Microbiologist

The magazine of the Society for Applied Microbiology ■ June 2012 ■ Vol 13 No 2 ISSN 1479-2699

Microbes and Sport

INSIDE

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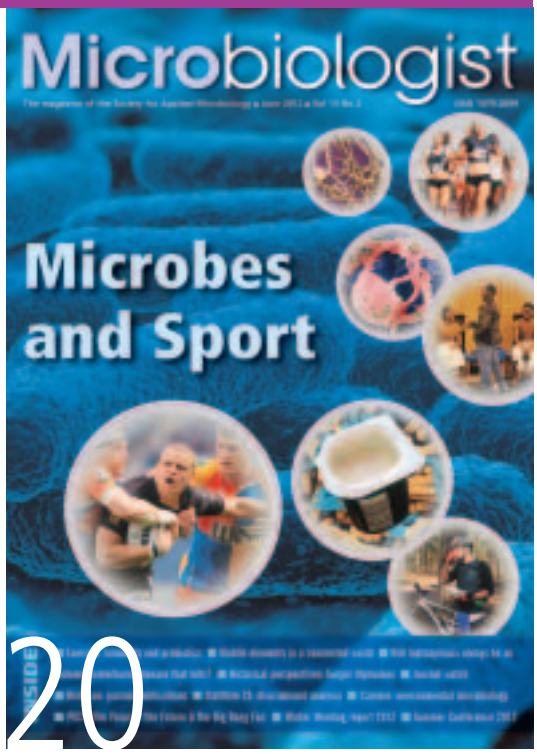
contents



Summer Conference 2012

Historical perspectives: fungal Olympians

information

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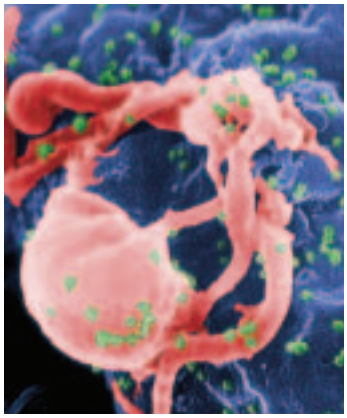
editorial

Lucy Harper discusses the ubiquitous nature of microbes



We all know that there are 10 times more bacterial cells in our body than human cells and that there are a billion microbes in just a pinch of soil. These were just a couple of examples of the ubiquity, importance and relevance of microbiology given by the first Chief Scientific Adviser to the President of the European Commission, Professor Anne Glover at our Spring Meeting this year. We were extremely pleased to award Professor Glover with this year's Procter and Gamble Applied Healthcare Microbiology Award, in recognition of her distinguished achievements in research and development in applied microbiology. She engaged delegates at the meeting with tales of her career (one which she didn't plan) and her fascinating work on bioluminescence and its use in researching neurodegenerative diseases like Alzheimer's and Parkinson's Disease. Look out for more from Professor Glover in future issues of *Microbiologist* and online.

The ubiquitous nature of microbes in soil is something which was exploited by the Olympic Delivery Authority (ODA), the public body responsible for developing and building the new venues and infrastructure for the London 2012 Games. The ODA used bioremediation to clean up parts of the London 2012 Olympic site which was previously contaminated with hydrocarbons. The bioremediation work of the ODA cleaned thousands of tonnes of contaminated soil for reuse on the Olympic Park site — yet another example of the importance of applied microbiology.

And sticking to the theme of the now-almost-upon-us London 2012 Games, this issue of *Microbiologist* looks at microbes in sport. We begin with an article from Professor Mike Gleeson, University of Loughborough, who describes the effect of exercise on the function of our immune system. He goes on to talk about the effect of probiotics in protecting athletes against upper respiratory tract infection. He says: "*The higher infection risk that comes with very high (some might say excessive) levels of exercise is the small price that athletes pay for being a potential Olympic gold medallist. There is accumulating evidence that the daily ingestion of Lactobacillus probiotics can reduce infection incidence in people with stressful lives*." You can read more on page 20.

There will undoubtedly be a massive influx of visitors to London 2012. Our second feature article looks at the effect of mass gatherings and international travel on the spread of infectious disease. Professor Mary Wilson, Harvard University, says: "*Mass gatherings, events such as the Olympic Games, the World Cup, and the Hajj, combine many risks for infectious diseases and other problems. Individuals and groups come together, often from diverse regions of the world. During the event, participants have close contact with large numbers of persons, sometimes in an indoor setting*." Professor Wilson continues: "*In recognition of the many potential health problems associated with mass gatherings, individuals with relevant experience and expertise are defining a discipline called mass gatherings health, a specialized, interdisciplinary area of public health that brings together individuals trained in public health, emergency and disaster planning, and travel medicine*." Read the full article on page 24.

Our third feature article is from Dr Jarlath Nally, University College Dublin, who looks at leptospirosis and water sports on page 28. Finally, we have an historical look at "Fungal Olympians" on page 31. Enjoy!

contribute

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

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

Lucy Harper

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

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

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benefits

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

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

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

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

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

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

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

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

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

membership options

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

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

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

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

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

■ **eAffiliate Membership:** This category of membership is open to microbiologists residing in Band I developing countries and is free of charge. It is an online only membership and provides access to the eAffiliate bursary only.

■ **eStudent Membership:** This category of membership is open to undergraduate students only. It is an online only membership and is free of charge. This category of membership does not provide access to the Society's grants or journals.

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

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

JOIN US!

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

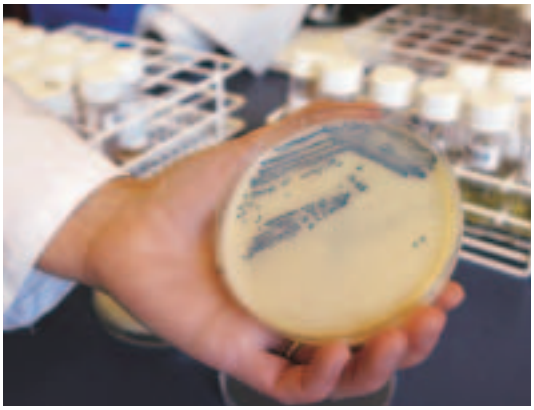
president's column

S*f*AM President, **Professor Martin Adams** talks about the virtual microbiology laboratory (VML) online

It is only natural that the application of modern molecular and computational techniques to microbiology attract so much attention; the opportunities they give us to understand, control and exploit microbial processes are enormous. It is clearly essential that both practising microbiologists and students are given every opportunity to become aware of and then use the huge power and potential these approaches give us. Generally speaking, this is not a problem, the excitement generated by new techniques and the commercial opportunities they afford mean that there is no shortage of enthusiastic people proselytizing on their behalf — presenting papers, putting on courses and conferences and producing other forms of published and online information. However, a risk that is run at such times of rapid and exciting progress is that attention given to existing, more traditional procedures can be neglected or marginalized in the rush to get to the more exciting stuff.

It's fair to say that many of the basic procedures and techniques that form the bedrock of practical microbiology today may come across as a tad old-fashioned. Certainly some, still widely used in microbiology laboratories today, though they may have a few modern whistles and bells attached, would not appear too unfamiliar to Robert Koch. Use of these techniques has persisted for good reason and failure to understand, master and apply them can render information obtained using state-of-the-art techniques worthless. Knowing that you have a pure culture, can maintain it in that state and grow it must be the starting point of many investigations.

For some time now we have been discussing within S*f*AM how we can support members with help in some of the fundamental skills that underlie so much else in microbiology. One early outcome of these discussions was the workshop in statistics run by Basil Jarvis at our Summer Conference in Manchester in 2009. Another, more recently introduced development, is that members now have access to a virtual microbiology laboratory (VML) online. The VML was developed originally by our colleagues at the University of Nottingham, primarily for use by their own students, but its wider value will be clear on a first (virtual) amble through the site. It covers a number of key basic practical techniques essential to microbiology: aseptic technique, the growth and isolation of microorganisms, common enumeration



techniques such as the standard plate count, most probable number techniques and absorbance, Gram staining, motility testing, biochemical tests, serotyping and basic microscopy. There is also a test yourself section which includes, among other things, the opportunity to calculate microbial counts based on raw plate count data — a skill that sometimes seems unnecessarily daunting to students.

Particular strengths of the virtual laboratory are the visual aids. The power of illustration is unquestionable — the venerable maxim that 'a picture is worth a thousand words' is variously attributed to ancient Chinese sages and advertising men in the United States. For those of you, like me, with a more far-reaching interest in popular culture, it may also stick in the mind as featuring prominently in the lyrics of a saccharine love song of the 1970s. In the virtual laboratory, video presentations are supported and enhanced by cartoon animations of the various procedures. This, in my view, is not an unnecessary redundancy but a powerful supplement to the videos since an animated diagram can often give a much clearer representation of what is actually going on. I speak as one who recently failed abjectly to make an origami *Tyrannosaurus rex* for my grandson by trying to follow a video.

Although I have emphasized the support provided for training in some well-established techniques, we recognize the wider value of the virtual laboratory and have plans to expand it to cover basic molecular techniques too. There is no substitute for hands-on practical experience, but the VML can help improve skills and give a better appreciation of practical techniques, particularly in situations where the expense of providing practical experience is prohibitive. So look out for further developments and, as always, we welcome feedback.

Martin Adams
President of the Society

ceo's column

Philip Wheat reports on the latest developments within the Society

Once again it is a pleasure to announce this year's annual *Environmental Microbiology* lecture. The lecture will take place at the Royal Society of Medicine in London, 8 October 2012 starting at 6.30 pm. This year's lecture will be given by Professor Sang Yup Lee from the Korea Institute of Science and Technology (KAIST) who will present "*Systems metabolic engineering for a green chemical industry*", full details of which can be found on page 17.

Professor Lee is a distinguished Professor at KAIST and he is attached to the Departments of Chemical and Biomolecular Engineering, Bio and Brain Engineering and Biological Sciences. He is also Dean of the College of Life Science and Bioengineering, Head of the Metabolic and Biomolecular Engineering National Research Laboratory, Director of the BioProcess Engineering Research Centre, Director of the Bioinformatics Research Centre and finally Co-director of the Institute for BioCentury. Amongst many other roles Professor Lee is also an Editor for the S*f*AM journal Microbial Biotechnology. He has co-authored over 350 peer-reviewed papers, edited over 50 books and his outstanding work has been recognized by numerous international awards.

The lecture is free of charge and open to all members to attend, you should receive an invitation from the President of The Society with this issue of *Microbiologist*. So that we can finalize arrangements for the event, please respond as soon as possible. Once again I would like to remind you that should you require financial assistance to attend, for such costs as travel and subsistence, why not apply for a **Scientific Meeting Attendance Grant**? For more information see the box to the right or visit: www.sfam.org.uk/en/grants--awards/scientific-meeting-attend-grant.cfm.

If you are unable to attend the lecture it will be available online within 72 hours of the event, a facility which members have informed us is a useful teaching resource. Last year's lecture has so far had over 2,000 hits.

I can report that as I am writing this column (end of March 2012) membership of The Society is at an all-time high. The total membership currently stands at over 2,100 members. This compares to just over 1,200 at the end of 2005, an increase of 75% in just over six years. This is good news, in particular, with the recent challenging economic climate in some parts of the world. Membership of S*f*AM provides tremendous value for money and this increase in membership goes some way to reflect that. I would like to thank all existing members who have recommended S*f*AM to potential new members.

Whilst I am discussing membership, I can report that, to date, we have recruited over 200 new eStudent Members (see *Microbiologist* December 2011, p9). Thank you to all our members who have promoted this category of membership in their institutions. If you would like further information on eStudent membership, please contact Julie Buchanan (julieb@sfam.org.uk). Finally, I can also report the success of the 3-for-2 offer to current members on renewing their membership. To date over 300 members have chosen to renew their membership by taking this option.

Philip Wheat
Chief Executive Officer

Scientific Meeting Attendance Grant or President's Fund? You decide!

Are you going to a scientific meeting? Do you need funding? Do you know which of our grants to apply for?

The **Scientific Meeting Attendance Grant** will fund your travel, accommodation and registration fees at any relevant scientific meeting, including S*f*AM meetings, up to a value of £300. This is ideal if you wish to attend a conference or one-day meeting/symposium but you're not presenting a poster or giving an oral presentation or contributing to the meeting in any other way.

The **President's Fund** is designed for you if you're presenting a poster or giving an oral presentation at a relevant scientific conference, meeting or workshop, including S*f*AM meetings. It will fund travel, subsistence and conference fees up to a value of £1200.

For more information about all our grants and awards, please visit: **www.sfam.org.uk/en/grants--awards/index.cfm**

SfAM AGM AGENDA 2012

The 81st Annual General Meeting of the Society for Applied Microbiology will be held on Wednesday 4 July at 4.45 pm at the George Hotel, Edinburgh.

1. Apologies for absence

2. Approval of minutes

Approval of minutes published in the September 2011 issue of *Microbiologist* of the 80th Annual Meeting held in Dublin in 2011.

3. Matters arising from the previous minutes

4. Report of the Trustees of the Society 2011

(i) Report of the Honorary President.

(ii) Report of the Honorary General Secretary.

(iii) Report of the Honorary Meetings Secretary.

(iv) Report of the Honorary Treasurer.

5. Adoption of the 2011 Annual Report

6. Election of new Members

(including Honorary Members), deaths and resignations.

7. Election of new Executive Committee Members

8. Any other business

We're listening

In 2009 we sent a survey to members asking you what more we could be doing to ensure you get the most from your membership of the Society. The results of this survey have guided the direction of many of our new initiatives. Since then we've:

- Increased the subsidy for members to attend our scientific meetings — now it only costs a member £250 to attend the three day Summer Conference (including accommodation).
- Increased the budget allocation to all our grants — now we're giving members more financial support for a wider range of activity.
- Increased membership benefits — now members get a 25% discount on Wiley-Blackwell books, online access to all five SfAM journals and more without seeing an increase in membership fees.
- Increased membership types — by introducing many new categories of membership, some of which are free of charge, we're sharing our membership benefits with a wider global network.
- Decreased membership fees — by offering three years membership for the price of two, Full Members can get even more value for their money.
- Provided better online connection — the new website, which went live in October 2011, provides members with the opportunity to connect with us and each other with just the click of a mouse. Online meeting booking and online grant application are two facilities requested by members and delivered by us. Our Facebook, Twitter and LinkedIn pages enhance the online experience for members and potential new members alike.

These are just a few examples of the ways we've implemented some of your requests. And we're not stopping there — we'll be sending you another survey during 2012, so if you want to influence our direction and have a say in future membership benefits, complete the survey to let us know.

All completed surveys will be entered into a prize draw.

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.

Argentina

H. Rubinstein

Bangladesh

F. Rahman

Belgium

P. Fickers

Brazil

C. Del Lama Marques; L. Marques

China

N.-Y. Zhou

Croatia

T. Pogacic

Cyprus

V. Wohrer

Denmark

J. Castro Mejia; B. Smets

France

S. Baron; C. Downing; H. Gueune; D. Hervio-Heath

India

Z. Bhathena; G. Paramasivam; D. Ranade; M. Sarma

Ireland

S. Bullman; K. Daly; D. Ferguson; E. Gabriel; B. Healy

Japan

Y. Taniuchi

Lebanon

D. Faour-Klingbeil

Mexico

A. Garcia Heredia

Nigeria

L. Kigigha; N. Kihula; A. Odunsi; E. Olukanni; I. Oyegbite

South Korea

D. Lee

UAE

S. Chir

UK

G. Absoud; J. Adetula; M. Afanasjeva; F. Akinwole; A. Al-Khayatt; T. Anwar; S.-J. Askin; A. Atuonwu; A. Awadh; N. Bamford; N. Barratt; A. Bartholomew; A. Basit; S. Batters; S. Bhandare; Z. Bi; L. Birse; R. Birtles; R. Borne; C. Brand; J. Broadbent; M. Bruser; M. Burgess; S. Butt; A. Caparnagiu; K. Carolan; H. Carr; P. Cassel; A. Chappell; K. Chopra; C. Churchwood; H. Ciesielczuk; R. Colledge; L. Conlon; F. Corby; G. Cotton; J. Deacon; O. Diribe; S. Doll; K. Dougal; J.-L. Douglas; C. Duignan; S. Dunsmore; G. Elliott; O. Elwick; L. Farrell; S. Farrell; M. Febrer; S. Fell; D. Fitzhenry; R. Fogerty; R. Fuchs; L. Furness; C. Gallina-Ramos; Z. Geberehiwot; A. Gillett; F. Gosney; J. Gouriet; L. Gray; K. Guest; M. Gurbo; L. Hall; F. Hassard; M. Hasso-Agopsowicz; N. Haughton; T. Hill; K. Holland; A. Hurpaul; W. Ind; H. Jacobs; A. Jaffer; B. Kainth; V. Kajander; V. Karpuk; S. Kelly; J. Khalid; B. Khan; S. Khanom; F. King; J. Knight; A. Knight; P. Krol; M. Kurpiewski; B. Lane; D. Lecky; H. Levitt; Y. Li; V. Logina; A. Lyne; L. Majchrzak; K. Mee; P. Mehrotra; A. Mikailionyte; G. Mills; P. Monk; B. Morgan; H. Morgan; O. Ogunleye; O. Okumbor; A. Olabode; A. Passley-Biggins; D. Peggs; R. Penzo; K. Preechasuth; S. Pregnolato; F. Raja; A. Raju; J. Ritchie; S. Roberts; A. Roberts; G. Robinson; P. Robinson; S. Rowan; O. Rumford-Warr; P. Russell; A. Ryan; E. Sadler; A. Salad; A. Salunkhe; M. Sanjeevan; I. Shabbir; P. Shanmuganathan; S. Shao; L. Sharif; N. Siddique; S. Silvera; H. Simmonds; D. Small; R. Soriano; B. Standen; R. Steel; H. Strudwick; Y. Syeda; E. Tapp; J. Tejpal; C. Tong; S. Valderama; G. van Keulen; P. Wallwork; A. Ward; S. Watkins; J. Watts; T. White; K. Whitehead; J. Williams; S. Wilson; G. Yemba; H. Yesilkaya; H. Yeung; A. Yusef; B. Zainuddin

Uruguay

A. Alvarez

USA

B. Bluhm; W. Crawford; A. Fernandes; M. Otte; E. Sanjuan

bioFocus

Mark Downs looks at journal publishing

Over 60 of our 92 member organizations are other learned societies, which we represent on issues such as funding, science and education policy, and careers and skills. These are all important, but increasingly, a key topic of discussion has been publishing. Learned societies have long histories of publishing high-quality scientific journals, books and reports. These publications not only serve their communities, but earn income to support the societies' charitable work. The surplus from publishing is often used to fund membership activity, engage the public, provide career and educational support, and to offer the high-level scientific oversight which these societies do so well.

Journal publications — using a peer review system to ensure quality and credibility — are a key part of the way the UK disseminates scientific research and knowledge, usually via university and other library systems. But a challenge to this long-established route is the argument that the public often has to pay twice to access this knowledge: once through the public funding of research, and then again through the need to pay for the journals which disseminate the outcomes. Government and other funders of research think it is time to change this model, to ensure anyone can access scientific publications funded through public money with no charge. This sounds eminently sensible, but does the ideology match the reality of publishing? As ever, the answer is not straightforward. If these publications are available for free, then how will they be edited, formatted, presented and stored in an accessible way? In practice, the peer review system and the important process of generating the final copy — the 'version of record' — cannot be delivered for nothing; it must be funded by a sustainable mechanism. Someone will have to pick up the cost even if it is not a traditional subscriber.

There are two open access systems that could allow the public to see articles for free. Firstly there is Gold Open Access, where the author pays a fee to cover publication; the money to support this might well have been included by funders in a grant. Then there is Green Open Access, where an author deposits the final draft of an article or data in a

searchable online archive, as a result of an agreement or after an embargo period. To construct a viable financial model for the latter system is tricky: how is the infrastructure paid for and maintained, and can we really risk losing the value of professional publications in terms of quality control, format, style and presentation? Gold Open Access also has its problems, particularly where publication happens after a grant has ended, where small or financially stretched funders simply won't pay the fee, and where the research is essentially unfunded (this is not uncommon).

Both Green and Gold systems are already operating in a limited way and many publishers, including learned societies, offer the option of Open Access Publications or a hybrid of Open Access and traditional publishing. It is clear that Open Access of some colour is here to stay, and there is an urgent need to produce a viable and sustainable financing mechanism to ensure a rosy future. But learned societies are concerned. Although many make their publications available for free after a set period anyway, if Green Open Access took off in a substantial way, subscriptions would fall and the drop in income could threaten the substantial charitable work they do. And even switching to the Gold system, which seems more likely, will lead to a period of change that will need to be carefully managed. What the Society and our Member Organizations would like is recognition from Governments that the changes in publishing are complex: learned societies have a critical role to play as charities in education, public policy and professional development, as well as being key publishers. It's important to see that these things are all linked: publishing revenue funds charitable work, which is critical to the future success of science in the UK and hence the economy. Policymakers need to take account of that as publishing models evolve. And before we start redesigning publishing, is it really an issue for the public as Open Access supporters claim? Who are the thronging masses who want to read specialist science publications? If there is such demand it has passed me by. And interlibrary loans for people with a real interest are still there. Open Access is a different model. But the jury is out on whether it is a better model.

This article first appeared in *The Biologist,* **Vol. 59**, No. 1, p64.

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

journalWatch

News about the Society's journals

Microbial Biotechnology

The following articles were the most downloaded articles from Microbial Biotechnology during January and February 2012:

Marine genomics: at the interface of marine microbial ecology and biodiscovery. Heidelberg, K. B., Gilbert, J. A. and Joint, I. **Vol. 3**, No. 5.

Crystal ball – 2011.**Vol. 4**, No. 2.

Bacterial persistence increases as environmental fitness decreases. Hong, S. H., Wang, X., O'Connor, H. F., Benedik, M. J. and Wood, T. K. (2012).

Strategies for discovery and improvement of enzyme function: state of the art and opportunities. Kaul, P. and Asano, Y. (2012). **Vol. 5**, No. 1.

Bio-palladium: from metal recovery to catalytic applications. De Corte, S., Hennebel, T., De Gusseme, B., Verstraete, W. and Boon, N. (2012). **Vol. 5**, No. 1.

Journal of Applied Microbiology

The following articles were the most downloaded articles from Journal of Applied Microbiology during January and February 2012:

Antimicrobial activity of essential oils and other plant extracts. Hammer, K. A., Carson, C. F. and Riley, T. V. (1999). **Vol. 86**, No. 6.

Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Dorman, H. J. D. and Deans, S. G. (2000). **Vol. 88**, No. 2.

A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Lambert, R., Skandamis, P., Coote, P. and Nychas, G.-J. (2001). **Vol. 91**, No. 3.

Dunaliella biotechnology: methods and applications. Hosseini Tafreshi, A. and Shariati, M. (2009). **Vol. 107**, No. 1.

Editorial. Gilmour, A. **Vol. 107**, No. 2.

Letters in Applied Microbiology

The following articles were the most downloaded articles from Letters in Applied Microbiology during January and February 2012:

Antifungal activity of thyme (*Thymus vulgaris* L.) essential oil and thymol against moulds from damp dwellings. Šegvić Klarić, M., Kosalec, I., Mastelić, J., Piecková, E. and Pepeljnak, S. (2007). **Vol. 44**, No. 1.

Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Nostro, A., Germanò, M., D'Angelo, V., Marino, A. and Cannatelli, M. (2000). **Vol. 30**, No. 5.

The probiotic bacterium *Lactobacillus plantarum* species 299 reduces intestinal permeability in experimental biliary obstruction. White, J., Hoper, M., Parks, R., Clements, W., Diamond, T. and Bengmark, S. (2006). **Vol. 8**, No. 4.

Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Pitcher, D., Saunders, N. and Owen, R. (1989). **Vol. 29**, No. 2.

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

Environmental Microbiology

The following articles were the most downloaded articles from Environmental Microbiology during January and February 2012:

Beyond the Venn diagram: the hunt for a core microbiome. Shade, A. and Handelsman, J. (2012). **Vol. 14**, No. 1.

Bacterial species may exist, metagenomics reveal. Caro-Quintero, A. and Konstantinidis, K. T. (2012). **Vol. 14**, No. 2.

Omics for understanding microbial functional dynamics. Jansson, J. K., Neufeld, J. D. , Moran, M. A. and Gilbert, J. A. (2012). **Vol. 14**, No. 1.

Targeted metagenomics: a high-resolution metagenomics approach for specific gene clusters in complex microbial communities. Suenaga, H. (2012). **Vol. 14**, No. 1.

Proteomics of extremophiles. Burg, D., Ng, C., Ting, L. and Cavicchioli, R. (2011). **Vol. 13**, No. 8.

Environmental Microbiology Reports

The following articles were the most downloaded articles from Environmental Microbiology Reports during January and February 2012:

Microbial respiration in ice at sub-zero temperatures (-4°C to -33°C). Bakermans, C. and Skidmore, M. (2011). **Vol. 3**, No. 6.

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

Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef. Littman, R., Willis, B. L. and Bourne, D. G. (2011). **Vol. 3**, No. 6.

Quorum-sensing quenching by rhizobacterial volatiles. Chernin, L., Toklikishvili, N., Ovadis, M., Kim, S., Ben-Ari, J., Khmel, I. and Vainstein, A. (2011). **Vol. 3**, No. 6.

Powering microbes with electricity: direct electron transfer from electrodes to microbes. Lovley, D. R. (2011). **Vol. 3**, No. 1.

The Society publishes two monthly journals: *Journal of Applied Microbiology* and *Letters in Applied Microbiology*. 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.

Felicity Howlett
Wiley-Blackwell

Winter Meeting 2012 report

Royal Society, London, UK, Wednesday 11 January 2012

Microbiological safety of imported food

Microorganisms and climate change

The Winter Meeting 2012 was held on the 11 January at the Royal Society in London. The day began with the Denver Russell Memorial Lecture before two taster presentations for the afternoon sessions on *Microbiological safety of imported foods* and *Microorganisms and climate change*

Martin Adams presenting Jean-Yves Maillard with a framed print

The Denver Russell Memorial Lecture was this year presented by Dr Jean-Yves Maillard of Cardiff University whose presentation was entitled **Biocides against emerging and new pathogens — a cause for concern?** He began by paying tribute to Professor Denver Russell, whose work has contributed a significant amount of knowledge to the field.

He went on to provide an historical look at biocides including mummification, fumigation using burning juniper branches against the plague, and the use of chlorine to prevent childbed fever by Semmelweis in the mid-1800s. He went on to describe today's uses of biocides including the use of disinfectants, the preservation of food and other materials, as well as the development of antimicrobial surfaces. He asked whether the development of antibiotic resistance is encouraged by the use of low concentrations of biocides and described a recommendation from the **Scientific Committee on Emerging and Newly Identified Health Risks** (SCENIR) 2009 "*Prudent use guidelines for biocides*". Jean-Yves then went on to talk about the varying degrees of susceptibility of different organisms to biocide activity. The adaptation of microorganisms was described, through the formation of spores, protozoal encystation and the formation of viral aggregates. The repeated exposure of microorganisms to low concentrations of biocides decreases their susceptibility to biocide treatment: very low concentrations of biocides change the susceptibility profiles of pathogens — but does this matter? Jean-Yves explained that in practice the increased use of biocides on surfaces and in products can promote antibiotic resistance. To summarize, Jean-Yves returned to the SCENIR recommendation: "*In view of the large and increasing use of biocides and the continuous increase of resistance to antibiotics, data and methods are needed to clearly characterize the risk.*"

Lucy Harper

The next two lectures of the morning were setting the scene for the afternoon's sessions, with Caroline Willis from the Health Protection Agency (HPA) discussing monitoring the **Microbiological safety of imported foods**. Fifty per cent of the UK's food is imported and the Food Standards Agency (FSA) strategic plan 2010 to 2015 states that imported foodstuff should be safe to eat. The UK relies on HACCP and not a system of end product testing, thus assessing the risk and implementing critical control points. Sampling methods

should be based on three things: statutory requirements, risk assessment (rapid alert system) and surveillance. In 2009 the HPA carried out sampling on 3,055 imported foodstuffs, examples of contaminants found were *Salmonella* spp. in vegetable and salad stuff, *Bacillus cereus* in bean curd, peppers and spices and moulds in raisins. Result interpretation is also an important part of the work conducted by the HPA in monitoring imported food with both European and HPA guidelines being used for this analysis; the European guidelines do not cover all food types and thus the HPA have compiled a more comprehensive list including testing parameters.

Next Andrew Nichols from the University of Plymouth discussed climate change and communicable diseases and what the risks are! The main dramatic weather conditions that can have an impact on infectious diseases are: heatwaves, storms, floods, fires and droughts. The effects of these are observed in agriculture, fisheries, ecosystems, economies, health and well-being. The diseases resulting from these changes in climate can be brought about via water, vector or food, although the impact in the UK is low. Water can result in an increased risk of disease due to flooding and heavy rainfall when combined with increased temperature, which can lead to outbreaks of toxic algal blooms, and cholera. Increasing ambient temperatures and flooding can also support vector species such as malaria. Outbreaks in countries, such as Greece, that are not commonly associated with malarial disease have been observed in recent years. Increases in temperature can also lead to increases in diarrhoeal diseases, in particular, salmonellosis. Surveillance, early warning systems, policy responses and risk assessments should go some way to the control of infectious diseases caused by climate change.

Katie Laird

Following a break for lunch and a chance to visit the trade show, delegates split into two groups for the afternoon sessions.

S*f*AM meetings secretary, Andy Sails, chaired the afternoon session addressing the **Microbiological safety of imported foods**. In her talk, **From the banal to the bizarre — microbiological hazards and imported foods**, the first speaker, Sue Jones, cited examples of some of the more unusual submissions to the HPA Food, Water and Environmental (F,W&E) laboratory at Porton.

Although HPA guidelines are used in addition to EC regulations for imported foods, exotic foods may not be adequately covered. Sue presented a case study of a local resident wishing to commercially produce a mudfish paste and a raw crab product. Despite samples passing tests for microbiological safety the production processes were deemed to be unsatisfactory, so the products were considered to be high-risk.

Oriental bean curds which are fermented using *Bacillus cereus* pose a dilemma as this organism may consequently be present in numbers which are classed as unsatisfactory. A further complication associated with *B. cereus* was also highlighted: the length of time it takes to reliably differentiate this organism from other *Bacillus* species is in conflict with the timescale of the Port Authority not wishing to detain the product.

Edible or decorative leaves were described as being 'under the radar' of regulations. Some of these are used for wrapping ready-to-eat foods and some are chewed rather than ingested. In both of these situations, however, the leaves have the potential to transmit pathogenic organisms to consumers.

The final group of products described by Sue involved contamination with *Salmonella* species. Infant salmonellosis has been associated with imported reptile feed and 5 to 7% of dog chews have been found to carry a wide range of *Salmonella* serotypes.

Christine Little, HPA Colindale, then spoke about **Salad days — foodborne outbreaks due to imported fruit and vegetables: hazards, vehicles and sources**. She explained that although the number of outbreaks associated with fruit and (uncooked) vegetables is smaller than those associated with meat products, the size of each outbreak can be substantially larger.

The year-round demand for fresh fruit and vegetables in the UK has resulted in them being sourced from many different countries through highly complex supply chains. Christine used two specific UK outbreaks to illustrate some of the pertinent issues. The 2010 outbreak of *Salmonella* Bareilly was found to be linked to consumption of bean sprouts. By examining the UK bean sprout distribution network the problem was traced back via caterers to a wholesale producer. The second example was the 2007 outbreak of *Salmonella* Senftenberg which arose from contamination of pre-packed fresh basil grown in Israel. This typified the situation where those affected do not recall the consumption of minor ingredients of a dish.

***Salmonella* and imported eggs and poultry** was the topic for the third talk, given by Sarah O'Brien, University of Liverpool. Two prospective population-based studies of infectious intestinal disease conducted 15 years apart (iid1 and iid2) were described. Following an initial telephone survey, participants were followed up for a year. Those who developed gastrointestinal symptoms were divided into two groups: one group were simply observed whilst the other had their routine clinical practice altered, including having samples taken. The results indicated that the observed decrease in cases of non-typhoidal salmonellosis is real. This decrease has been attributed to the success of industry-led control interventions.

Sarah then concentrated on *Salmonella* outbreaks associated with eggs. Recent online publication of foodborne outbreaks listed by type of cuisine has revealed a high odds ratio for *Salmonella* infections associated with raw shell eggs if the eggs are sourced from outside of the UK. This is compatible with the finding that UK flocks have a much lower prevalence of *Salm.* Enteritidis than those from other EU member states.

The final talk of the session was **Safety of imported foods — a commercial perspective**, presented by Kaarin Goodburn representing the Chilled Food Association, UK (CFA). The CFA was formed in 1989 in response to concerns regarding *Listeria* in foods. Kaarin emphasized some features of the food products that CFA regulate, such as having short shelf lives, being predominantly multicomponent, UK made and hand wrapped.

After summarizing the Association's strategy and membership requirements she explained that there were four layers of enforcement. Traceability was cited as a non-

negotiable part of chilled food management systems – both backwards (to the source) and forwards (to the customer). The traceability of raw materials is at the level of field identification, rather than just producer or country of origin. Kaarin concluded by saying that standards which are implemented to assure chill temperatures must be appropriate.

Louise Hill-King

Afternoon session B provided the opportunity for four eminent speakers to present their thoughts and data regarding the highly topical field of microbes and climate change. The first presentation by Dave Reay of the University of Edinburgh set the scene for the following presentations. He highlighted the pivotal, yet often overlooked role of microbes in sculpturing our climate and the possibilities for harnessing this ability to manipulate the global climate. During his talk, he overviewed the magnitude of our algae-filled oceans and their role in CO_2 cycles. He went on to elaborate how slight temperature shifts influence the function of our oceans as "carbon sinks" and the contribution of wetlands to global methane levels. Through increased understanding of geoengineering we have the potential to harness these microbial contributions to our evolving world. Dietary changes influence microflora and consequently gaseous emissions. Subsequently, agricultural management, such as application of ammonium sulphate to paddy fields, could reduce their methane emissions, and careful balancing of nitrifiers/denitrifiers to manipulate nitrification inhibitors, coupled with the use of microbial biofuels, have a powerful potential to reduce our impact upon the global climate.

The session focus then switched from influencing our climate to looking at the effects of climate change upon pathogens of human/veterinary importance. Marion Wooldridge of the Animal Health and Veterinary Laboratories Agency (AHVLA) enlightened us to how climate was only one of several influencing factors upon pathogen emergence and spread. Combined forces of climate, human demographics, politics and economics, technology and societal factors all contribute to changing disease patterns. These complex interacting factors all contribute to the evolving dynamics of infectious diseases providing new niches and opportunities for emerging infectious episodes. These complex interacting forces resulting in the new emergence of infection were illustrated with examples such as Crimean-Congo haemorrhagic fever virus (CCHFV) and its tick vector, *Hyalomma marginatum*, in Turkey and the Balkans, and the incursions of *Aedes albopictus* mosquitoes into temperate regions and subsequent new foci of infection with chikungunya virus.

This theme was further developed by Paul Gale, also from the AHVLA, who focussed on vector-borne viruses and the driving forces that influence their dynamics within Europe. Modelling studies have predicted that we will see incursions of infections such as CCHFV, Rift Valley Fever virus and African horse sickness from their current endemic regions. The example of CCHFV was further explored to model how these infections were being predicted to expand, such as through the spread of ticks on migratory birds. Tools such as the Geographical Information System (GIS) contribute towards these modelling efforts, further facilitated through European networking initiatives such as Epizone.

The session concluded with a switch in emphasis, this time looking from the perspective of the pathogen. Paul Hoskisson from the University of Strathclyde explored pathogen adaptation to their changing planet. He discussed how extreme virulence was likely to reflect recently emerged pathogens and that through co-evolution with their host, they would eventually adapt to less virulent forms. During his talk he also explored the largely anthropogenic forces that result in the emergence of novel pathogens through exertion of selective forces on the complex ecological microbial dynamics.

This lively and thought-provoking session gave all attendees plenty of "food for thought" for their journey home.

Sally J Cutler

Environmental Microbiology lecture 2012

The 2012 *Environmental Microbiology* Lecture will be presented by Professor **Sang Yup Lee**, Distinguished Professor and Dean of the College of Life Science and Bioengineering at KAIST (formerly the Korea Advanced Institute of Science and Technology).

Royal Society of Medicine, London ■ Monday 8 October 2012

Professor Lee will present: *Systems metabolic engineering for a green chemical industry*. It has been 20 years since the term metabolic engineering was officially introduced. Microorganisms isolated from nature are often inefficient, so metabolic engineering has been employed to improve microbial performance.

Recently, metabolic engineering has become more powerful and is now essential in developing superior microorganisms formed from the integration of systems biology with synthetic biology. In his lecture, Professor Lee will present the general strategies for metabolic engineering of microorganisms. These will be accompanied by many successful examples, including the production of chemicals, fuels and materials. Systems metabolic engineering will also be introduced as an essential technology in making any bioprocess competitive.

The lecture will take place on 8 October 2012 at the Royal Society of Medicine, London, 1 Wimpole St, London W1G 0AE. The lecture will begin at 6.30pm, with tea/coffee being served from 6pm. There will be a drinks reception following the lecture.

If you wish to attend the lecture, please contact emlecture@sfam.org.uk or complete and return your invitation slip which is included in this issue of *Microbiologist*.

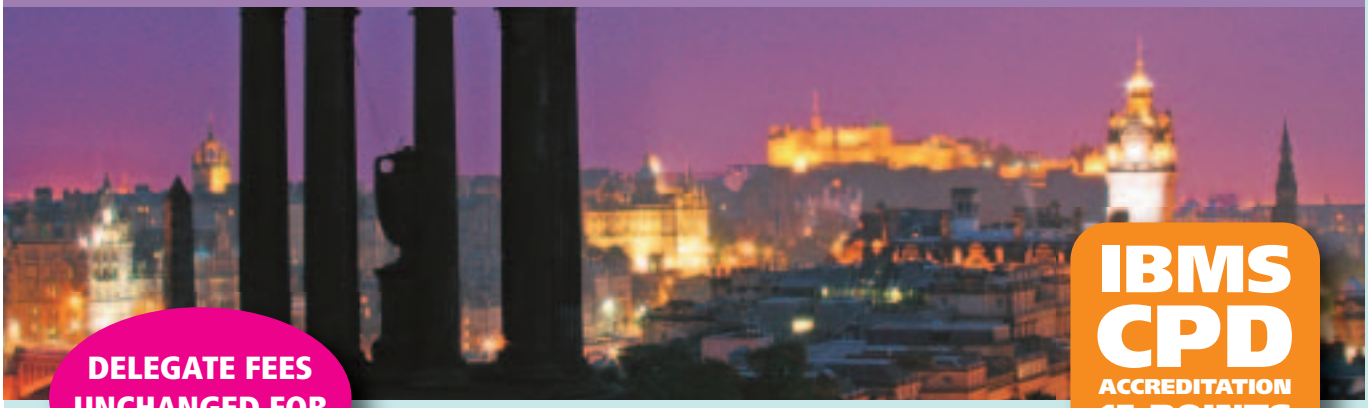
2 - 5 July 2012

Summer Conference 2012

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

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

The George Hotel, Edinburgh, UK ■ Monday 2 July — Thursday 5 July 2012



DELEGATE FEES UNCHANGED FOR 2012!

IBMS CPD ACCREDITATION 15 POINTS

Programme

Monday 2 July 2012

11.00-17.00 **Intellectual property workshop**

18.00-19.00 **Lewis B Perry Memorial Lecture**
Globalization of antimicrobial resistance
Didier Pittet, University Hospital in Geneva, Switzerland

19.00-20.00 **Drinks reception and buffet**

20.30-22.30 **Quiz night**

Tuesday 3 July 2012

Session 1: Microbial resistance to antibiotics and biocides

09.00-09.35 **Impact of bacterial resistance to biocides in the healthcare industry**
W. Rutala, University of North Carolina School of Medicine, USA

09.35-10.10 **Bacterial resistance and cross-resistance: overrated story or real concern?**
Jean-Yves Maillard, Cardiff University, UK

10.10-10.45 **Recent advances in antibiotic resistance in *Ps. aeruginosa***
Keith Poole, Queen's University, Kingston Ontario, Canada

10.45-11.15 **Tea, coffee and trade show**

11.15-11.50 **Evolution and dissemination of vancomycin resistance in Gram-positive cocci**
Patrice Courvalin, Institut Pasteur, France

11.50-12.25 **β lactamase resistance in Enterobacteriaceae**
Neil Woodford, Health Protection Agency, Colindale, London, UK

These preliminary programme times and titles were correct at the time of going to press

12.25-13.00 **Controlling antibiotic resistance through stewardship**
Rob Townsend, Sheffield Teaching Hospitals Foundation Trust, UK

13.00-14.00 **Lunch, trade show and posters**

Session 2: Natural and experimental adaptation in bacteria

14.00-14.35 **The various lifestyles of the *Burkholderia cepacia* complex species: a tribute to adaptation**
Eric Deziel, Centre INRS, Canada

14.35-15.10 **Adaptive evolution in Geobacter**
Pier-Luc Tremblay, University of Massachusetts, USA

15.10-15.45 **Extremophiles and halophiles: adaptation to high salinity environments**
Terry McGenity, University of Essex, UK

15.45-16.00 **Tea, coffee and trade show**

16.00-17.00 **Attended poster session 1**

17.00-18.00 **Student session**

17.00-19.30 **Trade show and competition**

Wednesday 4 July 2012

Session 2 continued: Natural and experimental adaptation in bacteria

09.00-09.35 **Studying and designing systems: towards orthogonal biochemical reaction networks**
Sven Panke, ETH-Zurich, Switzerland

09.35-10.10 **Engineered bacteria as pollution bioreporters**
Jan Roelof van der Meer, University of Lausanne, Switzerland

10.10-10.45 **Tea, coffee and posters**

10.45-12.00 **Attended poster session 2**

12.00-13.00 **Lunch and posters**

Session 3: Bioremediation

13.00-13.35 **Microbial resource management and environmental biotechnology**
Willy Verstraete, University of Ghent, Belgium

13.35-14.10 **Selection and manipulation of bacteria for rhizoremediation**
Juan Luis Ramos, EEZ, Granada, Spain

14.10-15.10 **Student presentations**

15.10-15.30 **Tea, coffee and posters**

SfAM Award Lectures

15.30-15.35 **Introduction to the New Lecturer Research Grant**
Martin Adams, President of the Society

15.35-16.10 **SfAM New Lecturer Research Grant Lecture**
Nick Jakubovics, School of Dental Science, Newcastle University, UK

16.10-16.15 **Introduction to the W H Pierce Prize**
Martin Adams, President of the Society

16.15-16.45 **W H Pierce Prize Lecture**
To be confirmed

16.45-17.15 **Annual General Meeting**

19.00 onwards **Drinks reception and conference dinner at The Hub**

Thursday 5 July 2012

Session 3 continued: Bioremediation

09.00-09.35 **Bioremediation of petroleum hydrocarbon contaminants in marine environments**
Ian Head, University of Newcastle, UK

09.35-10.10 **Exploiting fungi in bioremediation of hazardous chemicals**
Hauke Harms, Helmholtz Centre for Environmental Research, Germany

10.10-10.45 **Tea, coffee and posters**

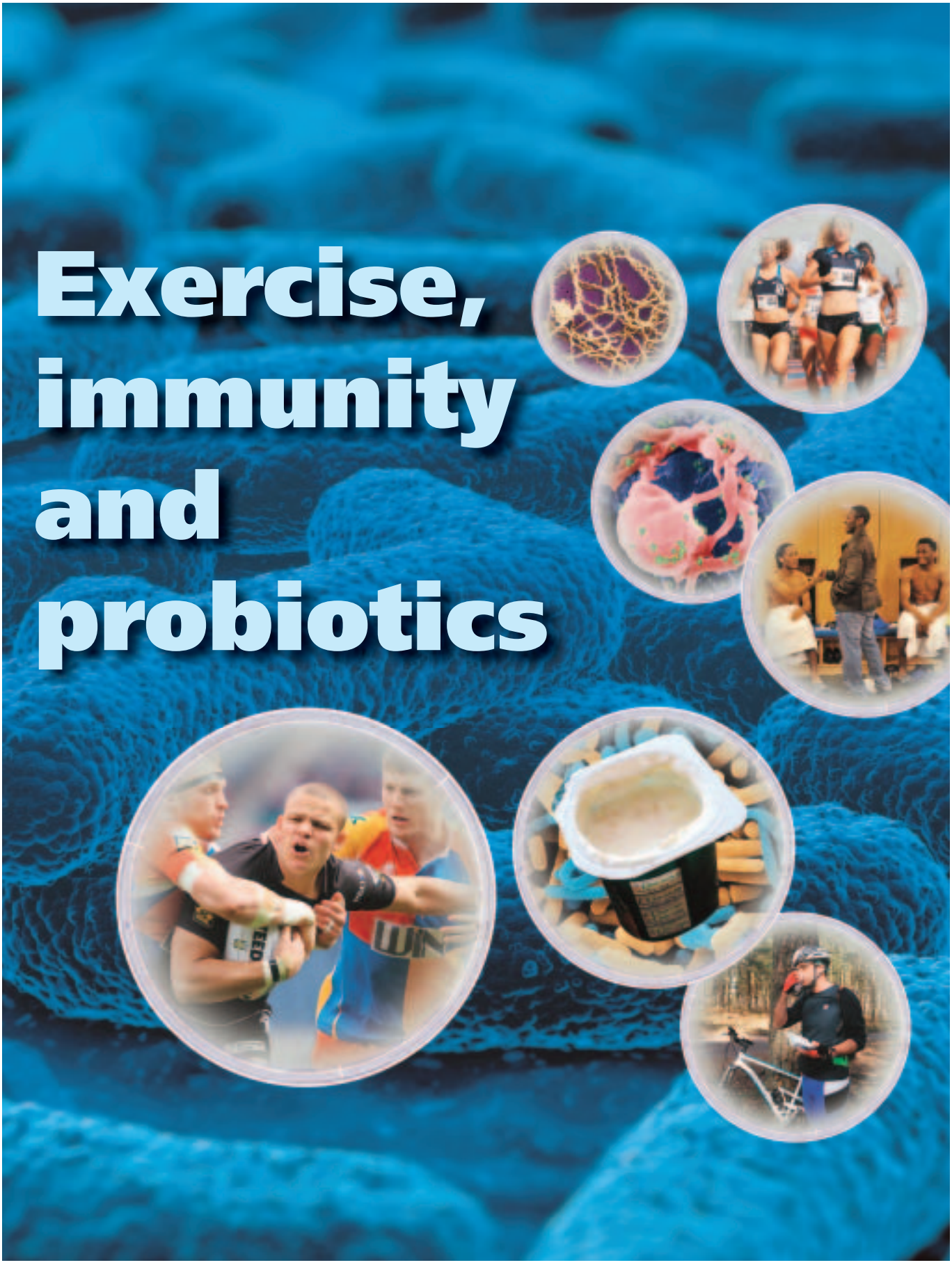
10.45-11.20 **Geomicrobiology and bioremediation**
Geoffrey Gadd, University of Dundee, UK

11.20-11.55 **Bioremediation of uranium from minewaters**
Lynne Macaskie, University of Birmingham, UK

12.00-13.00 **Lunch and close**

To book your place please visit: www.sfam.org.uk/en/events/index.cfm/summer_conference

Exercise, immunity and probiotics



How exercise affects your immunity and susceptibility to infection

We all suffer from colds at some time but recent research indicates that a person's level of physical activity influences their risk of respiratory tract infections such as the common cold, most likely by affecting immune function. Moderate levels of regular exercise seem to reduce our susceptibility to illness compared with an inactive lifestyle (Matthews *et al.*, 2002; Nieman *et al.*, 2011) but long hard bouts of exercise and periods of intensified training put athletes at increased risk of colds and flu (Walsh *et al.*, 2011 a & b).

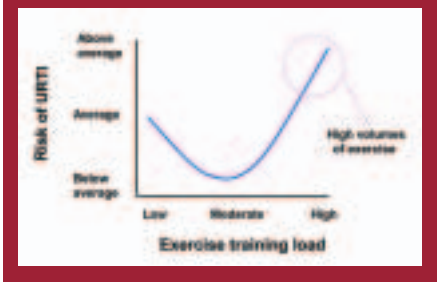
Immune function and infection risk

Infections of the nose, throat, windpipe (trachea) or the two airways that branch from the trachea as it reaches the lungs (bronchi) are the most common infections that people acquire. These upper respiratory tract infections (URTIs) include the common cold, sinusitis and tonsillitis. More than 90% of URTIs are due to an infection with a virus: the most common ones being human rhinovirus, adenovirus and coronavirus. The more severe and feverish symptoms of flu are caused by an influenza virus. The average adult has two to three URTIs each year and young children have twice as many. We are constantly exposed to the viruses that cause these infections, but some people seem more susceptible to catching URTIs than others. Every day our immune system protects us from an army of pathogenic microbes that bombard the body. Immune function is influenced by an individual's genetic make-up as well as other external factors such as: stress, poor nutrition, lack of sleep, the normal ageing process, lack of exercise or excessive training (Gleeson, 2005). These factors can suppress the immune system, making a person more vulnerable to infection.

Exercise and its effect on the immune system

Exercise can have both a positive and negative effect on the functioning of the immune system and can influence a person's vulnerability to infection. Researchers have found a link between moderate regular exercise and reduced frequency of URTIs compared with a sedentary lifestyle and also with excessive amounts of exercise and an increased risk of URTIs. A one-year study of over 500 adults found that participating in one to two hours of moderate exercise per day was associated with a one third reduction in the risk of getting a URTI compared with individuals that had an inactive lifestyle (Matthews *et al.*, 2002). Other studies have shown that when 40 minutes of moderate exercise is repeated on a daily basis there is a cumulative effect that leads to a long-term improvement in immune response. A study on over 1,000 people showed that individuals who exercise two or more days a week have half as many days off school or work due to colds or flu compared with those who don't exercise (Nieman *et al.*, 2011). Other factors that were correlated with a reduction in URTI risk included a high intake of fruit, being married, being male, having a moderate or high level of fitness and having a low level of mental stress.

Figure 1. *The 'J-shaped' model of exercise and upper respiratory tract infection (URTI) risk*



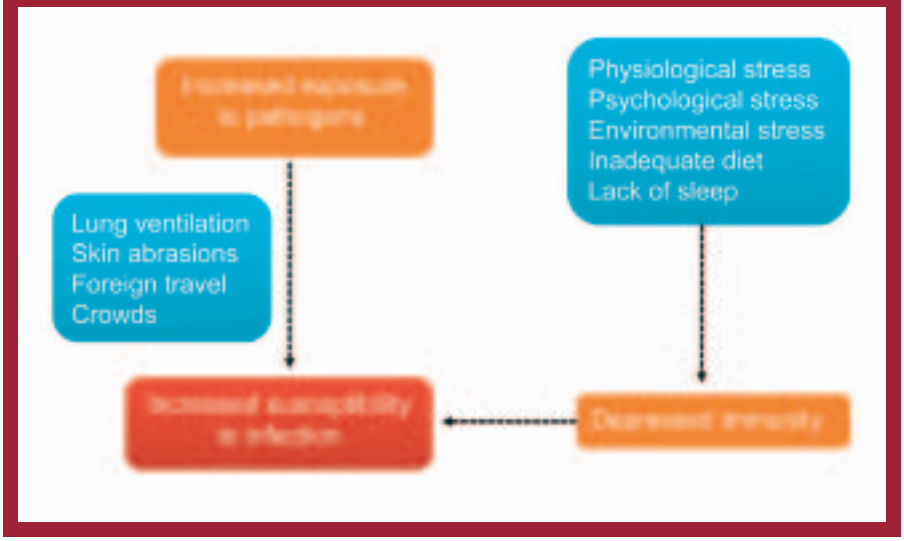
However, more is not always better in terms of exercise volume as other studies have reported a two-to-six fold increase in risk of developing a URTI in the weeks following marathon (42.2km) and ultra-marathon (90km) races (Peters & Bateman, 1983; Nieman *et al.*, 1990). A 'J-shaped' model (Figure 1) has been used to describe the relationship between the amount of regular physical activity that is undertaken and risk of URTI (Nieman, 1994). The increased sensitivity to infection with high levels of exercise may be due to a depression in immune system function of the individual. Studies have shown that prolonged strenuous bouts of exercise causes a temporary suppression of various immune cell functions and that performing such exercise on a regular basis with limited recovery can result in a longer lasting and more severe depression of immunity (Gleeson, 2005). This effect is likely due, in part, to increased levels of stress hormones like adrenaline and cortisol, and anti-inflammatory cytokines such as interleukins 6 and 10 that suppress white blood cell functions. After strenuous exercise, athletes enter a brief period of time in which they experience weakened immune resistance and are more susceptible to viral and bacterial infections, in particular, URTIs. Another problem for athletes is that their exposure to pathogenic (disease causing) microorganisms in the environment may be higher than normal (Figure 2) due to increased rate and depth of breathing during exercise (increasing exposure of the lungs to airborne pathogens), exposure to large crowds and frequent foreign travel (Gleeson & Walsh, 2012). Some of the reported sore throats may not be due to infectious agents but to non-infectious airway inflammation caused by allergies or inhalation of pollutants (Walsh *et al.*, 2011a). However, whatever the cause of respiratory illness symptoms, the worry for athletes is that exercise performance is almost always impaired.

Most people are more susceptible to colds in winter but numerous studies on athletes indicate that they tend to be most susceptible to picking up infections at times close to competition. This usually follows a period of intensive training and added mental stress with the anxiety of wanting to perform well. Even a mild infection can impair an athlete's ability to perform at the highest level. Preventing infections is therefore very important to them and they can help themselves by ensuring good personal hygiene, good nutrition and minimizing other life stresses (Walsh *et al.*, 2011b). Certain nutritional strategies have also been shown to limit immune depression during exercise and/or reduce URTI risk in athletes. These strategies include: avoiding micronutrient deficiencies, ingesting carbohydrate during exercise and taking flavonoid and probiotic supplements (Walsh *et al.*, 2011b).

Probiotic supplements to reduce infection risk

Probiotics are food supplements that contain live microorganisms which when administered in adequate amounts can

Figure 2. *Causes of increased infection in athletes*



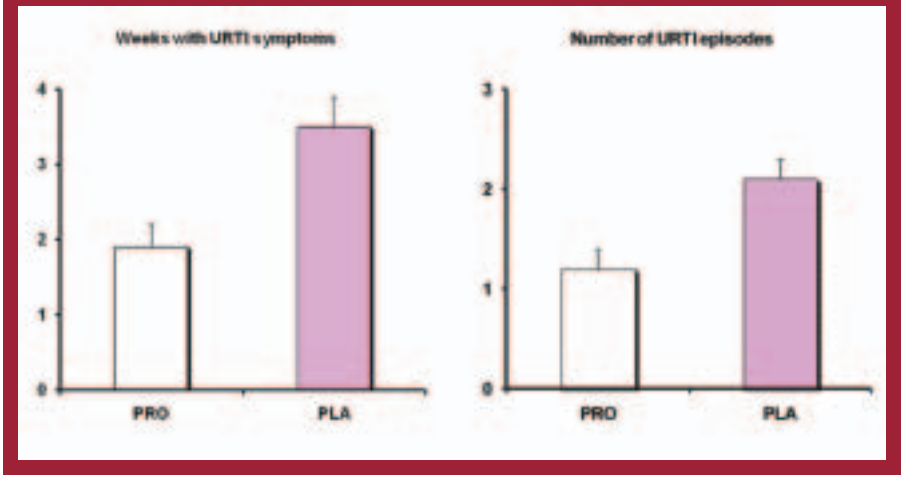
confer a health benefit to the host (WHO/FAO, 2002). There is now a reasonable body of evidence that regular consumption of probiotic strains that are proven to survive gut transit, can modify the population of the gut dwelling bacteria (microbiota) and influence immune function (Minocha, 2009; Borchers *et al.*, 2009) though it should be noted that such effects are dose- and strain-dependent. Probiotics modify the intestinal microbiota such that the numbers of beneficial bacteria increase and usually numbers of species considered harmful are decreased. Such changes have been associated with a range of potential benefits to the health and functioning of the digestive system, as well as modulation of immune function.

Probiotic mechanisms of action

Probiotics have several mechanisms of action. By their growth and metabolism, they help inhibit the growth and reduce potentially harmful effects of other bacteria, antigens, toxins and carcinogens in the gut, but in addition, probiotics are known to interact with the gut-associated lymphoid tissue, leading to positive effects on the innate and even the acquired immune system.

Studies have shown that probiotic intake can improve rates of recovery from rotavirus diarrhoea, increase resistance to enteric pathogens, and promote anti-tumour activity (Minocha, 2009). Some evidence suggests that probiotics may be effective in alleviating some allergic and respiratory disorders in young children (Leyer *et al.*, 2009).

Figure 3. *Effect of probiotic (PRO) compared with placebo (PLA) on upper respiratory tract infection (URTI) incidence in an athlete cohort (n=58). Data from Gleeson et al., (2011)*



In recent years it has become evident that some probiotics, particularly *Lactobacillus* (*L.*) strains, when ingested on a daily basis, can reduce URTI incidence in adults (Hao *et al.*, 2011).

Probiotic intervention studies in athletes

Although there are few published studies of the effectiveness of probiotic use in athletes, there is growing interest in examining their potential to help maintain overall general health, enhance immune function or reduce URTI incidence and symptom severity/duration (West *et al.*, 2009).

In a double-blind, placebo-controlled, cross-over trial in which healthy elite distance runners received the probiotic *Lact. fermentum* or placebo daily for 28 days with a 28-day washout period between the initial and the second treatment, athletes (n=20) suffered fewer days of respiratory illness and lower severity of respiratory illness symptoms when taking the daily probiotic (Cox *et al.*, 2010). The probiotic treatment also elicited a two-fold greater change in whole-blood culture interferon-γ production compared with placebo, which may be one mechanism underpinning the positive clinical outcome. In another study of athletes who presented with fatigue, impaired performance and a deficit in blood CD4+ (T-helper) cell interferon-γ production compared with healthy control athletes, this apparent T-cell impairment was reversed following a one-month course of daily probiotic (*Lact. acidophilus*) ingestion (Clancy *et al.*, 2006).

In a study on the effect of a *Lact. casei* probiotic supplement on URTI and immune and hormonal changes in soldiers participating in three weeks of commando training followed by a five day combat course, no difference in infection incidence between groups receiving daily probiotic or placebo was reported (Tiollier *et al.*, 2007). The study duration was probably too short for that but did show that the probiotic was associated with better maintenance of salivary immunoglobulin A (IgA) levels: saliva IgA decreased after the combat course in the placebo group, with no change over time in the probiotic group. A recent randomized, placebo-controlled trial in 58 Loughborough University athletes reported a lower incidence of URTI

episodes during a four-month winter training period in subjects receiving daily probiotic (*Lact. casei* Shirota) compared with placebo (Figure 3), and this study also reported better maintenance of salivary IgA in the probiotic group (Gleeson *et al.*, 2011). Importantly, in both athlete and non-athlete populations, falls in saliva IgA have been associated with increased URTI incidence (Walsh *et al.*, 2011 a & b). Another recent study using *Lact. fermentum* reported reduced URTI incidence among male but not female athletes during 11 weeks of training (West *et al.*, 2011).

From the research reviewed here, one cannot reach a solid conclusion of probiotic benefit for sportspeople. Nevertheless, there is now sufficient understanding of the mechanism of action of certain probiotic strains, and enough evidence from trials with highly physically active people to signify that this is a promising area of research with mostly positive indications at present. To date, most studies of probiotic interventions in athletes have been relatively small-scale and some large-scale, double-blind, placebo-controlled trials are needed to confirm likely probiotic benefits for athletes. At Loughborough we are currently conducting such a clinical trial in 250 athletes.

Conclusion

The message from current research is that moderate exercise has a positive effect on the immune system. So to keep colds at bay we should all go out for a brisk walk or participate in sports at least several times per week. Being active on a regular basis also comes with other health benefits including a reduced risk of developing metabolic diseases (e.g. type 2 diabetes) and cardiovascular diseases (e.g. coronary heart disease) later in life. The higher infection risk that comes with very high (some might say excessive) levels of exercise is the small price that athletes pay for being a potential Olympic gold medallist. There is accumulating evidence that the daily ingestion of *Lactobacillus* probiotics can reduce infection incidence in people with stressful lives like athletes.

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Mobile elements in a connected world

Global travel today in volume, reach, and speed is unprecedented in human history (Chen & Wilson, 2008). Almost a billion people traverse international borders each year (UNWTO, 2011). This movement by individuals coincides with many other global changes: population size of >7 billion, increasing density of human population (>50% urban), globalization of markets, including the food supply, migration of large populations because of sociopolitical, economic, and environmental factors, and global climatic disruption with an increasing number of extreme events, such as floods and droughts. Most of the recent population growth and that projected in the coming decades is in urban areas within developing countries in low latitude areas — the tropics and subtropics — areas characterized by high biological diversity and an abundance of human pathogens. Many large population centres are found in coastal areas — regions vulnerable to sea level rise with climate change — and seismically active areas.

What is the relevance of huge numbers of mobile people for human health, and specifically for infectious diseases? Travellers have long been known to be at risk from acquiring unusual infections during travel, especially during travel to tropical areas. In his book, *Death by migration: Europe's encounter with the tropical world in the nineteenth century*, historian Philip Curtin, lays out the relocation costs among European soldiers in the tropics between about 1815 and 1914 (Curtain, 1989). This "relocation cost" was used to describe the cost in deaths from disease associated with going to the tropics. Overall, the death rate from disease in soldiers who went to the tropics was at least twice that of soldiers who stayed at home.

Today, with the availability of vaccines and drugs, such as those used for malaria chemoprophylaxis and treatment of diarrhoea, travellers can usually remain healthy when visiting tropical and developing regions. Yet the flow of pathogens into travellers may not end with the traveller. As in the past, mobile populations have been extremely effective in moving pathogens and resistance elements around the world (Arguin *et al.*, 2009; Wilson, 2010). Study of microbes and immune responses in travellers can also be an effective way to sample distant geographic regions — areas that may lack good surveillance systems locally. Networks, such as the GeoSentinel Surveillance Network and the EuroTravNet, have been created to capture information that can be provided by mobile populations (Freedman *et al.*, 2006; Gautret *et al.*, 2009). The travellers are the sentinels and couriers who pick up and carry home (or to a new geographic setting) pathogens that can cause disease (Wilson, 2003). Important studies by such networks have documented that disease profiles vary by geographic region (Freedman *et al.*, 2006). In some instances, illness in returned travellers can provide an alert that can be used to warn local populations and to prepare those planning to travel.

Dengue, the viral infection spread primarily by *Aedes aegypti* mosquitoes, has been dispersed throughout the tropics and subtropics by humans who travel while viremic. Four different virus serotypes exist; infection with one

provides good protection against that serotype but only transient protection against the other three. So introduction of a new dengue serotype into a population already immune to one serotype can cause outbreaks. The *Ae. aegypti* mosquito is extremely well adapted to contemporary urban life and breeds in and around homes (such as in flower pots and discarded plastic cups). It enters homes and bites during the day, so bednets do not prevent bites. The confluence of several factors — rapid growth of human populations in tropical and subtropical urban areas often lacking infrastructure, increasing global travel, widespread infestation of urban areas with *Aedes* mosquitoes — and lack of a vaccine and effective mosquito control programmes — means that dengue infections continue to increase in number, geographic reach, and, in some instances, severity. Second infection with a different dengue serotype is one factor associated with more severe disease, and more populations globally have experienced dengue infections.

It is worth considering what elements are necessary to make a population receptive or vulnerable to the introduction and spread of a new pathogen carried by a traveller. For a vector-borne infection, such as dengue or chikungunya, a competent vector must infest an area and have access to susceptible humans. Environmental conditions must also be favourable. For a mosquito-borne infection, such as chikungunya, temperatures must be warm enough to allow the virus to replicate and disseminate in the mosquito and reach the salivary glands before the mosquito dies. This period of infection in the mosquito, the extrinsic incubation period, is extremely sensitive to climatic conditions, especially temperature. The outbreak of the viral infection chikungunya that occurred in northern Italy in 2007 occurred during the hottest period of the year (Rezza *et al.*, 2007). A competent vector, *Aedes albopictus*, already infested the area, having been introduced into Italy several years earlier. Introduction of *Ae. albopictus* into many countries has been via the used tyre trade (Reiter, 1987). Tyres shipped from one continent to another provide a protected environment for mosquito eggs. In a new environment, with warmth and water from rainfall, eggs hatch and mosquitoes infest the new environment.

Of course, while humans are changing the global milieu in profound ways, the microbes continue to replicate and evolve, finding ways to survive despite stressors, such as antibiotics, pesticides, and other agents. It appears that one reason for the recent explosive outbreaks of chikungunya infections is a mutation in the viral gene encoding the envelope protein (Tsetsarkin *et al.*, 2007). This mutation in the virus is associated with increased infectivity for *Ae. albopictus* and allows more efficient viral dissemination in mosquitoes, which may increase the efficiency of transmission. *Aedes albopictus* mosquitoes, which originated in Asia (Asian tiger mosquito), already infest many regions in Europe, Americas, Africa, and the Pacific Islands (Charrel *et al.*, 2007).

Travellers can pick up and carry resistant bacteria in the absence of symptoms. They can also carry home common bacteria with uncommon genetic elements conferring extreme resistance to antimicrobials. Tangden and colleagues in Sweden collected faecal samples (on rectal swabs) from travellers before and after international travel (Tangden *et al.*, 2010). Median travel duration was two weeks. They found that 24 of 100 travellers newly acquired extended-spectrum beta-lactamase (ESBL)-producing *E. coli* during travel. All of the *E. coli* produced CTX-M enzymes, primarily CTX-15, and were resistant to multiple antibiotics. Three travellers had taken ciprofloxacin for gastroenteritis; all acquired ESBL-positive *E. coli*. Travellers who reported gastroenteritis were more likely to have acquired CTX-M-producing *E. coli*, suggesting that faecally contaminated food or water may have been the source. The rate of acquisition of resistant bacteria varied by region of travel and was highest for those travelling to the Indian subcontinent (8/9 or 88%). Rates for other regions were lower: southern Asia (13%), Middle East (29%), and other Asian countries (32%). Faecal cultures were repeated six months later for those initially positive; 24% remained positive.

In Canada, Peirano and colleagues compared results of stool cultures from returned travellers and non-travellers with diarrhoea (Peirano *et al.*, 2011). Recent travellers were 5.2 times more likely to have samples positive for ESBL-producing *E. coli* than non-travellers. All ESBL-producing *E. coli* were positive for CTX-M genes. Highest rates of carriage were found in travellers returning from India (11/14), Egypt (19/38), and Thailand (8/38).

Globally mobile populations, including those travelling to receive medical care (medical tourism), have facilitated the rapid and wide dispersal of resistance genes (Wilson & Chen, 2012). The appearance of a new type of carbapenem resistance, carried by a mobile genetic element, the New Delhi metallo-beta-lactamase-1 (NDM-1), is of great concern (Kumarasamy *et al.*, 2010). First documented in 2006 (though the exact time and place of its first appearance are unknown), it has now been carried to many countries — and local nosocomial and community spread has occurred in some areas. It has been most commonly documented in the Indian subcontinent, in local hospitals (including in community acquired infections), and in individuals who have received medical care — elective or emergency — locally and then returned to other countries. NDM-1-producing bacteria have been identified in environmental samples (tap and other water samples) in Delhi, India. Through lateral genetic transfer this mobile genetic element has been able to spread to many Gram-negative bacteria, including *E. coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, among others. In the environment, it has been found in *Vibrio cholerae*. It is usually carried on a mobile plasmid and occasionally on chromosomes. Features of NDM-1-positive bacteria that are worrying are several. It is highly resistant; has spread rapidly to multiple countries; can transfer to many common bacterial pathogens; can be carried with commensal bacterial (persistence >one year documented); can be found in environmental sources; and is not detected by most laboratories (most lack the capacity to detect it). Globally, many healthcare settings lack surveillance and infection control capacity to detect and prevent its spread. Its emergence and spread along with the other resistance determinants pose a serious global challenge.

Mass gatherings, events such as the Olympic Games, the World Cup, and the Hajj, combine many risks for infectious

Figure 1. *Current known distribution of Aedes albopictus: December 2011*

diseases and other problems. Individuals and groups come together, often from diverse regions of the world. During the event, participants have close contact with large numbers of persons, sometimes in an indoor setting. Mass events can strain local resources. Access to safe food and water and sanitary waste disposal can be difficult. Outbreaks of gastrointestinal infections spread by contaminated food and/or water, such as shigellosis and campylobacteriosis, have been reported during and after mass events (Abubakar *et al.*, 2012). Infections that spread from person-to-person, such as influenza, meningococcal infections, and measles, have been reported many times. Because of its short incubation period and high transmissibility, influenza can cause outbreaks that begin during an event and spread rapidly within and beyond the mass gathering. Depending on the type of mass event and composition of the population, sexually transmitted infections may also be a problem. Outbreaks of meningococcal infection have occurred during the Hajj in the past; pilgrims then carried the outbreak strain to other geographic locations, leading to secondary spread in some areas (Lingappa *et al.*, 2003). This led to the requirement that all pilgrims planning to participate in the Hajj receive meningococcal vaccination. Influenza vaccine has also been used to reduce outbreaks of influenza at this mass event.

In recognition of the many potential health problems associated with mass gatherings, individuals with relevant experience and expertise are defining a discipline called mass gatherings health, a specialized, interdisciplinary area of public health that brings together individuals trained in public health, emergency and disaster planning, and travel medicine. Many sectors are involved in planning mass gatherings, including health, security, transportation, and public communications (Abubakar *et al.*, 2012; Khan *et al.*, 2012).

Even if health consequences are not apparent during the event, the impact of the mass gathering continues after the event ends. Infections with longer incubation periods, such as measles, brucellosis, HIV, and tuberculosis, may not become clinically evident until long after participants return home. In evaluating illness in someone who has participated in a mass event, it is necessary to consider the diseases endemic to the area where the mass

event took place — and also the microbial "baggage" of others who may have come from all over the world, and contacts that may have occurred during part of the travel loop — en route and during side trips. Ongoing work to provide insight into which diseases are most likely to spread in a mass gathering or disperse from a mass gathering will require integration of data from transportation systems, crowd behaviour modelling, and health surveillance information from local, national, and international levels. New sources of data, such as mobile phone technology, may allow real-time risk monitoring (Khan *et al.*, 2012).

Some infections can be easily introduced into a new place or population and others cannot. As noted above, vector-borne infections require the presence of a competent vector and suitable climatic conditions. Infections that are transmitted from person-to-person, such as influenza, meningococcus, TB, HIV, and measles, can be carried anywhere. Whether infection spreads in a new location depends on transmissibility of the pathogen and susceptibility of the population. Measles will not be introduced into a new area if the population is completely immunized — but many populations do not have high enough vaccine coverage to avoid all cases.

The vulnerability of a place and population to the introduction of a new pathogen will depend on multiple factors, including: environmental factors, access to clean water and sanitary waste disposal, living conditions, and host factors, such as: nutrition, immunization, behaviour, and access to early diagnosis and treatment. Areas with clean water and good sanitary waste disposal are not at risk from the introduction and spread of infections such as cholera (*Vibrio cholerae*). Global changes produce vulnerabilities that can allow rapid global spread of infections and mobile elements conferring resistance. At the same time, new tools have the potential to provide better surveillance and communication. Can we keep a step ahead of the pathogens? Can we keep up?

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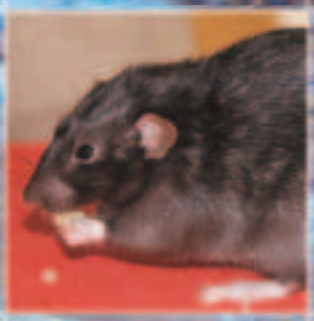
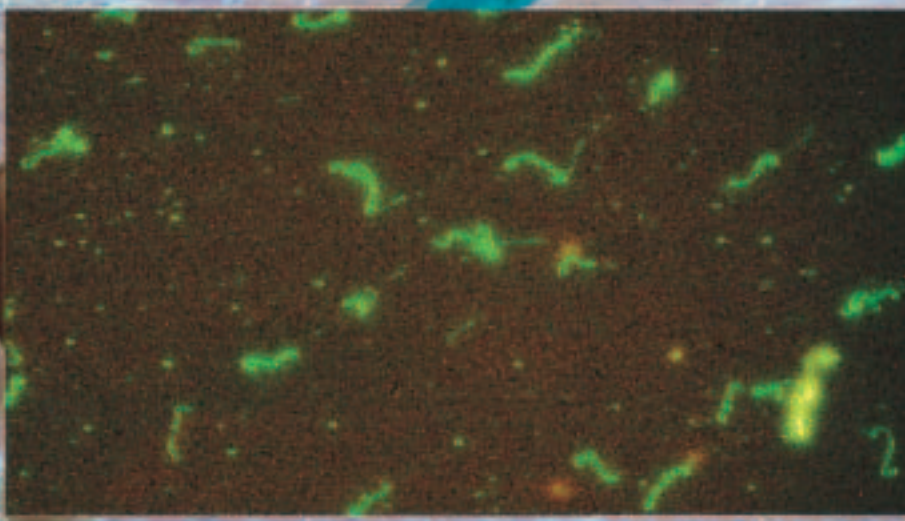
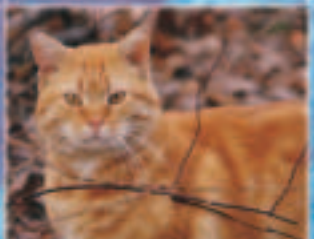
Will leptospirosis always be an endemic waterborne disease that kills?







A recent grant submission to understand pathogenic mechanisms of severe leptospirosis was returned to me after an unfavourable review. Notwithstanding other faults, one of the grant reviewers challenged "*whether there is a need for new treatments since there are a number of perfectly acceptable and cheap antibiotics available for prophylaxis and treatment of leptospirosis*." And yet, more than 500,000 severe (hospitalized) cases of leptospirosis are reported each year, with mortality rates ranging from 10 to 50% (Ko *et al.*, 2009). Recent outbreaks have occurred in Sri Lanka and the Philippines where more than 7,000 and 2,000 cases were reported in 2008 and 2009 respectively. Therein lies my disagreement with the grant reviewer; the perception that leptospirosis is easily recognized and, ultimately, easily treated.

There is also a perception that leptospirosis is a disease of developing, and not developed, countries. It is not. In any case, effective treatment requires a timely, sensitive and specific diagnosis which is not just limited to antibiotics. Effective public health interventions require a basic understanding of the disease itself. Whilst major outbreaks of leptospirosis are typically associated with flooding after severe weather or rapid urbanization, where slum settlements enhance conditions for epidemic rat-borne transmission of the disease; cases of leptospirosis continue to be detected at endemic levels in developed countries including the UK and Ireland. And most unfortunately, leptospirosis is still a life-threatening disease as exemplified in October 2010, when BBC News reported that Great Britain's double Olympic champion rower, Andy Holmes MBE, died at the age of 51 as a result of one of the more severe forms of leptospirosis.

So, why is leptospirosis endemic in developed countries?

1. Asymptomatic chronically infected wild and domestic animal species persistently shed pathogenic leptospires into the environment.

Pathogenic species of *Leptospira* colonize kidneys of reservoir hosts from which they are excreted via urine into the environment. The classic reservoir host is the rat, but a range of wild and domestic animal species can also act as reservoir hosts, including cattle and dogs. Reservoir hosts are typically asymptomatic, reflecting the unique biological equilibrium that exists between a specific serovar of *Leptospira* (of which there are over 250) and its respective reservoir host. For example, a rat can act as a reservoir host for *L. interrogans* serovar Copenhageni and can persistently excrete this bacterium at more than 10^5 leptospires per ml of urine without clinical signs of infection. In contrast, *L. interrogans* serovar Copenhageni can cause a most severe acute disease in dogs and humans. Reservoir hosts tend not only to be clinically asymptomatic, but can be serologically negative when tested using the gold standard microscopic agglutination test (MAT). This makes it inherently difficult to identify infected reservoir hosts as well as the actual source of infection in the first instance. Leptospires excreted into the environment can continue to survive in suitable moist conditions. If they dry, they die. They cannot survive in seawater. However, it has recently been demonstrated that leptospires form biofilms. Whether or not biofilm formation facilitates disease transmission remains to be determined.

2. Continued serological and culture studies are required to understand changing epidemiology.

Alternative detection methods include culture and/or molecular assays. Leptospires are fastidious bacteria and culture requires a high degree of expertise; this skill set is limited to a handful of laboratories in the UK and Ireland and so, culture of isolates from clinical samples is not routinely performed. Yet, cultures of leptospires from local geographical regions are not only essential for an optimal MAT, they are essential to develop effective vaccine strategies in domestic animals, and thus decrease the risk to public health. There is ample evidence to indicate that leptospires maintained in culture over time modify their virulence properties, and ultimately, such highly passaged isolates perform differently in experimental assays compared to isolates recently obtained from primary host tissue. Furthermore, there is also evidence to indicate that changes in epidemiology occur over time; culture of isolates is essential to confirm these changes, and to facilitate continued diagnostics and efficacious vaccination (Ellis, 2010; Hartskeerl *et al.*, 2011). Sensitive and specific molecular assays are available to facilitate detection and to quantify pathogenic species of leptospires in clinical samples and aquatic environments; however, cultured isolates are still necessary to understand the epidemiology of infection.

3. The differential diagnosis for leptospirosis should carefully consider case history.

Public health awareness is essential to control leptospirosis. Efficacious vaccination of dogs and cattle serves not only to prevent them from acute manifestations of leptospirosis, but also to inhibit the zoonotic transmission of infection. However, current vaccines do not protect from renal colonization as observed in reservoir hosts; these animals continue to excrete leptospires into the environment. A range of actions to reduce exposure to infection include recognizing potentially contaminated environments encountered during occupational exposure (contact with farms, handling animals, working in drainage ditches or sewers etc.) or recreational exposure (canoeing, caving, retrieving wayward golf balls etc.) and taking appropriate protective measures. These include reducing direct contact with contaminated sources (e.g. appropriate footwear), reducing potential routes of infection (e.g. covering cuts and grazes, avoiding ingestion of contaminated water during water based activities), and good hygienic practice (e.g. handwashing). However, it should be noted that clinical manifestations occur 5 to 10 days after exposure and that these can range from a mild non-specific fever, to the more severe icteric Weil's disease, to severe pulmonary haemorrhagic syndrome. It is incumbent on patients and clinicians alike to be aware of this, and carefully consider the significance of the clinical case history and possible exposure in the differential diagnosis, particularly if returning from recreational activities in tropical countries (Monahan *et al.*, 2009).

Due to the protean clinical manifestations and limited diagnostic services, very little is known about the true incidence of leptospirosis. It is estimated that 0.1 to 1 per 100,000 people living in temperate climates are affected each

year, with the number increasing to 10 or more per 100,000 people living in tropical climates. If there is an epidemic, the incidence can soar to 100 or more per 100,000 people. From 1998 to 2009, there was on average 51 (range=32 to 81) laboratory confirmed reports of leptospirosis in the UK (www.hpa.org.uk) and in Ireland, from 2004 to 2010, it was 20 (range=14 to 29) (www.hpsc.ie). It is generally accepted that current reports are a gross underestimate of true incidence and in response, the World Health Organization established the Leptospirosis Burden Epidemiology Reference Group (LERG) in 2009 (www.who.int/zoonoses/diseases/lerg/en). LERG is tasked with generating accurate estimates of disease burden to better direct adequate intervention, control and thus, prevention efforts.

"One Health" opportunities and approaches for the future

Domestic animal species and humans can both suffer similar forms of leptospirosis. For example, leptospiral pulmonary haemorrhage syndrome (LPHS) is increasingly recognized in human patients around the world. This is one of the most severe forms of leptospirosis which progresses in a rapid manner (hours) and is associated with mortality rates as high as 50%. There is a critical need to recognize and diagnose this clinical presentation in a timely manner to provide supportive therapies and treatments. Research to date suggests LPHS is a multifactorial disease, due in part, not only to the direct action of the pathogen on host tissue, but also the direct action of the host immune response. These observations suggest that in addition to antibiotics, immunotherapies need to be evaluated. Recent reports suggest that dogs and foals also suffer pulmonary involvement due to leptospirosis (Kohn *et al.*, 2010; Broux *et al.*, 2012). A "One Health" collaborative approach that integrates human health, animal health and environmental health can provide the necessary framework to facilitate a better understanding of the pathogenesis of LPHS and ultimately identify evidence-based treatments to reduce the mortality rates associated with respiratory complications of leptospirosis.

Humans interact with domestic and wild animal species and their respective environments on a daily basis. Prevention of leptospirosis is better than cure. Prevention strategies can include a risk assessment of potential sources of infection. Awareness, education and simple preventative measures can reduce risks of exposure to pathogenic species of *Leptospira*, particularly during the organization of large-scale activities where participants will be in contact with potentially contaminated water. Use of protective clothing to prevent skin abrasion during water activities helps reduce the risk of infection. Knowledge of the symptoms of disease allows for early diagnosis. Molecular methods can supplement culturing of *Leptospira* from areas associated with outbreaks for epidemiological purposes and provide assessment of suspect waterbodies prior to planned recreational events. Whilst the efficacy of prophylactic treatment remains to be addressed (Haake *et al.*, 2002; Ellis, 2011), awareness and implementation of some basic approaches to limit exposure to contaminated urine or contaminated water sources can be hugely successful to prevent the transmission of endemic leptospirosis.

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further information

The author has produced a short video of *Leptospira interrogans* observed by dark-field microscopy. Leptospires are cultured in liquid media. They have two periplasmic flagella that facilitate motility. They range in size from 10 to 20µm long and 0.1 to 0.2µm wide.

To view the video please visit:
http://www.sfam.org.uk/en/microbiologist/index.cfm

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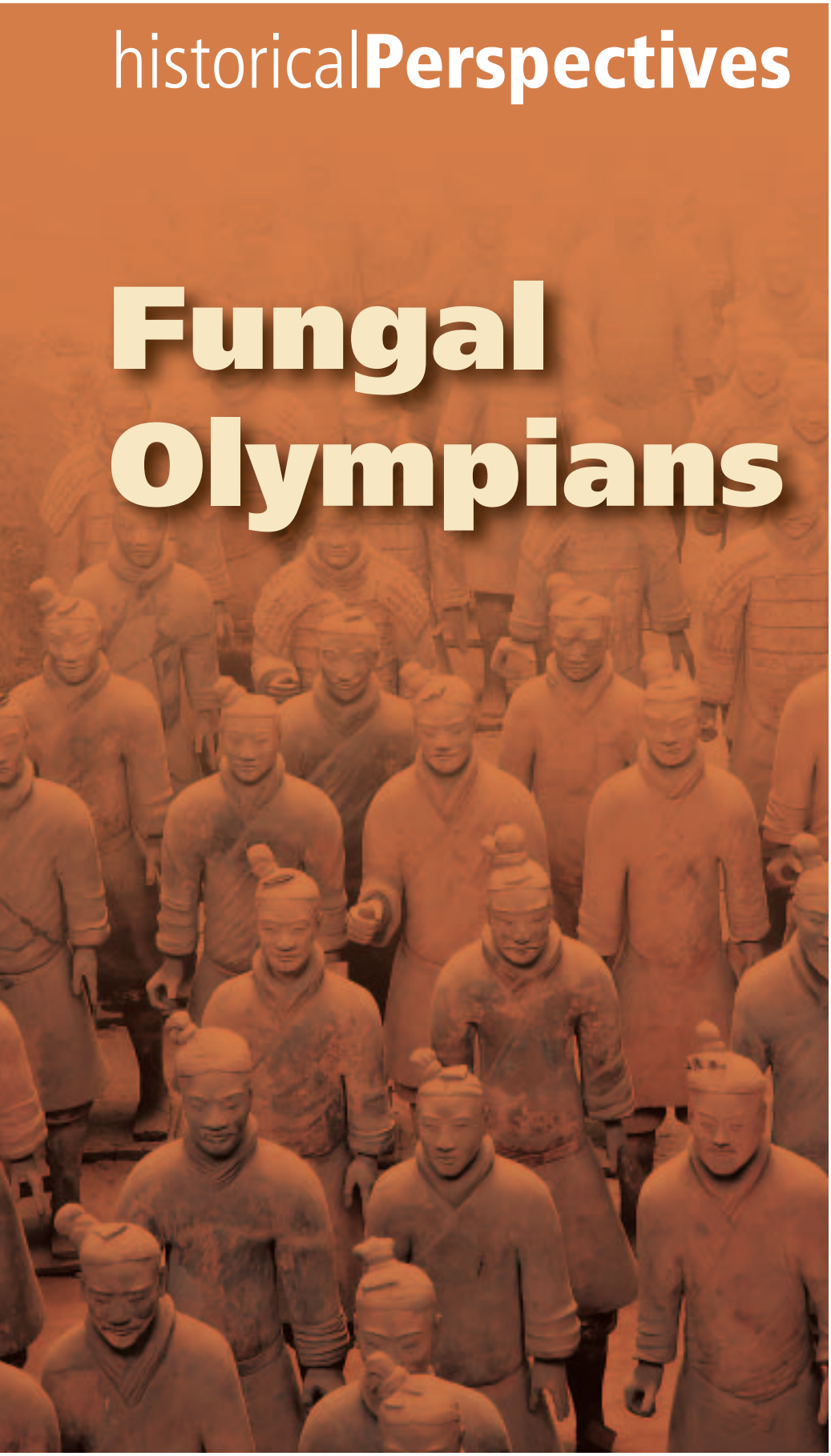
historical**Perspectives**

Fungal Olympians



The fungal kingdom is estimated to comprise between 1 and 10 million different species, covering a diverse range of habitats, from deep seabeds, deserts, soil, water and as saprophytes or parasites on humans, animals and plants. Evolutionary adaptation to such varied habitats has resulted in astonishing phenotypic and genotypic complexity and a remarkable range of macroscopic and microscopic features. The advent of the Olympics in 2012 presents an opportunity to examine a number of fungi that have made their infectious mark at various sporting events and also to discuss some fungal species which, whilst non-pathogenic to humans, should be recognized as "Olympians" in their own right.

Human infections

Dermatophytes

The most obvious fungal links to sporting events are the infections known as *tinea pedis* or "athlete's foot" and *tinea gladiatorum*, a skin infection seen mainly in wrestlers. These are just two of the diseases caused by dermatophytes, a group of keratinophilic fungi which are obligate pathogens, responsible for zoophilic and anthropophilic infections. These are generally confined to keratinized tissue, principally due to the inability of the causative fungi to thrive at 37^{o}C and penetrate viable tissue due to the immunological response in immunocompetent individuals. Occasionally, invasion of the subcutaneous tissue is observed in scalp infections resulting in painful suppurative lesions or kerion which may be accompanied by a secondary bacterial infection. Inflammatory reactions are caused by the host response to metabolic by-products and it is well documented that inflammatory lesions in humans caused by zoophilic or geophilic species are generally more severe than those caused by the anthropophilic dermatophytes. The various dermatophyte infections are named according to the area on the body on which they are found (Borman *et al.*, 2007).

Trichophyton tonsurans

Our first dermatophyte worthy of note is *Trichophyton tonsurans*. This is an anthropophilic species which has a worldwide distribution, although with a varied prevalence. It is usually

associated with *tinea capitis* where it can produce a scaly non-inflamed dermatosis resembling seborrhoeic dermatitis or an inflammatory disease with scaly erythematous lesions (Adams, 2000; Adams, 2002). In the UK it is responsible for only 5% of all dermatophyte infections, 70% of infections being associated with the scalp. In contrast, it is responsible for 45% and 55% of all dermatophyte infections in the USA and the Middle East respectively but causes 91% and 5% of scalp infections in those areas (Borman *et al.*, 2007). Its presentation in wrestlers with *tinea gladiatorum* is unusual as it produces a *tinea corporis* exhibiting the characteristic annular rings seen with other dermatophytes but only at points of body contact (Figure 6). It has been estimated that about 75% of wrestling clubs have been affected at some time, with 20% of wrestlers infected in some localities (Hedayati *et al.*, 2007).

Trichophyton rubrum

The second dermatophyte mould with close sporting links is *Trichophyton rubrum*. This organism, the most common cause of athlete's foot, was originally endemic to a small region of South East Asia, Africa and Australia. Colonization of these areas by Europeans, the advent of world wars and increased travel are purported to be paramount in the spread of this fungus to Europe and the USA (Robbins, 2012). It is a disease linked to affluence. Communal areas of swimming pools and gymnasiums are a prime source of infection and subsequent spread. Studies have shown that the organism can survive for a year on skin scales in the environment. Predisposing factors for athlete's foot are the wearing of occlusive footwear, exposure during recreation, individuals who produce less protective fatty acids and immune deficiency (Robbins, 2012). It is reported to be more common in men between the ages of late teens to 50-years-old but it is estimated that 70% of the population will be infected at some time and 17% of adults in the UK will be infected at any one time.

A tenuous link between fungi, athlete's foot and sporting events lies with the 2008 Beijing Olympics. At these games the pharmaceutical company Johnson & Johnson were allowed to exhibit a handful of the 8,000+ terracotta army statues that were found in 1974 by farmers digging a well in Xi'an Shaanxi province. The first emperor of China, Qin Shi Huang, ordered the building of the terracotta army 2,200 years ago, to protect him in the afterlife. In the 1990s it was noticed that 1,500 of the warriors had become "infected" with mould (Figure 5). A number of different species were isolated including some *Trichophyton* spp. which were treated with antifungal compounds developed by Johnson & Johnson in partnership with the museum scientists. The fungal contamination is

Figure 1. *Lactofuschin photomicrograph of Scedosporium apiospermum, showing characteristic conidia and annelidic conidiophores*

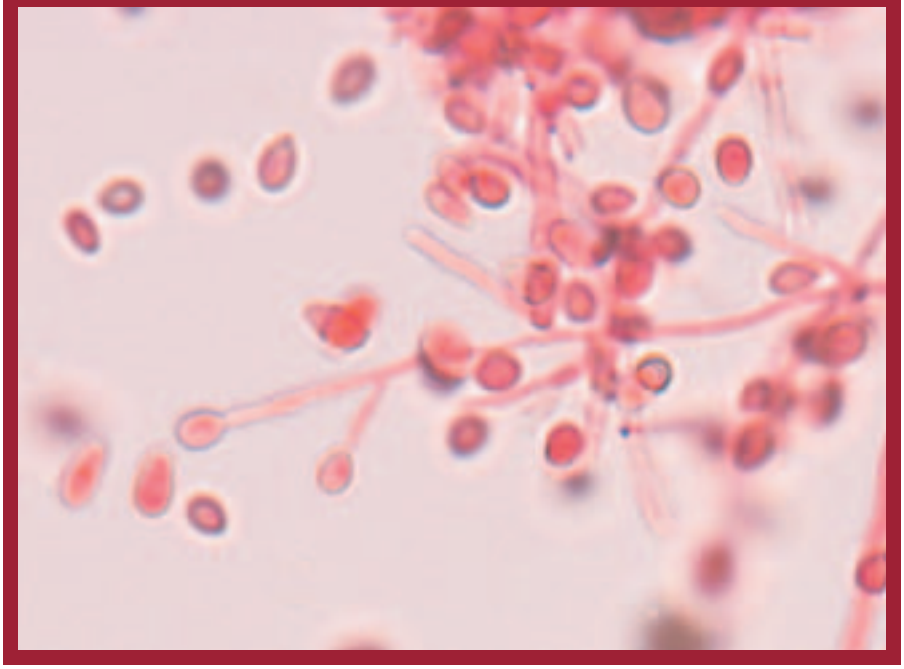


Figure 2. *Photomicrographs of various species of Mucorales. (A & B) Lichtheimia corymbifera; (C) Mucor sp. on the surface of tomatoes; (D) Mucor sp. sporangia; (E) Apophysomyces sp. sporangiophore; (F) Saksenaea vasiformis; (G) Syncephelastrum sp.; (H) Azygospore; (I) Zygospore*

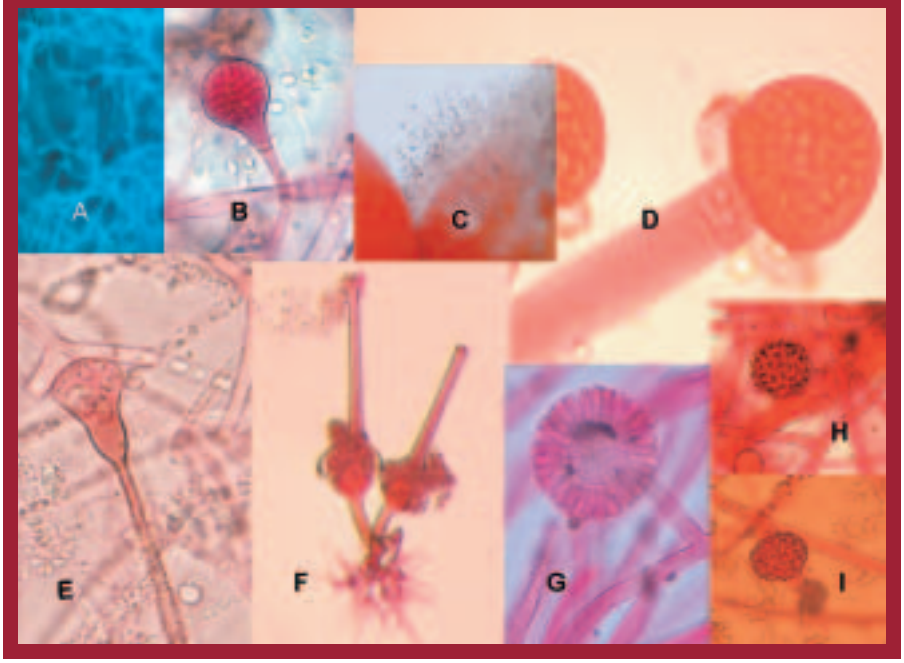


Figure 3. *Basidiocarps of Armillaria ostoyae*

believed to have been a result of mould carriage and increased humidity in the exhibition areas caused by the numerous visitors (Goldstein, 2008; Gittings, 2000; Leow *et al.*, 2008).

Scedosporium apiospermum

Scedosporium apiospermum made an appearance at the 15th Maccabiah Games in Tel Aviv, Israel in July 1997. At these games it was customary at the opening ceremony for the teams of athletes to take part in a parade which took them over the Yarkon River via a temporary bridge, into the stadium.

However, on this occasion the bridge collapsed propelling over 100 of the Australian athletics team into the river. This resulted in the immediate death of one person and the subsequent death of three other team members. At least 64 other athletes were injured in the accident. The three team members who died during hospitalization were initially thought to be suffering from the effects of chemicals used to eliminate mosquitoes from the river. Their symptoms included the signs of asphyxia which were evident just a couple of hours after admission. It was not until a post-mortem was carried out on the last patient to die, revealing lesions in multiple organs, that *S. apiospermum* was identified as the causative agent (Figure 1).

Clinical and histological diagnosis of *S. apiospermum* infection is difficult as features are indistinguishable from those of invasive aspergillosis. This organism is a well-known cause of infection following near-drowning accidents and even though neutropenia is a risk factor, infections in immunocompetent individuals have been demonstrated, mainly due to traumatic implantation or as a result of chronic lung disease. This fungus is often refractory to treatment (Cortez *et al.*, 2008; Tintelnot *et al.*, 2008).

Lichtheimia corymbifera, Saksenaea vasiformis, Rhizopus oryzae and Rhizopus microsporus

In terms of speed, "Team Fungus" haven't got a challenger that would upset the likes of Jessie Owens and Carl Lewis (gold medallists: 100m, 200m, long jump, 4x100m relay, 1936 and 1984 respectively), but the above fungi have certainly made their mark in terms of the speed with which they grow *in vitro* and their clinical affects *in vivo*.

They are all members of the Mucorales and are part of a cosmopolitan group of fungi numbering more than 600 species (Figure 2). Fungi of this order are typically fast-growing with aseptate, wide hyphae and most species produce upright sporangiophores on which sporangia filled with asexual spores are formed. Most are saprobes found in soil and on vegetation; a few are parasitic on plants and insects. They are opportunistic pathogens causing diseases including rhinocerebral, cutaneous/subcutaneous, pulmonary, gastrointestinal and disseminated mucormycosis. Predisposing factors for the acquisition of infection are neutropenia, poorly controlled diabetes mellitus, traumatic implantation of spores into wounds and the use of certain iron-chelating therapies. Rhinocerebral disease accounts for almost half of mucormycoses in which diabetes mellitus is the major risk factor (Ribes *et al.*, 2000).

The above fungi are angioinvasive causing blockage of blood vessels and subsequent necrosis which are important factors in their speed of spread. This is demonstrated *in vitro* where they will grow up to the lid of a Petri dish within a couple of days of incubation.

Other fungal Olympians

Pilobilus crystallinus

In the search for "athletes" worthy of being members of "Team Fungus" there is at least one fungal species that would dwarf the achievement of Norway's 2008 gold medal javelin thrower, Andreas Thotkildsen, who smashed the world record with a breathtaking throw of 90.57m. The mould in question is *Pilobilus crystallinus*, another member of the order Mucorales.

The normal life cycle of *P. crystallinus* involves herbivorous animals which ingest its sporangiospores whilst consuming vegetation. These then pass through the animals' digestive systems and are deposited in faeces where they germinate and develop into the vegetative organism. Although the sporangiophores are only around 4cm tall, the fungus can launch its sporangium to distances of up to 2m at speeds of up to 42.3 m/s (94.6mph). At best, a javelin on release can reach speeds approaching only 113km/h (70mph). The distance that the sporangium travels is equivalent to Andreas Thotkildsen throwing a javelin 360m. The power exerted by *P. crystallinus* in dispersing its sporangia is obtained by the disruption of a subsporangial vesicle which "explodes" when turgor pressure inside approaches seven atmospheres (Yafetto *et al.*, 2008). The direction of dispersal is governed by phototropism; the sporangiophore bends to point towards the sun. Presumably due to its saprophytic nature there have been no reports of this fungus causing infection in either man or animal. However, there are reports of lungworm larvae crawling onto *P. crystallinus* sporangia and

being dispersed along with the sporangiospores (Doncaster, 1981; Eysker, 1991).

Armillaria ostoyae

At the 2008 Olympics the German, Mathias Steiner, achieved a weightlifting super heavyweight gold medal, by lifting a total of 461kg. However, for size, his 6ft height and 140kg weight is no match for *Armillaria ostoyae* (Figure 3), from the Malheur National Forest in eastern Oregon, USA. This "honey fungus" was discovered in 2000 by scientists investigating the death of trees in dispersed areas of the forest. Molecular tests demonstrated that the damage was caused not only by the same mould species, but in fact the same mould. It covered an area of approximately 2,200 acres, was estimated to weigh 100,000kg and be between 2,400 and 8,500 years old (Barnard, 2000).

Amanita muscaria (fly agaric)

It is alleged that the final two fungi described here owe their participation in athletic events to their performance enhancing abilities. It is rumoured that Pythagoras took *Amanita muscaria* to give himself a hallucinogenic boost which enabled him to win a boxing gold medal.

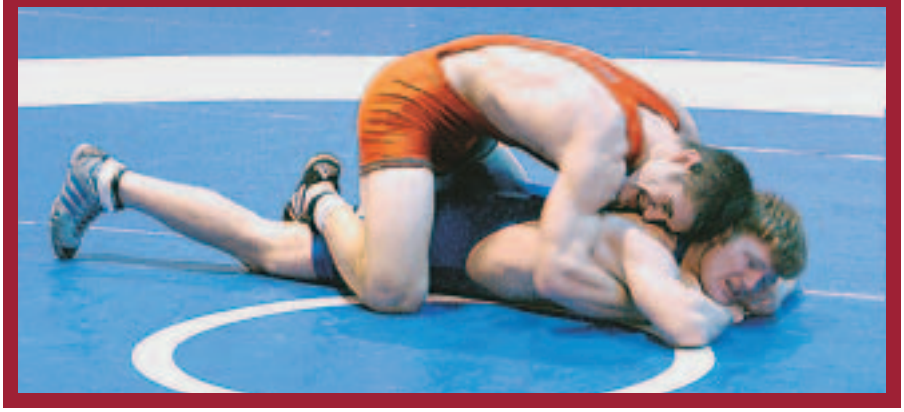
Amantia muscaria is a basidiomycete fungus and although originating from the subarctic and temperate regions of the northern hemisphere, has become a ubiquitous species. This fungus has a number of different coloured basidiocarps or caps. The variety that is most well-known has white gills and possesses white spots on a red cap (Figure 4). The basidiocarps of this species are depicted in many children's books. The name is believed to be derived from the practice of crushing the red caps into milk to be used to ward off flies and other insects. Although classed as poisonous, deaths from the ingestion of this fungus are rare. Most deaths are attributed to other *Amanita* spp., (Ferenc *et al.*, 2009), notably *Amanita phalloides*, which has a 50 to 90% mortality rate. A number of different cultures use *A. muscaria* in religious ceremonies (Crocq, 2007).

The two main active compounds in *A. muscaria* are muscimol and ibotenic acid. They are active on the central nervous system causing convulsive twitching, dizziness, "visions" and death-like sleep. On waking, individuals feel elation and are physically very active. The effects are unpredictable and vary

Figure 4. *Basidiocarps of Amanita muscularia — by Bernie Kohl*

Figure 5. *Terracotta Army — by David Castor*

Figure 6. *Greco-Roman Wrestling — by Staff Sergeant Christopher Campbell USAF*



between individuals and, as previously stated, deaths have occurred from their ingestion (Michelot *et al.*, 2003).

Ophiocordyceps sinensis

In 1993 female runners from Liaoning, China, broke half a dozen world records that had stood for years and won three gold medals at world track and field championships in Stuttgart. One runner of note, Wang Junxia, set four world records in six days in September 1993, knocking 23 seconds off the 3,000m record (Mackay, 2001). Their coach attributed the team's success to an "elixir" made with turtle blood and the fungus *Ophiocordyceps sinensis*.

Ophiocordyceps sinensis or the "caterpillar fungus" has long been used in traditional Chinese medicine (Yongjie *et al.*, 2009; Winkler, 2010). It is found in the plateaus of Tibet and the Himalayas, where it germinates inside the larvae of the ghost moth, eventually killing and mummifying them in their burrows. The fruiting body of the fungus emerges from the front of the larvae in the spring and can be seen protruding through the soil (Zhu *et al.*, 1998a).

It is thought that *O. sinensis* aids the body in utilizing energy more efficiently by increasing blood flow to the liver and other organs and by improving oxygen usage. *Ophiocordyceps* spp. possess antioxidant activity which may alleviate fatigue; other research has suggested that this fungus has antiviral, anticancer and immunomodulating activity (Zhu *et al.*, 1998b).

Regarding the enhancement of athletic performance, the Chinese athletes referred to previously tested negative for illegal substances although it remains possible that the "elixir" could have masked their presence. Interestingly, six Chinese runners were withdrawn by the same coach before the subsequent Olympic Games in Sydney.

It is hoped that the "Fungal Olympians" described here will give the reader a small insight into the diverse world of fungi and a glimpse of their unique characteristics.

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Dr Andy Borman, Adrien Sziekely, Dr Elizabeth Johnson.

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

Discriminant Analysis

StatNote 29

Introduction

Discriminant analysis (also known as discriminant function analysis or multiple discriminant analysis) is a multivariate statistical method of testing the degree to which two or more populations may overlap with each other. It was devised independently by several statisticians including Fisher, Mahalanobis, and Hotelling (Snedecor & Cochran, 1980). The technique has several possible applications in microbiology. First, in a clinical microbiological setting, if two different infectious diseases were defined by a number of clinical and pathological variables, it may be useful to decide which measurements were the most effective at distinguishing between the two diseases. Second, in an environmental microbiological setting, the technique could be used to study the relationships between different populations, e.g., to what extent do the properties of soils in which the bacterium *Azotobacter* is found differ from those in which it is absent? Third, the method can be used as a multivariate 't' test (StatNote 3, Hilton & Armstrong, 2005a), i.e., given a number of related measurements on two groups, the analysis can provide a single test of the hypothesis that the two populations have the same means for all the variables studied. This StatNote describes one of the most popular applications of discriminant analysis in identifying the descriptive variables that can distinguish between two populations.

Scenario

Azotobacter is a genus of aerobic nitrogen-fixing bacteria frequently found in soil. The organism is an important component of the nitrogen cycle in ecosystems and is useful to humans in the production of fertilizers, food additives, and biopolymers. It can also synthesize biologically important compounds, including plant hormones such as auxin, and can therefore promote plant growth. *Azotobacter* is common in soils which are neutral to weakly basic and is usually absent from acid soils. Hence, an investigator wished to determine the extent to which the presence or absence of *Azotobacter* in soils could be predicted from measurements of three variables, *viz.*, soil pH, total nitrogen (N) content, and the amount of available phosphate (P).

Twenty different soils were studied, in 10 of which *Azotobacter* was present while in the remaining 10 soils, *Azotobacter* was absent. Ten samples of each of the soils were collected and the pH, total N (total Kjeldahl nitrogen, nitrite-N, and nitrate-N), and available P measured in each sample, the latter by the Mehlich-2,3 method and expressed on a scale from 1 (low) to 60 (high). Data from the various samples were averaged for each of the 20 types of soil.

Data

The data comprise measurements of mean pH, total N, and available P for 10 soils with and 10 soils without *Azotobacter* and are summarized in Table 1.

Table 1. Soil pH, total nitrogen (N) (mg gm dry weight^{-1}) and available phosphate (P) (Mehlich score) in 10 soils with *Azotobacter* (+) and 10 soils without *Azotobacter* (-)

Azotobacter in soil	pH	Total N	Available P
+	6.3	1.46	6
+	6.2	3.20	2
+	7.1	5.10	5
+	7.2	2.05	1
+	6.0	6.14	3
+	7.1	9.12	4
+	7.0	8.30	5
+	6.6	2.56	9
+	6.4	1.08	10
+	6.8	2.51	8
-	6.0	3.35	5
-	5.9	4.68	6
-	4.3	8.80	4
-	4.9	9.10	3
-	5.1	11.24	2
-	5.3	7.30	1
-	5.5	6.10	2
-	5.1	5.15	4
-	5.0	4.76	5
-	4.9	8.32	7

How is the analysis carried out?

Examination of variables

Most of the major statistical packages such as SPSS and STATISTICA offer discriminant analysis as part of the multivariate analysis option. We will illustrate the analysis of our data using STATISTICA software (Statsoft Inc., 2300 East 14th St, Tulsa, Ok, 74104, USA).

The first step of the analysis is to examine the distribution of each variable, together with its mean and standard deviation (SD) (or median and quartile range), to provide a check against non-normality as discriminant analysis is a parametric analysis. There are insufficient data in the present example to make a specific test of normality (StatNote 1, Hilton & Armstrong, 2005b) but there are questions regarding two of the descriptive variables. Hence, pH is expressed on a logarithmic scale and available P on a fixed integer scale. Nevertheless, pH is usually measured to at least two significant figures while available P is measured on a 60 point scale, both variables being averaged over 10 subsamples from each soil and therefore, are likely to approximate a continuous scale. In addition, data can be transformed before analysis if there is concern about the degree of non-normality (StatNote 4, Hilton & Armstrong, 2006).

Correlation and regression analysis

The second stage is to examine the correlations between the three descriptive variables, *viz.*, pH, N, and P. Figure 1 shows the relationship between soil pH and total N for the two categories of soil, *viz.*, with and without *Azotobacter*. There are essentially two clusters of points: (1) those in which *Azotobacter* was present, characterized by higher soil pH and lower soil N and (2) those in which *Azotobacter* was absent, characterized by lower pH and higher soil N. The fact that two clusters of points are present associated with the presence or absence of *Azotobacter*, suggests that the discriminant analysis is likely to be successful. If the soils do not cluster according to the presence of *Azotobacter*, then it is possible that the data do not follow a bivariate normal distribution and as a consequence, the analysis is less likely to be successful. The relationships between soil pH and available P and between available P and N can be examined similarly.

Table 2. Step 0: Examination of variables not in the discriminant model before the analysis begins

Variable	Wilks' λ	Partial Wilks' λ	F to enter	Pr	T	1-T
pH	0.267	0.267	49.35	<0.001	1	0
Total N	0.777	0.777	5.18	<0.05	1	0
Available P	0.920	0.920	2.56	<0.05	1	0

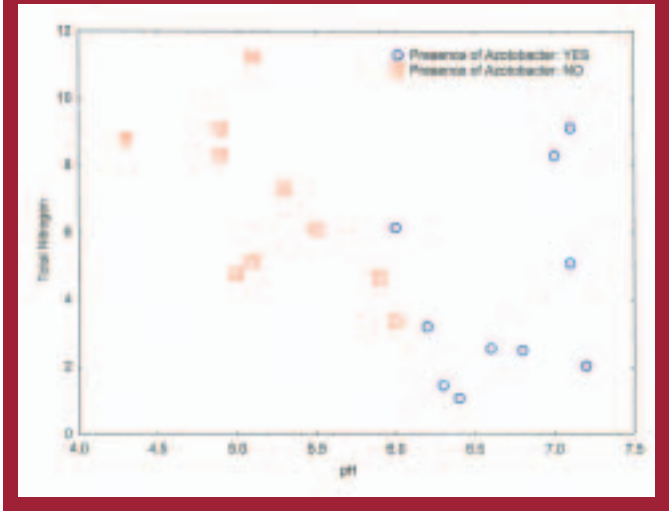
Abbreviations: Wilks' λ = Wilks' lambda, F = Variance ratio, Pr = Probability, T = Tolerance, N = Nitrogen, P = Phosphate

Table 3. Step 2: The first stage of the discriminant analysis

A. Variables in model						
Variable	**Wilks' λ**	**Partial Wilks' λ**	**F to remove**	**Pr**	**T**	**1-T**
pH	0.267	0.2673	49.35	<0.001	1	0
B. Variables not in model						
Variable	**Wilks' λ**	**Partial Wilks' λ**	**F to enter**	**Pr**	**T**	**1-T**
Total N	0.261	0.978	0.35	<0.05	0.975	0.025
Available P	0.258	0.968	0.55	<0.05	0.998	0.002

Abbreviations: Wilks' λ = Wilks' lambda, F = Variance ratio, Pr = Probability, T = Tolerance, N = Nitrogen, P = Phosphate

Figure 1. *The regression of soil pH and total nitrogen (N) in soils classified according to whether Azotobacter is present or absent. Essentially the soils cluster into two groups, i.e., those in which Azotobacter was present characterized by higher soil pH and lower soil N and (2) those in which Azotobacter was absent characterized by lower pH and higher soil N*



Discriminant analysis

Discriminant analysis uses a stepwise procedure to examine the relationships between the presence of *Azotobacter* and the three descriptive variables and therefore resembles stepwise multiple regression (StatNote 25, Hilton & Armstrong, 2011). Hence, variables are entered into the discriminant model one at a time, such that the variable making the most significant contribution is always the next to be chosen.

The analysis continues until: (1) all variables have been entered or removed, (2) the maximum number of specified steps in the analysis has been reached, (3) there is no other variable that can be usefully added to the model, or (4) any further variables not so far entered have a 'tolerance' (T) value less than that specified. At each step, the multiple correlation coefficient (R) is calculated (StatNote 25; Hilton & Armstrong, 2011) for each variable with all other variables currently included in the model. Hence, T is defined as: $T = 1 - R^2$ and is therefore, a measure of the 'redundancy' of the next variable given the variables that have already been entered into the model (StatNote 28, Hilton & Armstrong, 2012).

We can now proceed to the discriminant analysis proper. It is useful to examine the results of the analysis at each stage. First, in step 0 (Table 2), all the variables studied can be examined before any of them has been entered into the model. Four statistics are usually calculated for each variable. First, Wilks' lambda (λ) denotes the statistical significance of the current model and has a range of 1 (no power) to 0 (perfect power).

Hence, each value in the column represents l after each variable has been entered. Second, partial Wilks' λ is the unique contribution of each variable to the model. Since no variables have been entered at this stage, these values will be the same as those for Wilks' λ. Third, 'F to enter' and 'P' are calculated from Wilks' λ and denote which variable is likely to be entered first during step 1. 'F to enter' sets an 'F' value which has to be exceeded before a variable will be added into the model. By contrast, 'F to remove' sets a value such that after adding a new variable, a variable previously entered should be removed. The variable with the largest 'F to enter' is entered first, which in this case is soil pH. Fourth, T and R^2 are then calculated. At this stage $R^2 = 1$ and $T = 0$, as no variables have yet been entered into the model.

Interpretation

The analysis can then proceed to step 2 and the results are shown in Table 3. The first variable to be included in the model is soil pH, the overall discrimination between groups being indicated by a partial Wilks' λ of 0.2673. This value is quite low suggesting that pH has good discriminatory value. Note that $T = 1$ and $R^2 = 0$ at this stage because only one variable has been added to the model.

Variables not currently in the model can then be reviewed to check whether any further variables are likely to be added. In this case, neither soil N nor available P have an F to enter >1 and consequently, their inclusion will add little to the model. At this stage the analysis is terminated. However, if either total N or available P did have an F to enter >1, then the analysis would proceed to stage 2. In the present example, it is concluded that only soil pH effectively discriminates between the soils with and without *Azotobacter*, soil nitrogen and phosphate having little discriminatory power.

Conclusions

Discriminant analysis is a multivariate method of studying the degree to which two or more populations, defined by a number of descriptive variables, may overlap with each other. In its most popular form, the analysis uses a stepwise procedure to examine the relationships between a categorical variable, in this case the presence of *Azotobacter* in soil, and several descriptive variables such as soil pH, total N and available P. Variables are entered into the discriminant model one at a time such that the variable always chosen is the one making the most significant contribution.

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MiSAC annual competition 2012 sponsored by Yakult

This year's Microbiology in Schools Advisory Committee (MiSAC) Competition set the challenge to produce a one-page, illustrated news article for a healthy living magazine with the main aim of improving understanding of the contribution of the activities of microbes to healthy living. The broader aim was to use this knowledge for promoting the importance of healthy eating habits. Special sponsorship for the competition was generously provided by Yakult.

Fit for purpose: microbes and healthy living

Entries were invited from two groups: Key Stage 3 (KS3) and Key Stage 4 (KS4) (Secondary 1/2 and 3/4, respectively, in Scotland). More than 60 group entries from 55 schools and colleges yielded over 400 separate entries consisting of some 250 from KS3 and 160 from KS4. Nearly 500 students were involved from England, Wales, Scotland, Northern Ireland and Eire, and from British schools in Belgium and Germany.

Entrants were required to consider one aspect of the role of microbes in healthy living. A list of possible topics was provided as guidance, but choice was not limited to this list. The most popular subject was 'mycoprotein', most notably in the KS4 group, closely followed, particularly in the KS3 group, by 'the normal flora of the gut'. There was also considerable interest in 'modification of the normal gut flora (probiotics, prebiotics)'. Yeasts were the focus of the few who chose their own aspect of microbes' role in healthy living.

The Chairman and other members of MiSAC were joined on the judging panel by representatives from Yakult's Science Team, Science Director (and microbiologist) Dr Linda Thomas, and team member Leanne Hewitt, a nutritionist. The judges looked for those entries which met the required brief: written as an article, taking account of the intended readership, confining attention to one aspect, and incorporating the specified points - names and roles of microbes and their possible benefits.

Good entries were appropriately illustrated with attention-grabbing layouts together with evidence of good science and use of an entrant's own words. The requirement to give a name to the magazine, a title to the article and sources of further information provided an outlet for imaginative and witty talents. For the KS4 group, the continuing increase in quality and participation rate (a record 40% of all entries) was very much welcomed.

1st, 2nd and 3rd prizes were awarded and in addition, several entries were Highly Commended for scientific merit or design. All other student participants were awarded a certificate of entry, a much appreciated feature of the competition, and Yakult has kindly provided some microbiology teaching resources for each establishment. The results list and winning entries will be made available on the MiSAC Annual Competition (http://www.misac.org.uk/competition.html) and the Yakult's News and Events (www.yakult.co.uk/about-yakult/yakult-news-and-events/) websites.

The topics chosen for the competition are always linked to the National Curriculum but the requirements are framed to encourage students to look beyond this. It is hoped that this approach has proved to be an enjoyable experience that has also increased an interest in microbiology. MiSAC would like to thank all those students who entered this year's competition and express appreciation to their teachers for encouraging the students to enter the competition and providing them with guidance.

John Grainger Chairman, MiSAC

The Pharmaceutical Micro-Biology

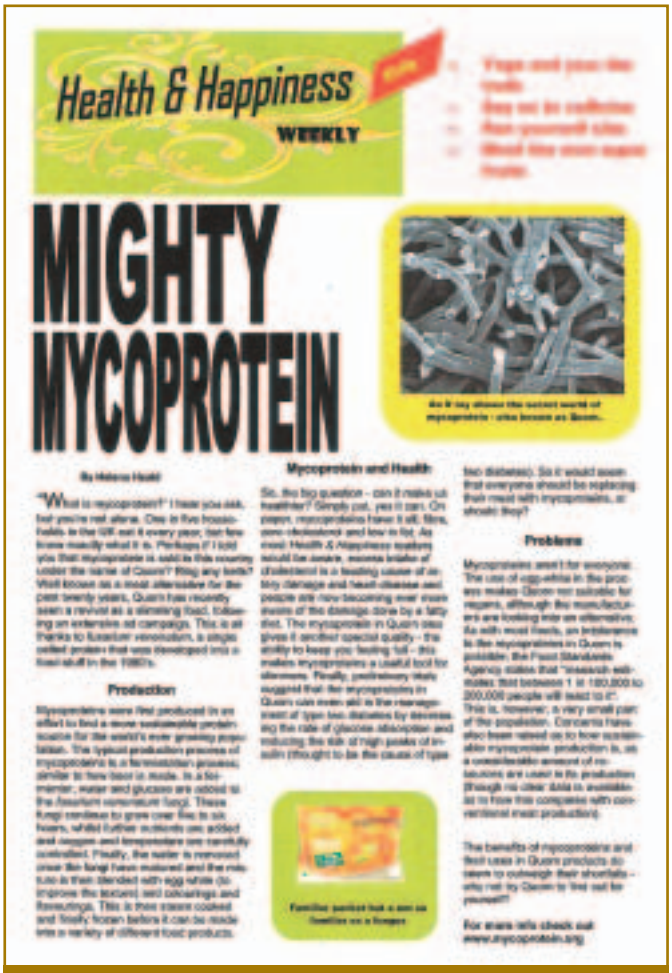
Micro-Algae: Small but Mighty!

By Nikhil Koria

What are micro-algae?

How can micro-algae improve your health?

KS3 1st: Nikhil Koria, King Henry VIII School, Coventry, on micro-algae



Health & Happiness WEEKLY

MIGHTY MYCOPROTEIN

Mycoprotein and Health

Production

Problems

KS4 1st: Helena Heald, Shrewsbury High School, on mycoprotein

MiSAC Competition Winners 2012

Key Stage 3, Secondary 1/2

First Prize:
Nikhil Koria, King Henry VIII School, Coventry.

Second Prize:
Amy Nolan, The Grange School, Hartford, Cheshire.

Third Prize:
Scarlet Mayes, St Nicholas' School, Fleet, Hampshire.

Highly Commended (scientific merit):
Mya Gahle, King Edward VI High School for Girls, Edgbaston, Birmingham;
Hannah Milne-Williamson, St Nicholas' School, Fleet, Hampshire;
Emma Wells, St Nicholas' School, Fleet, Hampshire.

Key Stage 4, Secondary 3/4

First Prize:
Helena Heald, Shrewsbury High School, Shropshire.

Second Prize:
Sophie Edwards and **Megan Gill**, St Nicholas' School, Fleet, Hampshire.

Third Prize:
Victoria Evans, Welshpool High School, Powys.

Highly Commended:
Hannah Banks, Waldegrave School, Twickenham, Middlesex (scientific merit);
Carl Chapman, Neston High School, Cheshire (design);
Rebekah Lee, Shrewsbury High School, Shropshire (scientific merit); **Milly Marshall**, Shrewsbury High School, Shropshire (scientific merit).

The Voice of the Future and the Big Bang Fair

Members of the PECS Committee have been involved in some brilliant events in the past few months. Here we tell you what we've been up to.

In the loop pecs

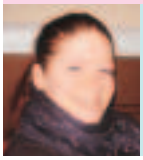
News from the S*f*AM Postgraduate and Early Career Scientist Committee

PECS NEWS

The PECS Committee are pleased to announce the student events at the S*f*AM Summer Conference, Edinburgh, Monday 2 July to Thursday 5 July 2012.

- **Monday 7–8.30pm:** An ice-breaker session with a competition to get everyone involved.
- **Tuesday 5–6pm:** A student-only session run by Sandra Smith of Fairplace Cedar to cover networking skills.
- **Tuesday from 7.30pm onwards:** PECS committee invite you to join them for a social evening out on the town.
- **Wednesday 4.45–5.15pm:** PECS committee will hold an open committee meeting to take input from outside of the group.

For more information check out the website or email: **pecs@sfam.org.uk**. We look forward to seeing you all there!



Jo Tarrant
PECS Events Officer

The Voice of the Future

There are many opportunities in science to take part in interesting events and we, Jenni, Jo, Amara and Emmanuel, were lucky enough to attend an amazing event at the House of Commons, called the *Voice of the Future*.

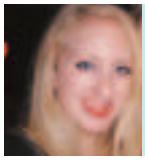
This event was organized by the Society of Biology as part of its National Science and Engineering week and was held on the 14 March. It was advertised as a 'unique opportunity for young scientists and engineers, including A-level students, to participate in a Science Question Time with Members of the House of Commons Select Committee on Science & Technology' but was so much more than that! We as young scientists were able to sit in the seats normally reserved for MPs and ask questions directly to them. Not only could the scientists interact with the Select Committee, but also with the Minister of Science, Rt Hon David Willetts MP, and the Shadow Minister for Innovation and Science, Chi Onwurah MP. This was a platform to engage with policy and decision-makers in this country and listen to their views and thoughts on the different issues that relate to science, engineering and technology. Questions were submitted before the event, and due to the massive response and the limited time that was allowed not all questions entered could be asked. Amara, Jo and myself were lucky enough to get ours chosen which made the experience even more special! Amara's question on freedom of scientific speech and libel reform provided for stimulating discussion. Jo's question was about the future issues for science in the face of Government spending cuts, a topical question in the light of the current economic climate. Mine was about the potential taxation of food to be documented to be bad for human health, but this was not looked on as a good idea by the Select Committee! Before the event Jo was considering different careers in science that she could do following her PhD, and after this opportunity she is now considering a career in science policy. Amara discovered through resources from the House of Commons library and the Parliamentary Office of Science and Technology, scientists are able to engage with MPs and provide information on science and environmental issues. These can provide potential job opportunities for early career scientists who don't want to pursue a career in research or academia.

Overall this was amazingly worthwhile and for us a once in a lifetime experience!

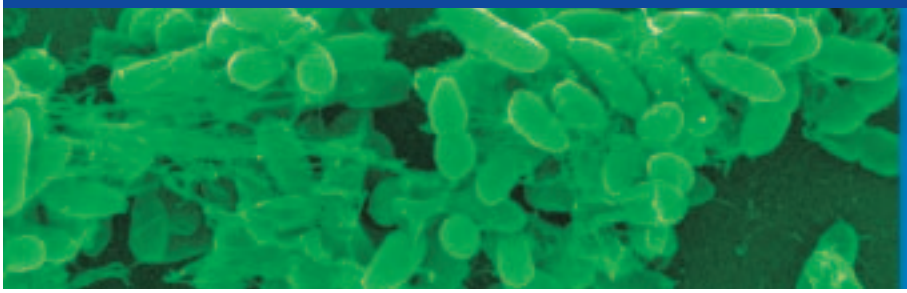
The Big Bang Fair

The Big Bang Fair was held at the National Exhibition Centre in Birmingham this year from 15 to 17 March. There was a massive variety of stalls at the fair including one by S*f*AM and the British Society for Immunology called Disease Detectives. The stand demonstrated different techniques for identifying pathogens in a sick patient. The most popular technique that was demonstrated was the latex agglutination test, using 'poo' (mushed up Weetabix and food colouring). This was a hands-on practical that most of the kids loved — once they had got past the grossness! Members of the PECS committee were invited to volunteer on the stand, which provided a great opportunity for developing communication skills with people of all ages and levels of scientific knowledge. It was the first experience of attending the BBF for Amara, Emmanuel, Jo and myself so we all learnt together becoming the self-proclaimed 'disease detectives'! Jo's favourite part of the event was interacting with the children who had an interest in science but had never experienced microbiology. Both Amara and Jo observed that science teachers really appreciated the stall because it gave them ideas about potential things to talk about in their classes. It was also important because not all children have the opportunity to work with scientific equipment such as microscopes, vortexes and centrifuges so this booth was a great chance to introduce different scientific techniques to interested kids.

Amara didn't know what to expect from this event, but believed that she gained useful experience that will help her the next time she has to do work in schools as a STEM Ambassador. We all found the experience mentally and physically challenging but would recommend this event to anyone interested in expanding their science communication skills, and looking to have great fun along the way!



Jenni Drever-Heaps
PECS Publications Officer



Environmental microbiology

Mike Dempsey tells us about his long and varied career in environmental microbiology

My interest in science began early, when my dad taught me some simple maths before I even started school! It developed properly later, during junior school, when he also taught me about electricity and simple circuits; and my favourite teacher (Mr Dobson) taught me about friction ("*without it, we'd slide off our chairs*!"), the fact that there were millions of atoms on the head of a pin and aerodynamics (we built simple gliders from balsa wood). I can still remember the buzz of excitement when I thought I had discovered an unusual triangle, until Mr Dobson sent me back to my desk to remeasure the angles more carefully — my first lesson in questioning the data. I also had a chemistry set, in the days when you could get chemicals from specialist toy shops, and pharmacists.

I can trace my interest in microbiology back to my 9th birthday, when my grandmother bought me a toy microscope. The first specimen that I examined was a blade of grass, on which I saw an oval, white object which I took to be a bacterium. I realized much later that my conclusion was wrong, when I learned at senior school about the resolving power of microscopes and the size of bacteria!

Thanks to an inspirational biology teacher (Mr Schorah) at senior school, we undertook a specialist A-level unit in microbiology. I can remember when we exposed agar plates to a variety of inocula and us all being fascinated and somewhat disgusted when we found haemolytic bacteria growing from a "smokers cough". I later discovered that Mr Schorah had completed a first degree in mathematics but got bored with the subject and took another one in botany. No wonder we spent so much time interpreting data which, after all, is what science is all about.

But it was at university that my interest in microbiology really took off. Not having reached the required grade in A-level chemistry for my original choice, biochemistry, I was offered a place in the Botany Department at Liverpool University, which ran a "unit course" system that allowed an astonishing amount of choice. I believe that one of the leading lights in the development of this excellent system was Tony Bradshaw, the erstwhile Head of the Botany Department. My initial reason for accepting the place was that, if I did well enough in the compulsory biochemistry units, I would ask to change degree for my second year. However, I found the biochemistry boring and was glad I had not been offered a place on the course!

In contrast, I found microbiology fascinating, at least the microbial ecology as taught by the redoubtable Tim Gray and Stan Williams, in the exciting and friendly botany department. I avoided medical microbiology, as I was much more interested in the useful things that 99% of microbes do, rather than the 1% causing all those nasty diseases! Although I wanted to focus on plant ecology for my honours year, I was unable to take that option as it was full (with seven students!); so I became one of four taking microbiology. My honours project gave me an introduction to chemostat culture, with the aim of investigating the effect of growth rate on the morphology of *Mycobacterium rhodochrous*: cocci, rods, or mycelia.

The economy was in recession when I graduated in 1975 and jobs were hard to come by. I was offered a place to train as an Environmental Health Inspector in Essex but the course was in transition between block and day release. By the time a firm offer was made, I was several weeks into the MSc in Biodeterioration of Materials at Portsmouth Polytechnic, where I decided to stay.

Following a highly successful MSc under the charismatic Gareth Jones, I stayed on to do a PhD in marine bacterial fouling under his supervision. This project was funded by Hempel Marine Paints and began with a course on antifouling paint technology at the company's headquarters in Copenhagen. The visit also involved an interesting evening with the marine testing station staff, sampling local fermentation products — followed by a breakfast of Underberg!

My PhD studies involved several aspects of marine bacterial fouling; including the enumeration of bacteria attached to different antifouling paints; developing a method to produce the type of copper ion that leaches from paints, so that I could measure its effect on the survival of bacteria suspended in synthetic seawater free of organic matter; a scanning electron microscopy study of attachment mechanisms and the morphology of biofilm development; and the influence of molecular and bacterial fouling on algal spore settlement.

The enumeration of bacteria attaching to antifouling paints threw up an interesting set of issues. I learned during the Copenhagen course that toxin particles migrate to the surface of a drying paint, so that the initial rate of leaching is high and it takes several weeks to stabilize at a lower level, which is maintained for about two years. I tried leaching gamma-irradiated panels in recirculating seawater, to find that bacteria colonized them within a few days. I then tried leaching in recirculating seawater containing glutaraldehyde, after discovering its disinfectant properties in the literature (this was also my introduction to the patent literature, as there was a patent on its use as a sporicidal disinfectant in hospitals). This time, glutaraldehyde polymerized on the paint surface, so these panels were also useless.

The third set of panels was exposed without leaching, on the basis that if bacteria were able to grow on them during

leaching, then the initially high rate of toxin release was not a problem. And, in any event, immediate exposure was what happened with a freshly-painted ship's hull. This was a valuable lesson in planning an experiment and ignoring perceived wisdom. It now brings to mind the motto of the Royal Society; *Nullius in verba* (take nobody's word for it).

The paint panels were exposed in the running seawater system at the polytechnic's marine laboratories on Hayling Island. I used traditional swabbing, dilution series, and pour plating – which was where I made my mistake. I was working in a small caravan, so I stacked the plates as I poured them. After incubation, I discovered that only the uppermost plate had growth throughout the agar. The next one down had a ring of growth around the periphery, and the rest had no visible growth. Puzzled by this observation, I delved into the literature on marine bacteria, and discovered Zobell's 1942 paper reporting that marine bacteria are psychrophilic and don't tolerate the temperature that agar is normally poured at (50°C). So, I had to cool the agar to 42°C before pouring plates and allow them to cool rapidly by placing them singly on the cool bench.

This work was carried out years before the development of culture-independent methods and was valuable experience in enumerating bacteria attaching to non-natural surfaces. The heat intolerance of marine bacteria was something that I had not learned studying terrestrial microbial ecology at Liverpool and the experience taught me to read the relevant literature before conducting new experiments!

The pour-plate method constrained the number of plates that I could handle and I have to thank Eric May for teaching me about the Miles and Misra "drop plate" technique. This was one of the few microbial culture techniques that I had not learned at Liverpool, and provides a good example of *continuing professional development*.

Having discovered that marine bacteria are able to colonize antifouling paints within hours of exposure in seawater, and that bacterial biofilms encourage the settlement of algal spores, it was time to defend my thesis. My director of studies, Gareth Jones, had selected one of the leading researchers on microbial attachment and biofilm formation to be my external examiner - the formidable Madilyn Fletcher, then at Warwick University. Time flew and before I knew it the grilling was over and all I had to do were "minor corrections" — phew, I'm a doctor!

Having spent about 18 months under-employed after finishing my PhD, I was worried that I would never put it to good use when a postdoc position came up at UMIST. It was in the department of chemical engineering, to develop a fluidized bed fermenter for the production of fuel ethanol by continuous culture using yeast. I didn't know what chemical engineering or this type of fermenter were, and I had never worked with *Saccharomyces cerevisiae*. However, I correctly identified that one of the key issues would be microbial adhesion, which I did know about. This was a great period of my career, doing nothing but research and no exams to take or thesis to write! Bernard Atkinson was the pioneer of attached biofilm systems and it was an inspiring time being a member of his biochemical engineering group.

Towards the end of this postdoc work, a permanent lectureship came up at Manchester Polytechnic and I was fortunate enough to be appointed with only a few months of the postdoc left. Following my appointment, I used my knowledge gained at UMIST to help design a new fermentation laboratory, and was therefore able to continue my research on fuel ethanol production. As interest in fuel ethanol waned, I switched interest to enzyme, antibiotic, and plant secondary metabolite production. However, a market survey by business studies students at the now Manchester Metropolitan University (MMU) discovered that the pharmaceutical industry was not interested in switching to fluidized bed fermenters – my first lesson in the harsh realities of the commercial world.

Fortunately the EU came to the rescue, as the Urban Wastewater Treatment Directive had just been published, which highlighted damage to the aquatic environment caused by discharge of ammonia released during secondary biological treatment. I therefore instituted a project to develop an expanded bed biofilm reactor, which used fluidized particles of glassy coke on which a biofilm of microorganisms grew to form bioparticles: in this case, a biofilm with nitrifying bacteria to oxidize toxic ammonia in secondary effluent to non-toxic nitrate. The success of this project led to the idea for a spin-out company, which I developed during 1999 to 2000 through the inaugural BBSRC/MRC Bioscience Business Plan competition, generously sponsored by Lord Sainsbury's Gatsby Foundation.

Following encouragement and support from colleagues at MMU (Dave McCormick, Ian Roberts, Lawrie Grant and Barry Plumb), Advanced Bioprocess Development Ltd. (ABD) was incorporated in August 2002. By December 2002, ABD had been accepted by Campus Ventures for business incubation. By May 2003, ABD had won a Department of Trade and Industry SMART Award, to build and operate a pilot-plant. This project was successful and ABD now has a full-scale prototype package plant operated at a UK water company site by a licensee.

During my time as MD of ABD, I have remained on the teaching staff at MMU, which has taught me valuable skills in time management, especially during times when both workloads peak! I have also gained plenty of valuable commercial experience, involving e.g. patenting, licensing, finance, insurance, and even some employment law. However, I could not have done this on my own and, as well as everyone mentioned above, I also owe a large debt of gratitude to Peter Jackson, erstwhile Chairman of ABD and still my "commercial brains". As he says, "Mike is technical; I am commercial." Although he also describes me as, "the most commercially-minded academic he has met!"

I joined SfAM in 2008 and was invited to join the Meetings Subcommittee in 2010, to help develop the environmental microbiology area of the Society. My first goal is to organize a meeting to celebrate the centenary of the activated sludge process, developed in 1913 to 1914 by Ardern and Lockett at Manchester's Davyhulme works. This process is responsible for helping to break the cycle of waterborne disease, not only in the UK but throughout the world. These days, we think of wastewater treatment as being for the protection of the aquatic environment and I am proud to be doing my bit to help this valuable work by developing a tertiary nitrification process based on expanded bed biofilm reactor technology.

So, a long and varied career but with microbiology always at its heart!

Mike Dempsey
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Prolonged stationary phase in Gram-positives: the Growth Advantage in Stationary Phase (GASP) phenomenon and stress responses

During the summer prior to the final year of my BSc Biology degree at the University of Northampton I was awarded a SfAM Students into Work grant. This allowed me to undertake a 10 week project in the university's microbiology laboratory enabling me to develop a range of practical skills as well as obtaining an insight into what research projects entail.

The aim of my research was to investigate long-term survival of meticillin-resistant *Staphylococcus aureus* (MRSA), meticillin-susceptible *Staph. aureus* (MSSA), vancomycin-resistant *Enterococcus faecium* (VRE) and vancomycin-susceptible *Ent. faecium* (VSE). The objectives were to determine if the Growth Advantage in Stationary Phase (GASP) phenomenon was occurring in *Staph. aureus* and *Ent. faecium* and whether antibiotic resistance, heat and acid adaptation increased as cells progressed through stationary phase.

The Growth Advantage in Stationary Phase (GASP) phenomenon is characterized by fluctuating numbers of viable cells when progressing through stationary phase. This is due to repeated rounds of population takeover which are the result of competition for resources. Certain cells within the population have mutations increasing their ability to harvest nutrients from dying cells; these cells divide when nutrients are ample and restore original cell numbers (Zambrano & Kolter, 1996). GASP was first identified in 1993 by Zambrano *et al.*, observations showed that aged cultures of *E. coli* were able to outcompete one day old cultures even when introduced as a minority (Zambrano *et al.*, 1993). Studies have shown that bacterial cells undergo genetic alterations throughout stationary phase resulting in mutant cells which have a competitive advantage over non-mutant cells from early stationary phase and are a different population to that of the parent cells. When subjected to extreme stress, subpopulations of cells expressing the GASP phenotype are able to outcompete the parent strain to remain viable (Finkel, 2006). A study by Kelly *et al.*, has shown that in *Campylobacter jejuni* rapid loss of viability followed by rapid recovery of cell numbers occurs within the stationary phase when subjecting cells to starvation conditions (Kelly *et al.*, 2001).

Although much of the research into the GASP phenotype has identified genetic mutations which are associated with amino acid catabolism (Zinser & Kolter, 1999), there are also reports of altered survival properties in aged cells compared to young cells which originated from the same culture. It has been demonstrated that as cells progress through stationary phase they became more resistant to stress (Martinez-Rodriguez *et al.*, 2004), this may be due to frequently encountered GASP mutations found within the *rpoS* gene (Finkel, 2006). The protein product of the *rpoS* gene is a regulator of gene expression and is widely used by both Gram-positive and Gram-negative bacteria to survive environmental stresses (Kazmierczak, 2005).

In order to identify GASP, growth curves were produced by taking samples of growing culture at hourly intervals for 80 hours; fluctuations in cell numbers over the 80 hours were identified and phases of growth ascertained for stress response testing.

At each stationary phase, cells were tested for antimicrobial resistance and their ability to adapt to both heat and acid stress. Adaptation assays for heat and acid were performed by determining the tolerance temperature and pH of *Staph. aureus* and *Ent. faecium* by subjecting them to a range of temperatures (50 to 75°C in 5°C intervals) and pHs (pH 1 to 5 in intervals of 1) for 10, 20 and 30 minutes. The tolerance was defined as the lowest temperature and time and highest pH and time at which approximately 2 logs of growth occurred. Adaptation assays required cells to be sublethally stressed for 10, 20 and 30 minutes followed by subjection to heat and pH tolerances to determine whether heat and pH adaptation had increased throughout stationary phase.

The results of my investigation showed that progression through stationary phase had no effect on antimicrobial resistance of *Ent. faecium*. VSE and VRE did not adapt following pre-treatment at sublethal pH (pH 5), yet VRE cells showed a natural increase in acid resistance as they progressed through stationary phase. Cells also showed an ability to adapt to heat with increases of 1.5 to 2 log following pre-treatment at sublethal temperatures (50°C). This increase was similar in cells from both early and late stationary phase suggesting that cells could adapt to heat irrespective of age.

Investigations into stress induced adaptation and antimicrobial resistance

of *Staph. aureus* as it progresses through stationary phase are currently ongoing.

I would like to thank S*f*AM for giving me the opportunity to undertake this project, it has given me first-hand experience of working in a research environment and allowed me to develop both subject specific and general skills.

Finally I would like to thank my supervisor Dr Katie Laird née Fisher for making the project possible and directing me in both practical skills and progression of the project. Likewise I would like to express my gratitude to Professor Carol Phillips for her feedback and opinions on the findings of my research and ways to further the project.

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Danny Sewell
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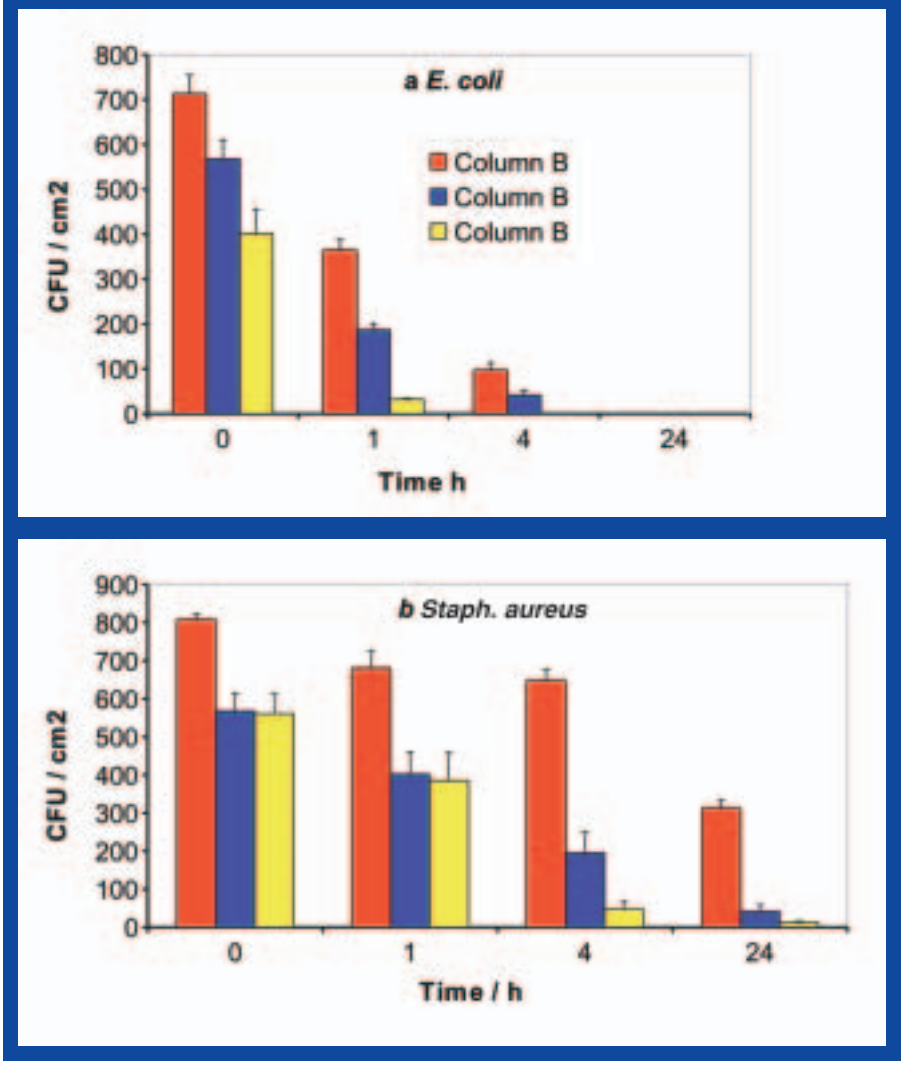
Evaluation of novel nano-structured antimicrobial surfaces

I am currently working my way through a Biological Sciences degree in the School of Environment and Life Sciences at the University of Salford. Upon completion of my second year, I was given an opportunity to undertake a project involving testing of antimicrobial surfaces by the S*f*AM 'Students into Work' scheme. When I heard of this project I thought it would be a great chance to see what working in a research environment would entail.

The project was run in parallel with a European community FP7 grant "Flexible production technologies and equipment based on atmospheric pressure plasma processing for 3D nano-structured surfaces" which involves more than 20 different partners across Europe. My project involved evaluation of novel antimicrobial surfaces. This project appealed to me because of its real-life application, to be able to provide surfaces in the hospital that can actively kill clinically relevant pathogens. Hospitals have been coming under increasing pressure to provide clean and safe environments for patients. This pressure to maintain high standards in sanitation is at an all-time high. It is now possible, with the advancement in technologies, to produce nano-structured surfaces which release antimicrobial silver, killing microorganisms on that surface.

Along with this project I was working indirectly with the company CVD Technologies. This company produces silica coatings incorporating silver nitrate and have an ultimate aim of a five log reduction in the number of microorganisms present on the surface within four hours. My role within this project was to assess a number of test bacteria on different surfaces. There is currently no formal protocol for this type of microbial testing so my first task was to produce a workable protocol which could be repeated. Firstly, it was necessary to devise a mechanism for

Figure 1. *Viability of test organisms on antimicrobial glass, (a) E. coli (b) Staphylococcus aureus*



distribution of the bacteria which would simulate the type of natural contamination that would occur within a hospital. With that in mind I decided to use a sprayer which would coat the surfaces with microorganisms, just as atmospheric microorganisms would settle on the surfaces in a hospital.

This was tested on three different samples of glass; an untreated control surface, a commercially available product, sample A, and sample B which was produced by CVD Technologies and was still under development. The first bacterium that was chosen to be tested was *E. coli*, which was grown in Tryptone-Soy broth, washed by centrifugation and resuspended in water. Different dilutions were prepared to allow different levels of contamination and 200µl sprayed to evenly cover a 3 x 5cm area. The samples were then allowed to dry.

Three methods were then compared for recovery of the organisms from the surface to test for viability. These were: a swabbing method commonly used in hospitals, a dipslide method and a contact plate method. This test was run over 24 hours, with; *E coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecium* and *Mycobacterium smegmatis*. Triplicate tests were performed and the means and standard deviations taken to produce the graphs. The results obtained with *E. coli* and *Staph. aureus* using the swabbing microbial testing method, which gave the highest recovery, are shown in Figure 1. There was a total kill of *E. coli* on sample B after four hours (Figure 1a) whereas *E. coli* was not killed on the control samples until 24 hours. The Gram-positive *Staph. aureus* was more resistant and it did not die on the controls even after 24 hours. The results showed that the most active test surface was sample B, the surface developed by CVD Technologies. The next step will be to prove this activity against more clinically relevant pathogens such as *Clostridium difficile*.

After completing my project I feel very privileged to have been a part of research that one day could aid in making our hospitals a safer and cleaner environment. Given the importance of this testing it was an opportunity for me to gain valuable knowledge and experience of this field. As a result of undertaking this project I will reap the benefits when I enter my final year having gained a substantial amount of knowledge about microbiology and microbiological techniques. I have gained some very important skills which will be used not only in my final year, but which will be taken with me throughout my working life. I have enjoyed being part of this project and would recommend this scheme to any student who is interested in getting a first-hand look at what working within a microbiology laboratory entails.

I would like to thank Dr Howard Foster for giving me the chance to be a part of this project and SfAM who supplied funding for the 10 weeks and gave me the opportunity to improve myself on such a useful placement.

Paul Fitton
University of Salford

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Microbial degradation of pesticides in soil

Herbicides, insecticides and fungicides are the three main groups of pesticides. In 2006, more than 22,400 tonnes of pesticides were applied in the UK (DEFRA, 2008) to protect crops against infestations and disease, and thereby promote increased productivity. Pesticides persisting in the environment can become a hazard, as in the case of the insecticide dichloro-diphenyl-trichloroethane (DDT). It was applied to destroy mosquitoes spreading diseases such as malaria and typhus and was efficient, but its persistent nature led to its entry into the food chain, where it was retained in fatty tissues of fish. Subsequently it accumulated in higher organisms and caused a major decline of many bird populations. Since the 1970s and 1980s lessons have been learned and such environmental catastrophes are much less frequent.

Pesticides are formulated to adversely affect the physiological function of the target pest, mainly insect, fungus or plant. It is inevitable that, during application, a sizable proportion of the applied compound will fall onto non-vegetated soil. In the soil, degradable compounds can immediately undergo a process of transformation and mobilization. Chemical hydrolysis, photolysis and phytodegradation can transform molecules into potentially less harmful or non-toxic metabolites. Simultaneously, environmental conditions may readily affect the movement of the pesticide. The pesticide may be volatilized into the atmosphere. Heavy precipitation may cause dissolved molecules, and those adsorbed to soil colloids, to enter watercourses. There may be mass flow of water through soil, transporting molecules that are not adsorbed to soil

organic matter. This can cause leaching into and contamination of groundwater and these processes can lead to a large dispersion of pesticides or metabolites into the environment.

Soil microorganisms have a remarkable ability to remove pesticides by normal catabolic activities. This capacity is, however, greatly influenced by the pesticide characteristics and the soil physico-chemical properties. Microbial degradation tends to occur most rapidly within the upper layers of the soil, where microbial biomass is highest and activity greatest. To encourage degradation, these microorganisms need to be available in adequate numbers and diversity, have appropriate enzymes, preferably aerobic conditions and favourable temperature, soil moisture content and pH. Bacteria, actinomycetes and fungi tend to be the main degraders.

Pesticide formulation is usually based on naturally occurring substances with the addition of one or several functional groups, the number and position of which can alter the biodegradability of the molecule. Such compounds represent a novel source of carbon and energy for growth of the microorganism. Biodegradation usually involves complete mineralization, although cometabolism can occur, whereby the molecule is transformed into a less, or more, toxic metabolite, but the microorganism is unable to derive energy from the process. A pesticide may also be recalcitrant to degradation.

Initial steps involved in biodegradation often include removing the toxic or added component to create a transformed molecule which may then be channelled into a common pathway to complete mineralization. These enzymatic processes are mainly intracellular, although large molecules must be cleaved first by extracellular enzymes to aid entry into the cell. Microorganisms possess a large array of enzymes, each restricted to a single type of reaction on a narrow range of substrates of similar structures. They acquire and evolve new catabolic pathways to degrade newly introduced xenobiotic chemicals by conjugation, the transfer of plasmids from one organism to a recipient strain (Top, Holben & Forney, 1995). Subsequent applications allow microorganisms to promote degradation of that pesticide.

Pesticide degradation in soil often follows a first order kinetics decay curve, and pesticides have specific half-lives (DT_{50}), associated with specific soil types and climatic conditions. Techniques such as HPLC can be used to assess DT_{50}, while use of ^{14}C labelled substrates increases sensitivity. Soil texture, pH and organic matter content, combined with the capacity of pesticides to adsorb onto clay minerals and organic matter, affect the degradation rate.

Readily water-soluble pesticides tend to remain at the surface of organic matter and clay colloids, allowing them to be highly mobile, and such pesticides in soil solution are easily accessible to microorganisms (Rowell, 1994). Hydrophobic non-ionic pesticides adsorb more strongly to organic matter particles, resulting in a reduced concentration of pesticide in the liquid phase. Such an adsorbed chemical or one isolated within nanopores will lead to very slow desorption of the chemical, and slow or limited mass transfer of pesticide to the microorganism, reducing bioavailability (Bosma *et al.*, 1997).

A pesticide which has a long contact time with the soil becomes aged and more resistant to extraction, indicating that if the soil is not disturbed there is reduced danger to the environment. Hence sorption and associated bioavailability play an important role in the ability of the microorganism to degrade the pesticide.

Many degrading organisms have been identified by enrichment and isolation on medium containing the pesticide as the sole source of carbon. Molecular methods, such as polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), sequencing, and stable isotope probing (SIP) can be employed to identify further uncultivated degrading organisms present in the soil (Mahmood, Paton & Prosser, 2005).

The rich diversity of microbial phylotypes found in soil provides a reservoir of ability to modify added chemicals. A wide range of variable soil characteristics and pesticide properties influence the rate of degradation and bioavailability to the microorganisms. In this way microorganisms fulfil an extremely important function in removing excess pesticides almost completely from the environment.

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RNA arbitrarily primed PCR as a tool for the measurement of gene expression

Analysing gene expression (i.e. mRNA of specific genes) allows microbial function to be assessed. Numerous techniques for analysing mRNA expression in the environment are potentially available, including competitive RT-PCR, Northern blots, ribonuclease protection assays, *in situ* hybridization and microarrays. These techniques have been widely used to assess gene expression in pure cultures, and are increasingly exploited to determine gene expression in environmental systems. However, many of these methods rely on prior knowledge of the target genome and as comprehensive information on soil genomes is extremely limited, with less than 1% of all bacteria thought to be culturable (Torsvik & Ovreas, 2002) this leaves many soil genomes unaccounted.

RNA arbitrarily primed PCR (RAP-PCR) was developed to analyse a large genome where no previous information

about the genome is required, thus they are particularly suited for analysis of environmental systems where little is known about the genome sequences present. RAP-PCR is a variation of differential display PCR (DD-PCR), a similar technique widely used to analyse differential gene expression in eukaryote cells. In RAP-PCR, mRNA is extracted from samples that have been differentially treated (e.g. exposed to the presence of a pollutant or not), this is then amplified with an arbitrary primer (a primer with any particular sequence 10 to 15bp in length) to create cDNA. The cDNA is then amplified in a PCR reaction with two arbitrary primers, resulting in a range of amplicons of differing lengths which are then separated on a polyacrylamide gel and visualized by techniques such as autoradiography, fluorescent primers, silver staining or SYBR green staining (Liang, 2002; Taroncher-Oldenburg & Anderson, 2000). A differently expressed band is determined to be one which appears in one set of mRNAs and not in another. Once identified as differentially expressed bands, they are generally excised, sequenced and given a putative identity using BLAST or FASTA. Once the band has been sequenced, changes in its expression levels between samples can be analysed using real-time PCR, as the intensity of the band on the gel is not a reliable indicator of the level of its expression.

RAP-PCR is a very powerful procedure for analysing community genomes, as it is both rapid and of low-cost with a large percentage of genomes screened using a limited number of primers. Pure culture studies have estimated a high gene percentage coverage using RAP-PCR with probabilities of detecting rare transcripts in a 4mbp genome at 0.05% (Walters *et al.*, 2001) if 5,000 bands are detected however, with an undefined genome size in the soil, calculating the genome coverage is much more difficult. RAP-PCR and DD-PCR have identified a wide range of genes from both pure cultures and environments, including those involved in the biosynthesis of amino acids (Palumbo *et al.*, 2005), metabolic enzymes, methane and cyclohexanone monooxygenases, salinity regulated genes, a *CrosRS* regulatory system and genes induced by mitomycin C.

Gene analysis in the environment is subject to a number of drawbacks (Saleh-Lakha *et al.*, 2005). Technological limitations and high costs minimizes the amount of DNA and RNA analysis that can be carried out, while environmental limitations influence the quality and quantity of nucleic acids and lack of full data on the soil genome affect a full assessment of all metabolic activities in the environment. While RAP-PCR tries to address most of these drawbacks, and is a very powerful technique for detecting changes in gene expression, one significant problem is the lack of information available on sequences detected in such a study, with best matches often returning from bacterial genomes which are fully sequenced (Bhaya *et al.*, 2000; Brzostowicz *et al.*, 2003) or with 16S rRNA sequences which appear to dominate bacterial genomic databases. With a large number of sequences returning with 'no significant match' in Blast analysis, other than monitoring genetic expression of the gene with real-time PCR, a full conclusion as to the correct function of these genes cannot be drawn. While it has been shown that these are actively expressed genes and thus of importance to activity in the soil community, until an improved genomic database is available, the full role of many of these sequences remains unclear.

Originally RAP-PCR was applied to pure cultures of fungi and bacteria but over the last number of years it has extended into environmental studies. My work with RAP-PCR involved analysing the differential gene expression in a soil microcosm exposed to 2,4-dichlorophenol (2,4-DCP), in order to identify microbial functions associated with the early stages of its degradation. Changes in RNA fingerprints generated by RAP-PCR were observed over a 21 day time course. A wide variety of differentially expressed genes were detected, with a number of novel nucleotide sequences identified. Overall, most sequences detected (> 50%) were either found to have an unknown gene function or found not to have a significant match to a known sequence. The genes detected and assigned a putative match probably represented most areas of microbial cell function including cell metabolism, cell transport, cell division, cell motility, amino acid synthesis, and stress protein production. No sequence matched with genes known to be directly involved in the degradation of 2,4-DCP. Relative abundances of a number of genes identified in this study were subsequently quantified by real-time PCR and indicated different levels of expression at each time point, with the most significant changes occurring two days following application of 2,4-DCP to the soil, with genes both being unregulated and down regulated at this time point.

I would like to thank the Society for Applied Microbiology for awarding me a President's Fund grant which enabled me to attend the 12th ISME conference in Cairns, Australia in August 2008 and present a poster detailing the work described here.

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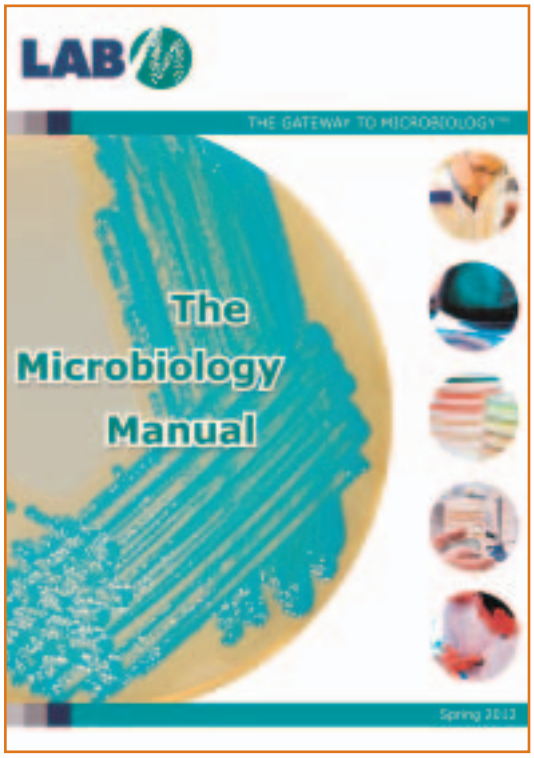
corporate news

The latest news, views and microbiological developments from our Corporate Members



New Lab M technical manual now available

With the continuing expansion of its microbiological culture media portfolio, Lab M has revised the associated technical manual which is now fully available. The Spring 2012 edition will reflect the fresh new branding brought in during 2011 when the company launched its information-rich 'gateway to microbiology' e-commerce website. Offering detailed specifications for all Lab M products including: dehydrated culture media, Harlequin™ chromogenic media, anaerobe isolation media, supplements and additives, this latest edition of the Lab M technical manual is available to download from our website or on request by email.

Lab M's Managing Director, Ian Morris, said, "*Lab M is undergoing an exciting period of further growth and development, and the company's product portfolio is moving forward all the time. We have been revisiting all our supporting documentation and this has given us the opportunity to advance the look and feel of our materials to reflect our ever-widening global perspective, as well as ensuring they remain as useful and easy-to-use as possible.*"

Lab M's ongoing success in both domestic and export markets is driving the company forward into a period of rapid growth and transformation. Fully integrated research, development, quality assurance and manufacturing enable Lab M to provide a range of precise, rapid and dependable methodology for the detection, isolation and identification of micro-organisms.

further information

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Neogen

In business since 1982, Neogen has grown to more than 700 employees in multiple domestic and international locations developing, manufacturing and marketing a diverse line of products dedicated to food and animal safety. The company's Food Safety Division markets dehydrated culture media, and diagnostic test kits to detect foodborne bacteria, natural toxins, genetic modifications, food allergens, drug residues, plant diseases and hygiene concerns.

Neogen's Contract Laboratory Services provides the food, beverage and animal feed industries with a complete range of laboratory analysis to ensure the safety, quality and legality of manufacturing operations. Continual expansion and investment in new technologies means that our food safety testing is covered by UKAS accreditation and carried out by highly trained scientific analysts.

Neogen's success has created an increased awareness outside the testing industry resulting in being named NASDAQ's Global Select Market and repeatedly named as one of *Forbes Magazine's* Best 200 Small Companies.

Since its founding, Neogen has pioneered rapid diagnostic testing. We have taken testing from complicated, expensive off-site methods, to simple and accurate, cost effective trusted on-site testing. Neogen's continuing focus on developing and performing tests for food safety testing and analysis and dedication to confidential customer service and support have earned a special trust and place in the worldwide food manufacturing community. We value and treasure this trust. And, with each day, we dedicate ourselves to providing you with products and service that enable you to manufacture and process trusted products.

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In the 1970s Don Whitley brought the technique of spiral plating to the UK after a visit to the USA. As a microbiologist himself, Don realised the substantial time and cost savings that spiral plating offered. Not only does the technique simplify manual and automatic counting of spiral plates, it eliminates the need for serial dilutions. It also saves incubator space, offers up to 69% labour-saving, and reduces laboratory waste and the cost of consumables.

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Laboratory testing for *Clostridium difficile* has received a great deal of attention over the last 12 months with changes to the recommended testing practices, methods and availability of kits and reagents.

Pro-Lab Diagnostics are proud to offer a full range of solutions, including the Prolisa GDH (ELISA), the ProFlow GDH (EIA Lateral flow) and ProFlow C.Diff Tox AB (EIA Lateral Flow) and the Portrait Rapid Molecular Platform. Demonstrations and samples are available on request, as are places at training seminars being held.

Pro-Lab Diagnostics is also expanding its range of innovative laboratory equipment with the additions of the BactiZapper bench top loop steriliser using infrared heat, and a range of economical hot plates and vortex mixers.

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Cherwell Laboratories — specialist microbiological solutions

Cherwell Laboratories offers a range of high quality products for environmental monitoring and validation of sterilisation processes including an extensive selection of prepared microbiological media plus microbial air samplers and biological indicators. The company works with the pharmaceutical and related industries to provide specialist products and solutions to meet both industry requirements and specific customer needs.

For example, the SAS Super Isolator, part of the microbial air sampler range provides an ideal solution for isolator cabinet and filling line monitoring. To help minimise contamination risks, the SAS Super Isolator features a stainless steel sampling head which is located permanently inside the isolator cabinet. The sampling head is then connected through the isolator cabinet out to the control unit, therefore reducing the need for the sampler to be continually passed into the cabinet. For added convenience, individual heads can also be supplied enabling a single control unit to be used for multiple sampling heads.

The SAS Super Isolator system has also been used to meet the needs of filling line monitoring for pharmaceutical manufacturers. Bespoke sampling heads enabled the introduction of viable samplers into the grade A environment reducing the need for interventions when performing environmental monitoring.

further information

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Effective environmental sampling in food production and in healthcare

Environmental sampling is an essential tool for monitoring the effectiveness of hygiene control measures in the food industry. Residues of organic material on food contact surfaces provide nutrients for bacteria which can combine to form biofilms which may not be completely removed during cleaning, and it is essential to monitor the microbial load to ensure cleaning is effective, and pathogens are absent.

Polywipes™ from Medical Wire are sterile premoistened blue sponge swabs for easy and effective sampling of surfaces for the enumeration of microorganisms, and the detection of pathogens such as *Listeria* and *Salmonella*. The sponge format is convenient for sampling larger flat or irregularly shaped surfacessuch as machines, vats, work tables, and conveyor belts.

Bright blue Polywipes™ are manufactured using special processes to ensure maximum recovery and optimum detection of microorganisms. Each sponge is premoistened in a phosphate buffer, and individually sealed.During sampling, any antimicrobial substances in the sample are instantaneously diluted to levels well below lethality.

Polywipes™ are also now becoming an essential tool in clinical environments for monitoring the control of healthcare associated pathogens such as *Clostridium difficile* and MRSA.

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BioConnections, celebrating 20 years

This year marks the 20th anniversary of BioConnections. The product range has continued to increase over the years providing products for clinical, veterinary, industrial and pharmaceutical microbiology labs.

Isolation of your microbe can be achieved with both traditional and chromogenic culture media, screening of both your aerobic and anaerobic isolates can then be undertaken using our range of bench reagents. Further identification of your isolates continues with Diatabs for biochemical profiling and you can finish with serological confirmation of your isolates with our large range of antisera.

With increased awareness of antibiotic resistance our Neo-Sensitabs kits facilitate the phenotypic detection of Antimicrobial Resistance Mechanisms (ARMs) such as Extended Spectrum Beta Lactamases (ESBLs) and *Klebsiella pneumoniae* Carbapenemases (KPCs).

For the rapid detection of certain enteric bacteria, viruses and parasites we offer a range of immunochromatographic test methods.

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AHVLA Scientific introduces an innovative range of RT-PCR controls to its specialist reagents range

AHVLA Scientific has developed several Tuff® RNA which encode target sequences for a number of RT-PCR assays for animal viruses. These can be used as positive PCR or process controls for diagnostic molecular tests, avoiding the risk of using an infectious virus.

Tuff® RNA are recombinant Cowpea Mosaic Virus that are stable, transportable at 4^{O}C and can be incorporated into your routine testing schedule as a positive control assuring the correct performance of specific RT-PCR assays. As the target RNA is encapsidated, the problem of RNA degradation associated with naked RNA or *in-vitro* transcripts is eliminated.

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The most important benefit of Tuff® RNA is that it is not infectious to animals or plants thus avoiding any potential risk of using infectious viruses as controls. We hope these novel controls will enable laboratories to more easily carry out a range of routine diagnostic investigations.

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Thermo Fisher Scientific improves water sample collection with new containers

The new range of Thermo Scientific Sterilin Water Sampling Bottles better protect samples against contamination and other damage after collection.

Designed for either chlorinated or non-chlorinated water sampling, the new bottles are available in two designs – square and rectangular, and both styles improve handling and traceability over standard containers. The over-cap design of the bottles reduces the risk of contamination as well as bottle-neck damage that can occur during transit. Tamper-evident seals also reduce the need for re-testing.

The square bottles, available in 500mL and 1000mL volumes, have a lower profile than many other models, making them ideal for use in restricted-access areas. Rectangular bottles, available in 350mL and 500mL capacity, have a flatter style that is useful when storage space is limited.

Thermo Scientific Sterilin Water Sampling Bottles are lightweight and durable, ideal for use within industrial applications. Manufactured from polyethylene terephthalate (PET), these bottles are available dosed with a buffered sodium thiosulphate (Na Thiosulphate) solution or undosed. Product specifications can also be tailored to individual customer requirements.

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information

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

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

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

NCIMB expand oilfield microbiology services

NCIMB Ltd has expanded its range of oilfield microbiology services to include use of the molecular qPCR technique to enable rapid quantification of the groups of microorganisms involved in corrosion. The new technique will complement existing services offered by the Aberdeen-based company.

NCIMB's Oilfield Services Manager Cheryl Richardson said "*Corrosion caused by the proliferation of microbes such as sulphate-reducing bacteria (SRBs) is a serious issue for the oil and gas industry and can cause costly failures and shutdowns. It is therefore important to monitor the presence of these organisms so that remedial action can be taken if their numbers start to rise. The qPCR method will enable us to give our clients rapid results if they are, for example, monitoring response to adjusted levels of biocide within the water injection system, whereas MPN (most probably number) counts may still be the most appropriate method for routine monitoring*".

NCIMB Ltd offers a range of microbiological and chemical analysis services to the oil and gas, food, pharmaceutical and personal care industries as well as housing the biggest reference collection of industrially and environmentally valuable microorganisms in the UK.

further Information

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The first direct detection kit for pathogenic *Listeria*

Technical Service Consultants new **SwabSURE ListeriaP** is an innovative, Campden BRI validated, ISO 18593 compliant, easy to use colour-change testing system for presumptive detection of pathogenic *Listeria* from food contact and environmental surfaces. ListeriaP permits easy differentiation between pathogenic *Listeria monocytogenes* and *Listeria ivanovii* from other background microorganisms including *Bacillus* spp., *Enterococcus* spp., *Micrococcus* spp., *Klebsiella* spp., and other *Listeria* species like *Listeria innocua*., simplifying the testing process involved in meeting safety and regulatory requirements such as HACCP.

The pre-moistened foam swab optimises sample collection whilst the unique TSC neutralising buffer inactivates any disinfectant residues including QAC's, phenols, peroxides and most sanitisers, enhancing cell recovery. The selective media with its patented chromogenic substrate reliably detects *Listeria monocytogenes* down to 1 CFU by changing colour from straw to turquoise within 24 to 48 hours. This visual screen reduces unnecessary and costly subculturing and further identification needs, eliminating false positives, a common problem with other traditional methods.

To order your Swab kit for the detection of pathogenic *Listeria* quote order number SS-L01. For further details, validation results or to request a free sample pack of 5 tests please contact us.

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SCIENCE SHOWOFF

Science Showoff comes to Edinburgh!

For one night only, and in association with the Society for Applied Microbiology, Science Showoff comes to Edinburgh!

On July 3rd at the Voodoo Rooms, the night where anyone can communicate any kind of science in any way is in town, looking for 10 performers ready to make the stage their own. Maybe you'd like to tell us science jokes, do a science demo, play science songs, show a science video, talk about your research, perform a science play, or anything else at all that fits in nine minutes?

If you're more of a watcher than a performer, then do join compere **Steve Cross** ("solid" — internet opinion) for a night of surprisingly high-quality sharing of all the latest science communication ideas.

Admission is free, but we strongly suggest you donate to our local charity, chosen specially for the event.

The Voodoo Rooms, Edinburgh, UK ■ Tuesday 3 July 2012

***Steve Cross** — photo by Benjamin Thompson*

For more information about this event please contact the Society Office on 01234 326661

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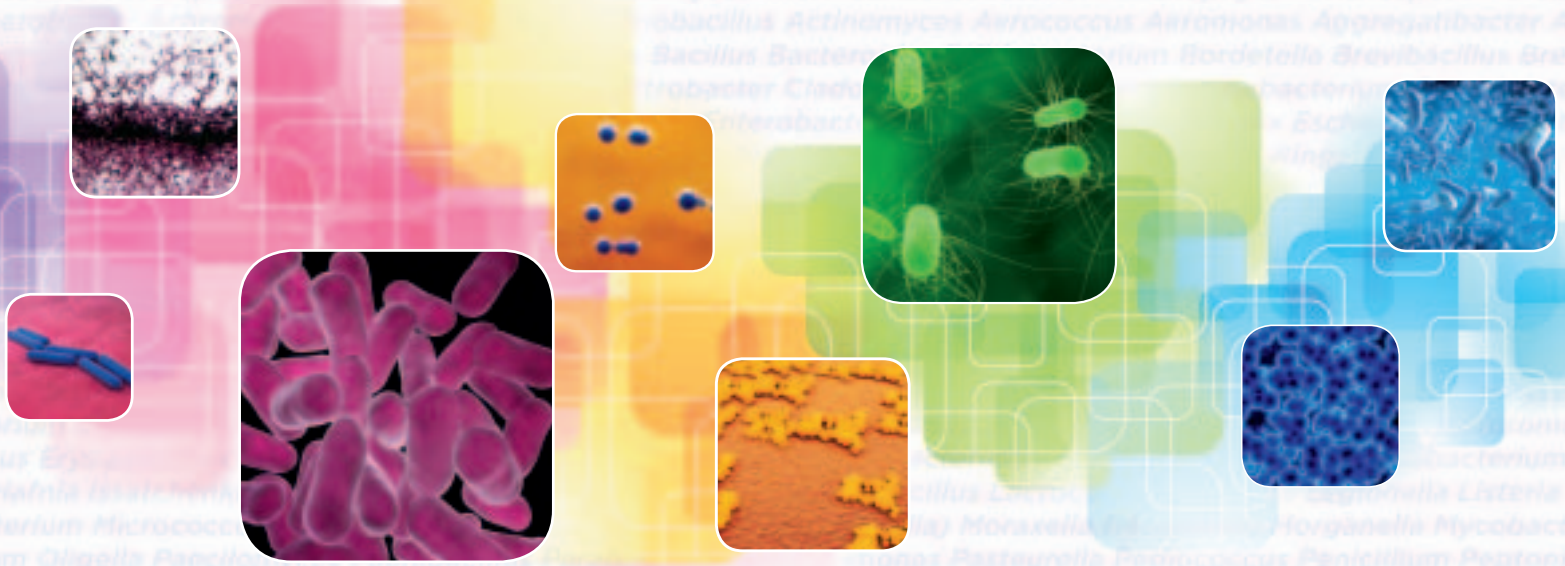

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