (e.g. yeasts) and hygiene testing. In addition to the name and principle for each method, it gives the time taken for the test, the name of the manufacturer along with contact details, comments on validation status of the method, and references for further reading.

As well as these methods publications, *A code of practice for microbiology laboratories handling food, drink and associated samples* (Guideline No. 9), now in its third edition, will help food microbiologists to run their laboratories safely and efficiently and help ensure that they generate results that are valid and meaningful.

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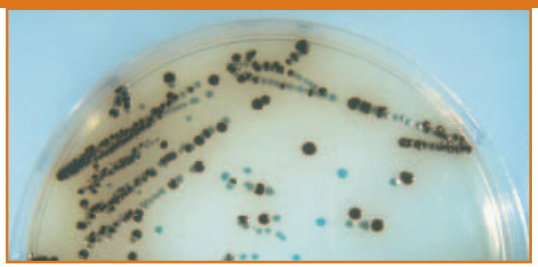

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Fungal Toxin Mystery Solved Using Biolog's Phenotype MicroArray™ Technology

An important breakthrough in fungal toxin biology has been made possible through the use of Biolog's Phenotype MicroArray technology. This major advance is described in two recent publications from a group at CSIRO in Queensland, Australia. The work by Donald Gardiner and his collaborators has recently been published in online editions of the journals Fungal Genetics and Biology and Microbiology.

The fungus *Fusarium graminearum* is a major pathogen of wheat. It is the causative agent of head blight and results in substantial worldwide crop losses. Central to the infection process, the fungus produces a trichothecene toxin called deoxynivalenol. Although the toxin is produced at high levels during wheat infection, researchers have never been able to induce *Fusarium* to produce significant levels under laboratory culture conditions. This inability has hampered study of head blight disease and approaches to disease prevention.

Now, this decades-old mystery has been solved. The CSIRO researchers used Biolog Phenotype MicroArray plates to culture the fungus simultaneously in hundreds of different micro-scale culture conditions. In a single experiment, they quickly screened hundreds of conditions and determined that key factors for turning on toxin synthesis are the presence of specific nitrogen compounds (arginine, agmatine, putrescine) as well as low extracellular pH.

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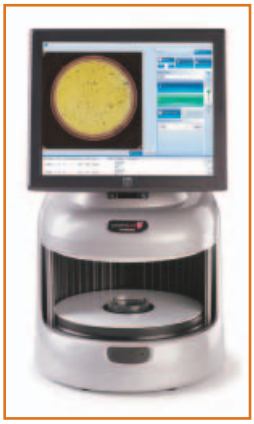

Launch of New Automated Colony Counter and Zone Sizing System with Unique HD Imaging

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Neogen Europe has received approval from the AOAC Research Institute for its rapid and accurate test for yeast and mould.

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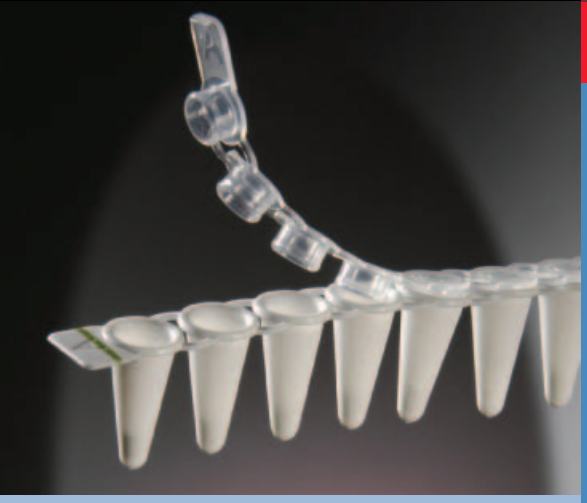

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