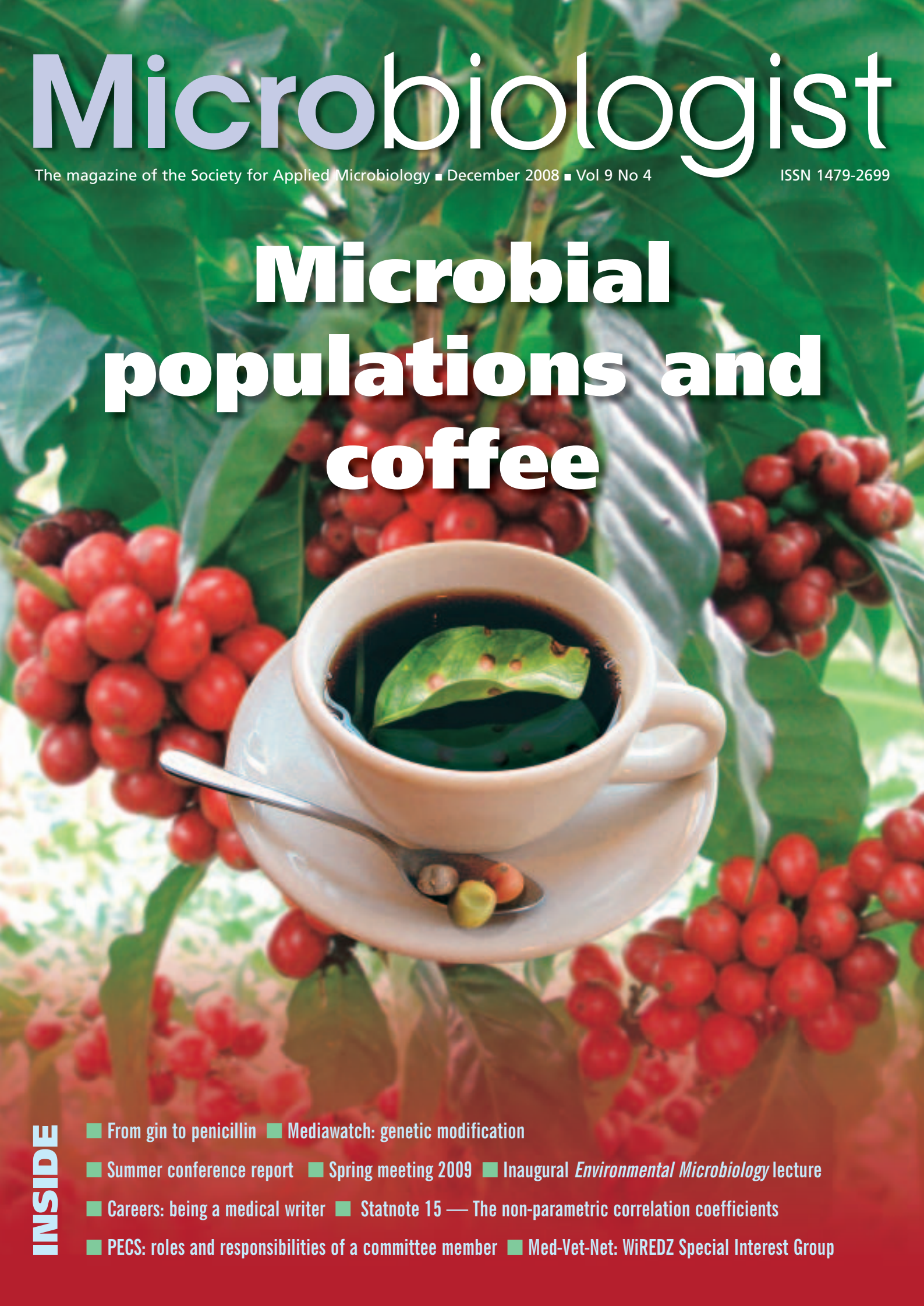
Microbiologist
The magazine of the Society for Applied Microbiology ■ December 2008 ■ Vol 9 No 4 ISSN 1479-2699

Microbial populations and coffee



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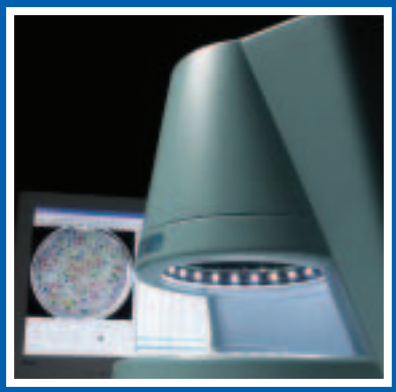

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Microbiologist the magazine of the Society for Applied Microbiology

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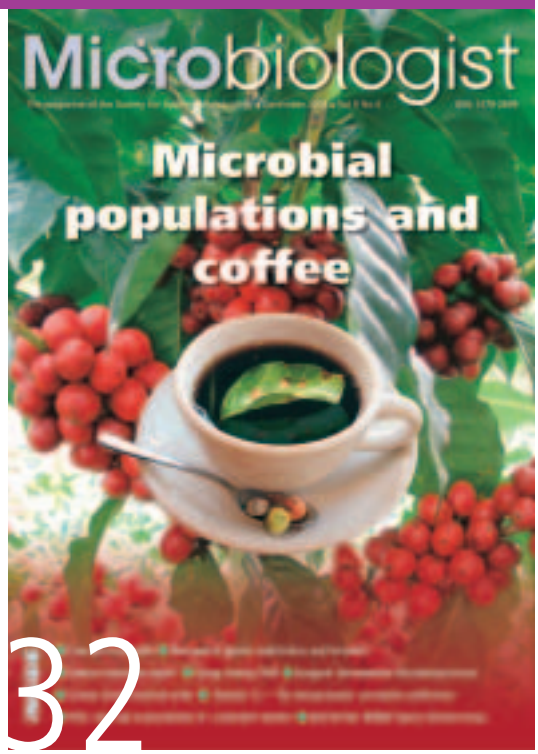

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Contributions: These are always welcome and should be addressed to the Editor at: lucy@sfam.org.uk

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

Design and print:
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Society for Applied Microbiology,
Bedford Heights, Brickhill Drive, Bedford
MK41 7PH, UK

Tel: +44 (0)1234 326661. Fax: +44 (0)1234 326678

www.sfam.org.uk

editorial

Lucy Harper has some great news for coffee drinkers

The theme of this issue of *Microbiologist* is drink. The first of our feature articles looks at one of the world's commodities — coffee.

Anyone who knows me will know that I can't get through the morning without at least one cup of coffee, and I'm sure I'm not alone. A recent study which followed the coffee drinking habits of 129,000 men and women over a period of twenty years, found that those who drank four or five cups a day were less likely to die of heart disease. Looking at men and women as separate groups, the study found that women who drank four or five cups of coffee per day were 34% less likely to die of heart disease (heart attacks, stroke or arrhythmia) and – after other factors such as smoking and obesity had been taken into account, 26% less likely to die of any cause. And the news is even better for men: the study found that men who drank more than five cups per day were 44% less likely to die of any cause and after other factors such as smoking and obesity had been taken into account, they were found to be 35% less likely to die. This is great news for coffee drinkers - according to this study, the more coffee you drink, the lower your risk of mortality (http://www.newscientist.com/channel/being-human/drugs-alcohol/dn14151-guzzling-coffee-may-cut-heart-disease.html?feedId=drugs-alcohol_rss20).

But did you know the many roles that microorganisms play in the production of coffee? Well in this issue of *Microbiologist*, you'll find out where the potential for microbial contamination of coffee lies at each stage of coffee processing, as well as the beneficial contribution microorganisms make towards coffee production (see page 32).

As well as coffee, we look back in time at the production of gin, beer and solvents (the latter of course not produced for human consumption) at Three Mills in London. The site at Three Mills has seen a varied an interesting history, from use as a distillery producing gin, to a solvent production plant and then much later, a film studio which was the site of the first series of the reality TV show, Big Brother! See page 36 to find out more.

The *Sf*AM Communications Award for 2009 is now open for nominations, so if you know someone you think has made a positive contribution to the public understanding of applied microbiology, then please send in your nomination (see page 11 for details). Also, for our younger members, turn to page 46 for details of how you could become more involved in the society through the postgraduate and early career scientist committee (PECS).

Finally, all that is left for me to do is wish you all a wonderful festive season and happy and healthy 2009!

Lucy Harper

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**

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

contact point

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

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

society office staff

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

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

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

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

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

executive committee

COMMITTEE MEMBERS 2006 - 2008

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

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

HON MEETINGS SECRETARY: Professor Martin Adams, School of Biomedical & Molecular Sciences, University of Surrey,
Guildford,Surrey GU2 7XH
email: m.adams@surrey.ac.uk

HON TREASURER: Professor Valerie Edwards-Jones, Research Development Unit, Manchester Metropolitan University,
Lower Chatham St, Manchester M15 5HA
email: v.e.jones@mmu.ac.uk

ORDINARY COMMITTEE MEMBERS UNTIL JULY 2009

Professor Carol Phillips, School of Health, The University of Northampton, Boughton Green Road, Northampton NN2 7AL
email: Carol.Phillips@northampton.ac.uk

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

Professor Joanna Verran, Manchester Metropolitan University, Dept Biological Sciences, Chester Street, Manchester M1 5GD
email: j.verran@mmu.ac.uk

ORDINARY COMMITTEE MEMBERS UNTIL JULY 2010

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

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

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

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

ORDINARY COMMITTEE MEMBERS UNTIL JULY 2011

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

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

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:

- Access to our four peer-reviewed Journals: *Journal of Applied Microbiology*, *Letters in Applied Microbiology*, *Environmental Microbiology* 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 73 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
- Eligibility to nominate a candidate for the SfAM Communications Award
- Fostering cross disciplinary research
- A 25% discount on the extensive Wiley-Blackwell collection of titles

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

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

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

JOURNALS: The Society publishes two monthly journals: *Journal of Applied Microbiology* and *Letters in Applied Microbiology*. We also produce this quarterly colour magazine, *Microbiologist*, which contains features, topical news stories and full details of our meetings. The Society is also a partner with Wiley-Blackwell in the monthly journal *Environmental Microbiology* and launched a new online journal in 2008; *Microbial Biotechnology*.

Wiley InterScience® is an online service provided by Wiley-Blackwell that gives Full and Student Members FREE access to the online versions of the Society's four journals: *Journal of Applied Microbiology*, *Letters in Applied Microbiology*, *Environmental Microbiology* and *Microbial Biotechnology*. Members can also submit papers directly to our journals via an online submission service. For more information about Wiley InterScience® or online manuscript submission, please visit **http://www3.interscience.wiley.com/cgi-bin/home.**

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. We also hold joint ventures with other organisations on topics of mutual interest.

WEBSITE: The website is the best source of detailed information on the Society and it's many activities. It has fully interactive membership areas where you can find archive issues of *Microbiologist* and 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* and *Microbial Biotechnology* for 2008, copies of *Microbiologist*, preferential registration rates at Society meetings and access to the members areas of the website.

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

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

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

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

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

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

JOIN US!

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

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

www.sfam.org.uk

microbreak

six word story winner

In the September issue of *Microbiologist* we invited all you budding writers out there to write a story in six words but with a microbiological link or theme.

Our inspiration was Ernest Hemmingway, who, it was said in the 1920's, bet that he couldn't write a complete story in just six words. He won the bet.

Thank you to all who entered. This proved an extremely popular competition and really seemed to capture your collective imagination. I'm delighted to announce that the winner of is **John Quinn** of Queens University, Belfast with his entry:

"Young Fleming's aseptic technique worried us..."

In the eyes of the judges this did indeed tell a very pertinent and important story in the history of microbiology.

Congratulations to John who will receive a £30 amazon voucher for his efforts.

The ten best entries will appear on the S*f*AM website so visit **www.sfam.org.uk/news** to see more imaginative and entertaining six word stories.

mailbox

write to: **lucy@sfam.org.uk**

subject: President's Fund enables opportunity
from: Victoria Davenport

I would like to thank the Society for Applied Microbiology for funding my attendance at the international Pathenogenic *Neisseria* Conference (IPNC) in Rotterdam this September.

My PhD student, Liku, gave a very polished presentation at this international forum which led to some interesting comments and further collaboration.

The value of attending such events can never be truly estimated, but I believe it has put me as an independent researcher, and UWE as a research organisation, on the international *Neisseria* map. Attendance has enabled dissemination of our novel and exciting research, which I trust can only have a positive impact on future grant applications. Thank you very much for enabling this opportunity.

subject: Praise for online lecture broadcast
from: Clifford Simmons

Yesterday afternoon I spent an hour watching the online broadcast of the S*f*AM/Wiley-Blackwell annual *Environmental Microbiology* lecture by Professor Rita Colwell.

At the end, quite apart from the excellent quality and interest of the lecture itself, I was stunned and sat for some time in waves of admiration for the innovative and enterprising efforts of the involved members of our Society in arranging, recording and distributing the video to the general membership. The whole idea and presentation was very much in keeping with the superior quality, preparation and presentation of our monthly journal which really is a one-off quality product amongst such publications.

What a genuinely super Society we have! I truly feel privileged to be a member!

I don't know who was responsible for the original idea, for the recording itself, or for arranging the ultimate distribution to members, or the involvement of Wiley-Blackwell personnel in all these arrangements, but please convey my real appreciation of a great and accomplished effort to all involved. I have such admiration for all their endeavours.

As a retired member, now in my eighties, I seldom make it the more outlying venues of the annual conferences, but I do still enjoy attending the Winter meetings in central London.

In the meantime, may I ask, is this the first such online broadcast? Are there more video recordings of lectures planned for future email distribution?

Judging by this first one I have, they are such a joy and if there are more I would like to collect them to create a superb collection.

president's column

Our new president, **Geoff Hanlon** pays tribute to Dr Margaret Patterson

When I joined the *Society for Applied Bacteriology* (as it was then known) as a junior academic in the early 1980s I would never have anticipated that one day I would be writing a column addressing you as its President. It is a pleasure and an honour to be asked to act in this capacity and I thank those people who have put their trust in me.

Although you are reading this edition of the *Microbiologist* with Christmas approaching, the demands of deadlines mean that I am writing the article in September and this is the first opportunity I have had to pay tribute to the work of my predecessor Dr Margaret Patterson. During her three year presidency the Society has seen many changes, most notably its incorporation as a company limited by guarantee, whilst at the same time retaining charitable status. This major task has been seamlessly steered through to completion by Margaret and Phil Wheat and hopefully the impact on individual members should have been negligible.

External affairs activities have increased during her period of office due to, among other things, our involvement with the Biosciences Federation and our association with MedVetNet. Society communications systems are now much more streamlined with the website offering a variety of new features as well as the *Micropod* podcast initiative together with online membership and conference applications. We have seen a marked increase in the level of funding available to members in the form of grants and the addition of new grants such as the New Lecturer Research Grant and the Communications Award.

I won't go into all the details of Margaret's many other achievements whilst in office, suffice it to say the Society is now in as sound a position, both financially and administratively as it has ever been. At this time the global financial markets may be in a worryingly unstable state but fortunately for us, Society income has trebled in the last 10 years due mainly to the excellent health of our journals. Our reserves continue to grow and this financial security is reflected in an increase in Society activities. It is clearly now my job to make sure this upward momentum is maintained and with the support of excellent office staff and a great committee I have every confidence that this can be achieved.

One of my first tasks as President was to work with Phil Wheat on a committee away day to help define the strategic objectives of the Society for the next three years. The discussion was centred on three themes, namely; membership, communications and meetings. It is premature to expand on the detail of the outcomes of that meeting here as many of the useful points raised need further deliberation and refinement. However, I feel it might be of value to share some of the thoughts we had concerning meetings, particularly the main summer conference.

You will all be aware that our current Society has its origins as the *Society of Agricultural Bacteriologists* which was formed in 1931 by a group of dairy microbiologists. Since then the range of members' microbiological interests has broadened considerably to embrace food, water, environmental, clinical, pharmaceutical and so on. We can genuinely claim to be *the* Society for Applied Microbiology and I am keen to ensure that all members feel that their Society adequately represents their interests. Herein lies the problem since if we hold a summer conference — say on the theme of water microbiology (as we did in Belfast this year) — then no matter how successful the event there will inevitably be a substantial proportion of the membership who feel disenfranchised. We have, of course, been aware of this for some time and in order to try and address the problem our winter meeting now runs as a one day event with two separate strands while the recently introduced and highly successful spring meeting is specifically geared towards the clinical/biomedical microbiology area. In addition, this autumn many of you attended the first of what we hope will be an annual *Environmental Microbiology* lecture (see page 30).

The summer conference is however, our flagship event which we hope gives an opportunity for all members to attend, meet with old friends and find inspiration in a relevant, good quality scientific programme. One item under consideration is that instead of having a single topic conference we run this meeting with a number of different themes reflecting the diversity of members' interests. Maybe popular topics such as food microbiology could run each year and perhaps we should have an enhanced social programme. I would be grateful for your opinions on this subject as this is your Society and your views are vital in shaping our future direction. Please feel free to contact me directly on g.w.hanlon@brighton.ac.uk.

One of the aspirations of the *Society of Agricultural Bacteriologists* back in 1931 was to be a *friendly* group of like-minded individuals who would meet to enjoy each others' company. Despite all the many transitions over the years we have always been known as a very friendly Society and as we continue to grow we must hold onto that original objective as our main focus.

I am looking forward very much to the three years of my Presidency and hope that I will get the opportunity to meet with many of you at the various events the Society now offers. All that remains is for me to wish you a very joyful and peaceful Christmas and a happy New Year.

Professor Geoff Hanlon
President of the Society

ceo's column

Philip Wheat reports on the latest developments within the Society

This time of year is the time to reflect on events of the past 12 months. Once again 2008 has been a very busy, successful year for the Society. We have been involved in many activities, some well established events and meetings, as well as some exciting new initiatives. The year began with a well-attended one day meeting at the Royal Society, London on 9th January. This meeting covered the topics of quality assurance and the microbiology of alcoholic beverages. The 2009 winter meeting will be held once again at the Royal Society on the 14th January and will cover Enterobacteriaceae in foods and rapid methods in microbiology. The meeting will be jointly sponsored by the International Life Sciences Institute (see page 25).

The next event in 2008 was the 2nd broadening microbiology horizons in biomedical science meeting held at Aston University, Birmingham, UK. The event was oversubscribed so if you are thinking of attending the 2009 event, which is to be held on 22nd April at Aston University (See page 28 for the full programme), I would strongly recommend you reserve your place as soon as possible.

The third meeting of the year was the summer conference which this year was held in Belfast. The meeting topic was water microbiology and the event was attended by leading scientists in the field (see page 20 for a full report of this meeting). This event also saw the presentation of the very first *Sf*AM Communications Award. Next years' summer conference will be held at Manchester Metropolitan University from the 6-9th July 2009. The title for the meeting is "fur, feather and fever – zoonotic challenges of the 21st century" (see page 28 for a preview of the programme). I do expect this meeting to be popular so early booking is once again recommended. In addition, student members should apply early for studentships to attend the meeting. Full members (who have paid at least two subscription fees) might also like to apply to the President's Fund to enable them to attend. Full details of studentships and President Fund can be found on our website (www.sfam.org.uk/grants.php).

The final event for 2008 was the inaugural *Environmental Microbiology* lecture which was held at the Royal Society of Medicine, London. The lecture was entitled *"climate, oceans, global warming, and cholera"* and was given by Dr Rita Colwell, distinguished Professor at the Universities of Maryland and John Hopkins, USA.

The event was attended by over one hundred members and invited guests. In addition, within 48 hours the event was also available online (see page 30 for a report of this lecture). It is pleasing to report that within three weeks of the event being available in this format, the site had over 1000 downloads. Planning is already under way for the 2009 event so watch out for details in forthcoming issues of *Microbiologist*.

As well as these events another challenge during 2008 has been the change in status of the Society to become a new charity and company limited by guarantee.

I have personally enjoyed meeting with new and existing members at all the exhibitions we have attended during the year. The Society is definitely seeing a rise in the number of people joining and we are also seeing a reduction in the number of members that decide not to continue their membership. This is good news for the Society and it's membership alike.

After looking behind us, it's now time for me to wish you all a restful and prosperous time over the Christmas and New Year period. Looking forward, I hope to see you at a society event in 2009!

Philp Wheat
Chief Executive Officer

In Memoriam: Professor Peter Gilbert

Photograph by kind courtesy of Manchester University

Peter Gilbert passed away on Monday the 18th August 2008. Peter was a larger than life character who was Professor of Microbial Physiology in the School of Pharmacy and Pharmaceutical Sciences at Manchester University, where he had been a member of staff since 1978. He was a loving father, husband and caring member of the academic staff at Manchester where he cared passionately about his subject and university education. As an academic, Peter was second to none. His name was synonymous with the fields of biocide mode of action and biofilms. Indeed, he was more often than not the first name on the list of potential speakers for any conference organiser. We will sorely miss his engaging, dynamic, sometimes provocative but always thought provoking talks.

During his career he authored more than 170 research papers, 80 review articles and tutored over 70 PhD students. He also organised 10 national and international meetings himself and he served on the S*f*AM committee where he made a valuable contribution to the activities of the Society.

Despite his outwardly gruff protestations, he always had students' and colleagues best interests at heart. There has been many an occasion over the years when he went out of his way to guide a student through a crisis or provide invaluable fatherly advice, as no doubt many will testify. He had a wicked sense of humour and was a genuine character who will be sadly missed by his friends and colleagues.

Peter is survived by his wife, Diane, and three children, Jessica, Sarah and David, and two children from his first marriage, Andrew and Helena.

We would like to thank **David Allison** and **Andrew McBain** for their assistance with this obituary

membership matters

S*f*AM member appointed new Chief Executive of VLA

We would like to extend our congratulations to Professor **Peter Borriello** who has been appointed Chief Executive of the Veterinary Laboratories Agency (VLA). He took up this position on 1 October 2008 following the retirement of Professor Steve Edwards at the end of September.

Like many organisations operating in the animal health market, VLA faces a number of challenges in the coming years. Peter will bring with him a wealth of experience from the public health arena to help address these challenges.

SfAM Communications Award call for nominations!

The S*f*AM **Communications Award** aims to recognise individuals who have communicated their work/applied microbiology to the general public.

The overall aim of this award is to raise the profile of applied microbiology and S*f*AM. The award will be for £1000 and nominations must be from Full Ordinary or Student members with a deadline in April each year. Nominations should be in writing, providing detailed information about all relevant media/communications work of the nominee. Nominations should be made by members of S*f*AM but nominees do not have to be members of the Society. Nominees could include:

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

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

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

The closing date for applications is 10 April 2009.

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

Membership Changes

NEW MEMBERS

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

Australia

V Green; E Kokkoris

France

M Binet

Nigeria

H Goodluck; E Ugoji

Norway

G S Johannessen

Rwanda

A Mbabazi

South Africa

T-L Botes; P Venter

United Kingdom

A Ashcroft; D Bean; J Cieniewska; S Cutting; N C Elviss; N Fairweather; H E Gordon; S C Grace Rachel; K Grant; F Ituah; D W Jones; L Lasecka; P X Marques; D Nnane; A Okechuku; O Olaoye; B Panchal; M Patterson; S Pranav; L Thomas; N R Williamson

USA

J Kinzelman; A Rodriguez-Palacios; L Santiago-Connelly

LOSSES

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

Professor Peter Gilbert; Full Ordinary Member since 1977

Mr J A Stott; Retired Member; joined 1968

Sponsor a new Member and win a £50 Voucher of your choice!

If you feel you could be our next winner for 2009, and would like some promotional material to help you recruit new members please contact Julie Wright, Membership Co-ordinator on 01234 326661 or email julie@sfam.org.uk.

SfAM welcome new committee members

Christine Dodd

My first degree was a BSc in Biological Sciences at the University of Leicester and I continued at Leicester for my PhD in Microbiology in the newly formed Microbiology Department. After research positions in the universities of Durham and Newcastle upon Tyne, I came as a post-doc to the University of Nottingham in 1985, joining a newly revived Food Microbiology group in the then Faculty of Agriculture at Sutton Bonington. I was appointed as Lecturer in Food Microbiology in 1989 and after several promotions and site name changes was appointed Chair in Food Microbiology in the Division of Food Sciences in 2006.

My research work is centred on ensuring the safety of the food supply. This involves characterisation of microbial populations in food products and production environments at the species and sub-species levels, and examining the factors influencing their introduction and survival. I have had a long association with the Society and was previously a committee member from 1990-1993. I was the local organiser for several conferences and won the W H Pierce prize in 1993. I am looking forward to serving on Committee again.

Leon Gorris

I am from The Netherlands and obtained my PhD degree in Microbiology at the Catholic University of Nijmegen. After a post-doc at the same University, I joined the Agrotechnological Research Institute in Wageningen (NL) where I established the Food Safety & Applied Microbiology department, with research in areas such as mild food preservation, combined processing ('hurdle technology'), biological crop protection, detection of micro-organisms and health-aspects of bioactive natural compounds.

From there I joined Unilever in 1998, first heading the Microbiology & Preservation department at Unilever's Food R&D facility in Vlaardingen (NL). Since 2002, I have been with Unilever's Safety and Environmental Assurance Centre, which is in Sharnbrook and thus actually very close to SfAM headquarters in Bedford. I am currently a science area leader, responsible for continuously developing our skills and technologies in risk assessment, which we apply to product innovation and issue management.

Since 2003 I have held the European Chair in Food Safety Microbiology at University of Wageningen, which is a part-time (20%) professorship. I am a member of the International Commission on Microbiological Specifications for Foods (ICMSF), heading their delegation to the Codex Alimentarius committee for Food Hygiene and coordinating ICMSF's input to the various Codex committees. I have been working with a variety of organisations, for example, the International Life Science Institute (ILSI), the International Association for Food Protection (IAFP), FAO and WHO, on food safety management and the role of risk assessment therein. I have been a member of SfAM for many years and am very happy that I have been given a chance to serve on Committee.

journalWatch

News about the Society's journals

Journal of Applied Microbiology
2007 Impact Factor 2.501

The following articles published in 2008 were the most downloaded articles from Journal of Applied Microbiology between July-September 2008:

Inactivation mechanisms of lactic acid starter cultures preserved by drying processes. C. Santivarangkna, U. Kulozik, P. Foerst. **Vol. 105**, No. 1, July 2008

Summer meeting 2007 - the problems with fresh produce: an overview. M.P. Doyle, M.C. Erickson. **Vol. 105**, No. 2, August 2008

Novel alternatives to antibiotics: bacteriophages, bacterial cell wall hydrolases, and antimicrobial peptides. A. Parisien, B. Allain, J. Zhang, R. Mandeville, C.Q. Lan. **Vol. 104**, No. 1, January 2008

Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. G.T. Macfarlane, H. Steed, S. Macfarlane. **Vol. 104**, No. 2, February 2008

Isolation and partial characterization of a bacteriocin produced by *Pediococcus pentosaceus* K23-2 isolated from Kimchi. M.S. Shin, *et al.*, **Vol. 105**, No. 2, August 2008

Letters in Applied Microbiology
2007 Impact Factor 1.623

The following articles published in 2008 were the most downloaded articles from Letters in Applied Microbiology between July-September 2008:

Effect of lactic and citric acid on the stability of B-aflatoxins in extrusion-cooked sorghum. A. Méndez-Albores, *et al.*, **Vol, 47**, No. 1, July 2008

Evaluation of the PCR method for identification of *Bifidobacterium* species. S.Y. Youn, J.M. Seo, G.E. Ji. **Vol. 46**, No. 1, January 2008

Preliminary characterization of exopolysaccharides produced by a marine biofilm-forming bacterium *Pseudoalteromonas ruthenica* (SBT 033). P. Saravanan, S. Jayachandran. **Vol. 46**, No. 1, January 2008

Isolation and characterization of ethanol-producing yeasts from fruits and tree barks. R.S. Rao, B. Bhadra, S. Shivaji. **Vol. 47** No. 1, July 2008

Comparison of three DNA extraction methods for *Mycobacterium bovis, Mycobacterium tuberculosis* and *Mycobacterium avium*. A. Amaro, E. Duarte, A. Amado, H. Ferronha, A. Botelho. **Vol. 47**, No. 1, July 2008

Environmental Microbiology
2007 Impact Factor 4.929

The following articles published in 2008 were the most downloaded articles from Environmental Microbiology between July-September 2008:

It's all relative: ranking the diversity of aquatic bacterial communities. Allison K. Shaw, Aaron L. Halpern, Karen Beeson, Bao Tran, J. Craig Venter, Jennifer B. H. Martiny. **Vol. 10**, No. 9, September 2008

New feel for new phyla. Michael Y. Galperin. **Vol. 10**, No. 8, August 2008

Resource availability influences the diversity of a functional group of heterotrophic soil bacteria. Silke Langenheder, James I. Prosser. **Vol. 10**, No. 9, September 2008

Roles of type IV pili, flagellum-mediated motility and extracellular DNA in the formation of mature multicellular structures in *Pseudomonas aeruginosa* biofilms. Kim B. Barken, Sünje J. Pamp, Liang Yang, *et al.*, **Vol. 10**, No. 9, September 2008

The quest for biofuels fuels genome sequencing. Michael Y. Galperin. **Vol. 10**, No. 10, October 2008

Microbial Biotechnology
The fifth issue of Microbial Biotechnology is now freely available to download online. Visit: www.microbial biotech.com

Editor's Choice:

Microbial-based motor fuels: science and technology. Lawrence P. Wackett. **Vol. 1** Issue 3 Page 211-225, May 2008

Tracing explosives in soil with transcriptional regulators of *Pseudomonas putida* evolved for respond. Junkal Garmendia, Aitor de las Heras, *et al.*, **Vol. 1** Issue 3 Page 236-246, May 2008

PEGylation of bacteriophages increases blood circulation time and reduces T-helper type 1 immune res. Kwang-Pyo Kim, Jeong-Dan Cha, *et al.*, **Vol. 1** Issue 3 Page 247-257, May 2008

Environmental Microbiology Reports – a new journal for 2009!

From January 2009, *Environmental Microbiology* will no longer publish Brief Reports; instead, Brief Reports will be published in a new sister journal, *Environmental Microbiology Reports*. The new journal will be an online only journal dedicated to the rapid publication of important new findings that can be adequately documented by a limited amount of text and displays.

The journal will be identical in scope to *Environmental Microbiology*, will share the same editorial team and submission site, and will apply the same high level acceptance criteria. The two journals will be mutually supportive and evolve side-by-side. For more information, visit: www.env-micro.com

Lucy Collister
Wiley-Blackwell

BIOSCIENCES FEDERATION

bio **focus**

Richard Dyer introduces a new organisation to represent the biosciences

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

Prospectus for New Organisation to represent the biosciences — incorporating the Institute of Biology and the Biosciences Federation

This is a reduced version of the prospectus. The full version, which contains more information on the finance, structures and immediate goals of the New Organisation, is available from the BSF website

The excitement of modern biology is palpable to all on a daily basis. Uniquely important issues are frequently discussed by the media and the public. How many in this country are not aware of the debates about stem cells, loss of species through global warming and modern agriculture, the teaching of biology in schools or what our diet is doing to our bodies?

But how do the biologists join these debates about biology? If they do at all, it is through a myriad of possible routes and representing too many organisations. The need for a unified voice for all the biosciences has never been greater. Our vision is to provide that unified voice in debates about the development of policy and best practice in education, career development, legislation and the funding of research. In undertaking these roles, strong outreach to all the regions of the United Kingdom will provide strong local foci of relevant interest for all the membership including teachers, research scientists and regional organisations.

Background

The exciting diversity of the biosciences has led to the formation of very many special interest groups where scientists and others with shared interests and mission productively work together. There are, for example, scores of Learned Societies and Medical Research Charities, as well as many individuals with a passion for biology whether it is on a professional or personal basis. Although it is difficult to be precise about figures, these organisations alone probably comprise more than two hundred separate and independent organisations, while there may be tens of thousands more individuals, some with no affiliation. This landscape is unique for the biosciences: physics, chemistry, mathematics and engineering are represented by very few organisations, which are wealthy and influential. Whilst special interest groups undoubtedly bring advantages of focus to research or fundraising, the fragmentation of the biosciences leads to huge disadvantage in other areas – for example, in outreach to schools or in representing biology to Governments and Funders. In order to ameliorate this problem various groupings have come together under an umbrella organisation where matters of common interest can be dealt with more effectively than by a multiplicity of individual actions. But in the biosciences this increased effectiveness is diluted because there are several umbrella organisations (e.g. the Institute of Biology and Biosciences Federation) and therefore some of the problems associated with fragmentation remain.

Recently the Councils of the Institute of Biology (IoB) and the Biosciences Federation (BSF) proposed that a New Organisation (NO)

should be created that will embrace the activities and strengths of both BSF and IoB, and add new activities that will benefit UK biosciences and provide greater value to the membership. This proposal was unanimously endorsed by a joint meeting of the IoB and BSF at the Royal Society in May 2008.

Implementation group

In order to move this ambitious plan forward, the BSF and IoB have established a joint Implementation Group (IG) chaired by Prof Sir Brian Heap. Other members of the IG are Dame Bridget Ogilvie, Prof Dame Nancy Rothwell, Prof Malcolm Press, Prof Keith Gull, Prof David Coates, Mr Alan Johnston, Dr Alan Malcolm and Dr Richard Dyer. Members of the IG are unanimously agreed on the following key issues:

- Membership of NO will be open to both individuals and organisations from any sector of the biological sciences.
- The Council of the NO shall comprise a Chair and 12 members — four of whom will be elected by the individual membership, another four from the institutional membership and the final four shall be nominated by Council to ensure that there is a good balance of representation and that the Council is fit for the purposes expected of a Charity in the 21st century.
- The NO shall have a Royal Charter and continue to offer chartered status and Fellowship to individual members.

Currently the IG is preparing the papers that will need to be put to the memberships of BSF and IoB for the approvals necessary for these proposals to be implemented.

Finance and structures

The NO can be launched successfully with the present combined incomes of the BSF and IoB. In 2007 the corporate subscription income for BSF was £235k and the membership income for IoB was £860k. For 2009 the total subscription income for the two organisations will be circa £1200k. In addition, the IoB raises about £200k from other sources and the BSF about £100k per annum from member organisations for identified projects. This *à la carte* funding is an important element for future development.

A full Business Plan for NO was produced mid November 2008.

The IG is not focused on the detail of the structures that may be set up within NO: that will be a responsibility of the first Council and new Chief Executive. However, the IG is determined to suggest some overarching principles for the organisation of NO and in particular that it is a flat structure with the ability to make rapid decisions.

Immediate goals for NO

Membership

There will be an immediate drive to increase the number of individual and institutional members. Currently some large areas of the biosciences are poorly represented in both the BSF and IoB.

The NO will retain a Royal Charter and offer chartered status to its individual members. An immediate goal will be to strengthen the standing of this qualification by introducing a structured "Career and Professional Development" programme. The aim will be to increase the esteem of all qualifications, including Fellowship.

Outreach

Structured outreach to schools and the public more generally will be built through the regional groups that already exist in the IoB. There is considerable scope for exciting new ventures.

The NO will also focus on increasing outreach to the membership as a whole through regional scientific meetings and high quality lectures and debates. The leadership, both executive and non-executive, will ensure that the activities of NO are not solely based in London or SE England. Finally there is the prospect of substantially increasing the effectiveness of outreach through the media – both directly and by partnering with the Science Media Centre.

Policy Work

Both BSF and IoB have been largely reactive to policy initiatives coming from Governments and Funders. Although these responses are important it is equally important to be proactive in order to set the national agenda. In this context national means UK as a whole and the four countries of the union.

Why now?

Both IoB and BSF could do many of the activities that are proposed for NO. But in undertaking these activities IoB and BSF would not engage all the heartlands of the biosciences. To meet the challenges of today, biology needs a single voice. Our subject needs an organisation like the Royal Society of Chemistry or the Institute of Physics which has the respect of the community as a whole and where individuals are proud to be members because of the standards maintained and the quality of project delivery. With your support NO can achieve this status rapidly: this is urgently needed and the current opportunity must be embraced

Richard Dyer
Chief Executive
Biosciences Federation

Genetic modification

Bernard Dixon OBE re-examines GM ten years after it first erupted in the media

mediawatch

microbiology in the news

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

our policy on the media

We will:

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

It's now around ten years since controversy erupted in Britain over genetically modified (GM) food. Following allegations about the danger of eating GM crops, and then about cultivating them, sensational headlines precipitated nationwide hysteria.

Until that time, GM tomato puree and cheese made using recombinant chymosin had both been selling well in supermarkets. But overnight, in response to the furore rather than any real evidence of hazard, retailers and wholesalers backed away, assuring the public that their products were GM-free. *"We are corrupting nature,"* wrote the Chairman of Iceland, the first supermarket to renounce GM, *"and we have sufficient evidence to show that nature fights back — salmonella, listeria, BSE"* (Walker, 1997).

A decade later, it is instructive to re-examine the whole episode — not least because evidence has never been published to support the original claims of toxicity that triggered the frenzy. However, in contrast to the UK, GM crops have long been cultivated and eaten elsewhere around the world. Over a billion acres have been planted in the USA since 1996.

Although the saga has not been primarily microbiological, it has contained microbiological elements, and it certainly holds lessons for microbiologists working in areas with the potential to trigger public, media and political controversy. Chief among these are the twin errors of assuming that benefits of novel technology do not require advocacy because they are self-evident, and of believing that allegations of danger, however ill founded, can be ignored. A third mistake is for proponents of a new technology, aghast at the antics of opponents, to go to the other extreme, exaggerating its benefits and dismissing all criticism out of hand.

Anxieties about GM appeared sporadically in the UK media from the early 1980s onwards, perhaps because this single issue acted as a lightning conductor for three wider concerns — about intensive agriculture, the power of multinational companies and scientists "interfering with nature" or "playing God". Other key factors were the UK outbreak of bovine spongiform encephalopathy (BSE) in the late 1980s, its link with human variant Creutzfeldt-Jacob disease (CJD), and then serious outbreaks of *Escherichia coli* 0157 infection in Scotland. Although nothing to do with genetic modification, these developments fostered public distrust of food scientists and were often coupled together in media discussion of GM.

It was, however, a *World in Action* television programme in August 1998 that precipitated the nationwide furore. The programme mentioned experiments by the distinguished lectin expert Arpad Pusztai at the Rowett Research Institute, Aberdeen, which had apparently demonstrated that when rats ate GM potatoes their growth and immune system were impaired. Subsequent press coverage indicated that Pusztai had inserted into the potatoes a gene coding for an insecticidal lectin, though details were unclear. On the programme, Pusztai stated that he would not eat any GM food, at least until it had survived similar tests.

Regrettably, Pusztai's results were preliminary and unpublished. Moreover, even if the very worst interpretation were placed on the work, it could hardly justify the condemnation of *all* GM food. Discoveries of potentially useful but toxic antibiotics have never been taken to damn antibiotics in general.

Some scientists have blamed the media solely for GM hysteria. Journalists were certainly responsible for headlines such a "Mutant crops could kill you" which heralded anti-GM campaigning by several newspapers.

An especially significant headline was "Smeared GM expert vindicated" (Lean, 1999) which greeted publication of a paper by Pusztai and a collaborator a year after the TV programme (Ewen & Pusztai, 1999). Most reporters missed or ignored the fact that the paper did not actually support Pusztai's original claims. It was about something quite different: the microstructure of the small intestine. The editor of The *Lancet* unwisely decided to publish this paper, against peer review advice, not because he believed the findings were generalizable, but simply to place them in the public domain.

Even the British Medical Association was swept along by the hysteria. It issued a report (BMA, 1999) concluding that "transgenic products may adversely affect people suffering from allergies." Amplified through the media, this assertion led to the spectre of a huge rise in unexpected allergies if GM foods were widely consumed.

Yet the single reference which the BMA used to back its claim was to a paper (Nordlee *et al.*, 1996) by researchers who had introduced the 2S albumin gene from Brazil nuts into soya beans. They found that an immunoglobulin in serum from eight of nine individuals, already known to be allergic to Brazil nuts, bound to proteins of similar molecular mass extracted from the beans. Three out of four of the subjects showed positive skin prick reactions.

Thus a screening test on a well-recognized allergen, carried out specifically to exclude hazards of this sort, was transformed in the public mind into the threat of unforeseen allergies lurking in our food. The BMA did not point out that one of the most valuable potential applications of genetic modification to plants is to render them safe by deleting allergen genes.

Meanwhile, the focus of the GM furore was shifting from alleged food toxicity to alleged environmental damage. The key event here occurred in May 1999 when newspapers carried large coloured photographs of a monarch butterfly. Headlines were typified by "GM pollen that can mean a cloud of death for butterflies" (Derbyshire, 1999), implying that GM plants in general threatened butterflies in general.

The articles stemmed from the appearance of a brief report (Losey *et al.*, 1999) claiming that monarch larvae, placed on leaves dusted with pollen from corn modified genetically to produce *Bacillus thuringiensis* (*Bt*) toxin, ate less, grew more slowly and suffered higher mortality than those from leaves dusted with untransformed corn pollen. The authors said these effects were probably attributable to *Bt* toxin in the pollen, and that this threatened monarch butterflies

within range of pollen from the US Corn Belt.

However, critics pointed out that these laboratory findings could not be extrapolated to field conditions, while the paper itself was seriously incomplete. Crucially, the authors did not compare the GM pollen with *Bt* toxin itself nor with other widely used pesticides which transgenic plants are intended to replace.

In contrast to a decade's research that has still failed to demonstrate any toxicological risks of GM products, there are conceivable dangers in cultivating plants with transgenes that might spread elsewhere and be expressed in other plants. But farmers already have a well-established method of raising crop varieties whose pollen grains must not be allowed to reach certain compatible relatives. Even when there are considerable real dangers (not conjectural ones), they simply grow the plants far enough apart to prevent cross-fertilisation (Royal Society, 1998). Thus industrial grade oilseed rape, which contains a substance (erucic acid) toxic to humans, is planted distant from varieties for human consumption.

The other allegation against GM crops on environmental grounds is that they threaten biodiversity. This has not been supported by field tests. Farm-scale trials in the UK demonstrated reductions of 60-80% in weed biomass in the presence of GM herbicide-tolerant sugar beet and spring canola, but an increase of 82% with herbicide-tolerant maize (Freckleton *et al.*, 2003). But these trials did not deliver a verdict on GM in general. They simply confirmed that weed killers kill weeds. The principal investigator said that he would have expected the same results with conventional, non-GM herbicide-resistant varieties (Firbank, 2003).

Unfortunately, as attitudes have hardened over the past decade, rejection of GM food on environmental or toxicological grounds has become less important than rejection *per se*. Like radioactivity, the epithet GM has been demonised for its own sake, as something inherently odious. I have myself spoken to people so deeply influenced by this idea that they could scarcely believe that GM microorganisms have been used for many years to make life-saving drugs such as interferons, human insulin and erythropoietin.

Things could have been very different, if the proponents of GM plants had, back in the 1970s and early 80s, publicised their potential agricultural, environmental, nutritional and medical benefits, and anticipated and addressed public concerns. A vivid example of the latter was the use of antibiotic resistance genes as markers in the transgenic process (Goldstein *et al.*, 2005) and the possibility that they might move from plants to pathogens. The researchers who developed this technique presumably had no idea that it could become a significant factor in the clamour against GM food. It was simply a convenient laboratory technique.

In April 1988, the First International Conference on the Release of Genetically Engineered Organisms (REGEM 1) took place in Cardiff. Despite its title, the meeting dealt with plants too, and was designed not just as a scientific conference but also as an event with unusually broad media participation and with a public interface in the form of booklets written on both molecular biological and ecological aspects of GM. Planned in recognition of the fact that novel technology can proceed only to the extent that it enjoys public trust, it was seen as the first of a series of such meetings, intended to discuss GM in a transparent way as research proceeded and new possible applications emerged.

REGEM-1 did not, however, set the mould for future conferences. Although highly successful, the initiative was a one-off exercise. The organisers felt their job was done, and did not require repetition. They were wrong, weren't they?

further reading

■ Bernard Dixon's book; *Power Unseen — How Microbes Rule the World*, consisting of vignettes of 75 different micro-organisms, with accounts of the many ways in which they affect human affairs and the biosphere, is now available as a print-on-demand book after being out-of-print for several years. It is published by PFD and can be obtained through Amazon.com.

His latest book, *Animalcules*, will be conventionally published by the American Society for Microbiology in December 2009

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Bernard Dixon OBE
Bernard Dixon is the former editor of *New Scientist* and a regular contributor to *Lancet Infectious Diseases*

Med-Vet-Net Wildlife related Emerging Diseases and Zoonoses (WiREDZ) Special Interest Group

With the increasing realization that wild species are significant reservoirs for both zoonotic disease and new and emerging diseases, surveillance in wildlife has become even more important. Wild animals are increasingly recognized as hosts and vectors for zoonoses and new and emerging diseases. Global examples such as Nipah, Ebola and West Nile Virus and European examples such as rabies, Avian Influenza Virus and Hantavirus appear regularly in the press and are of concern to both scientists and to the person on the street.

The importance of wildlife in the evolution and transmission of zoonotic diseases has been recognized by Med-Vet-Net, and with their support a Special Interest Group (SIG) was set up to discuss wildlife-related zoonotic and new and emerging diseases. New and emerging diseases of wildlife must be considered as potential zoonoses, as this is how they are first assessed. The SIG title **WiREDZ** reflects this scope: **W**ildlife **R**elated **E**merging **D**iseases and **Z**oonoses.

This Special Interest Group covers any WiREDZ of relevance to the European context but the overall aim is to look for changes in disease epidemiology, and changes in zoonotic potential. Preliminary discussions among the SIG project group occurred at a conference in the UK (Central Science Laboratory) in November 2007. Subsequently, the objectives of the WiREDZ SIG were discussed at the Med-Vet-Net Lyssavirus Special Interest Group meeting held in Madrid, December 2007. As a result of discussions the following objectives and tasks were considered:

1. to provide a visible contact platform for institutes and researchers working on WiREDZ; and
2. to provide a think tank and a discussion platform for future research and collaborative projects.

WILDLIST contact database: your chance to collaborate

The primary objective of WiREDZ is to develop a register of people working on WiREDZ throughout Europe – this is the Med-Vet-Net WILDLIST. This will help collaboration, and link those who are working with specific wild species (mammals, birds, vertebrates) and those groups working on specific wildlife diseases throughout Europe. The Special Interest Group and WILDLIST will have good links to other projects and organizations.

If you are working on diseases of any wild species in Europe, even if this is only part of your job, we kindly ask you to log just six simple pieces of data on to the web-based WILDLIST: http://www.medvetnet.org/wiredzreg

1. Your name
2. Your email address
3. Your institute
4. Your institute's email address
5. The wild species you work with, e.g. bats, rodents, deer, ungulates, foxes, rabbits etc.
6. The wildlife diseases you work with, e.g. rabies, *Salmonella*, brucella, vector-borne diseases, West Nile fever, Flaviviruses.

In time, this website will be developed but our principal priority is to connect workers throughout Europe with others that are working in the field of wildlife disease.

Your email address will be secure, because it will not be displayed on the web: any enquiries will be made through a web form and only genuine messages forwarded to you.

Please could you pass this on to your colleagues who also work with wildlife diseases.

Discussion platform and think tank

A SIG meeting is planned for December 2008 in Budapest. The meeting budget should cover attendance by approximately 20 delegates, with a small number of places for self-supporting delegates (up to a maximum of 30 delegates in total). The intention is to invite several delegates from countries in Europe where little is currently known about systems in place (if any) for wildlife emerging disease and zoonosis surveillance. This meeting will provide an opportunity to discuss WiREDZ work in European countries, particularly those countries where systems and approaches to WiREDZ work are not currently known. At this meeting we wish to promote involvement of all European countries. There will be short workshops on three important WiREDZ which we believe most European countries monitor in wild species: rabies, Hantavirus and tularaemia.

Teresa Belcher
Communications Director
Med-Vet-Net

med-vet-net

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

information

For more information about Met-Vet-Net, visit:
www.medvetnet.org/
or contact Teresa Belcher on:
+44 (0)1908 698810

For more information about WiREDZ, please visit:
http://www.medvetnet.org/wiredz
or contact Dr Paul Duff, Veterinary Laboratories Agency, UK or Dolores Gavier-Widen, SVA, Sweden: email:
info@medvetnet.org

information

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

You can also find details of this years' Summer Conference on page 28 of this issue of *Microbiologist*

Summer Conference 2008 Report

The microbiology of water in work rest and play

Wellington Park Hotel, Belfast, Monday 7 to Thursday 10 July 2008

Another successful S*f*AM summer conference took place at the Wellington Park hotel, Belfast from 7 – 10 July 2008. The meeting kicked off with the Lewis B Perry memorial lecture presented by Dr Bernard Dixon OBE who discussed the UK GM controversy (see page 16). The theme of water was unfortunately reflected by the weather which persisted all week. But this didn't dampen spirits and delegates enjoyed the scientific sessions on recreational waters, industrial and commercial uses of water, management of contaminated water and potable water. The social programme was also enjoyed by all and concluded with an excellent meal at Stormont which was topped off with an after dinner speech from the first winner of the S*f*AM Communications Award, Professor Richard James of Nottingham University (see *Microbiologist*, Vol. 9, No. 3 page 17).

Recreational waters

The recreational waters session was opened by Dr Julie Kinzelman of the Racine Health Department, Wisconsin, USA who presented an overview of the role of recreational water in human disease. In her presentation, Dr Kinzelman described the significant burden of disease associated with recreational water use and highlighted the substantial financial burden to the nation. The factors influencing the health risk to individuals include the nature of the hazard, the characteristics associated with the water body i.e. are conditions right for the persistence and or growth of pathogens, the immune status of the user and the type of exposure. She also highlighted the extensive range of diseases associated with recreational water use including gastroenteritis, acute febrile respiratory illness (ARFI), ear and eye infections, skin rashes, meningoencephalitis and renal disease. A particularly thought-provoking fact Dr Kinzelman shared with us is that *"on average a person has 0.14 grams of faeces with them at all times" and that "swimming is essentially communal bathing"* leaving the audience considering *"would they dare go for a dip?"*

Next, Dr Adam Fraise from University Hospital Birmingham presented an overview of the risks associated with communal bathing and hydrotherapy pools. He began by describing an intriguing outbreak of multi resistant *Pseudomonas aeruginosa* infection in a paediatric oncology unit in which molecular typing demonstrated that the likely source was contaminated bath toys. He then moved on to describe an outbreak of *E. coli* O157 associated with a swimming pool which resulted in eight confirmed cases and three hospital admissions. The causes of the outbreak were determined as a faulty pump and lowered chlorine levels in the water. The free living amoeba *Naegleria fowleri* which

occurs in soil, lakes & rivers and proliferates at high temperatures (45°C) can cause fatal meningitis. Infection is often associated with a history of swimming in warm water with symptoms occurring two-to-five days after exposure although the incubation period can be up to two weeks. Symptoms include fever, anorexia, headache, vomiting, and most patients die within one week of onset. Interestingly, this organism was found to be colonising the roman baths at Bath and led to the closure of the baths to bathers to prevent exposure to this most unpleasant pathogen. Dr Fraise also highlighted the potential risks associated with hot spas and described an outbreak of Legionella in the spa pool of a leisure club which resulted in 78 confirmed cases and several hospital admissions due to pneumonia.

The risks of recreational exposure to *Leptospira* were described by Dr Jarlath E. Nally of University College Dublin. His presentation began with a description of the history of the infection and a reminder that "leptospirosis is considered to be the most widespread zoonotic disease in the world". Acute infection in humans occurs 5-10 days after exposure and symptoms reflect the systemic dissemination of the spirochete, ranging from a mild febrile illness to the more severe icteric Weil's disease, characterized by renal and liver failure. He then described several large outbreaks of disease associated with recreational water use in athletic competitions including triathlons and kayaking. In closing, Dr Nally reminded us that the disease is preventable by limiting exposure by avoiding contaminated water, wearing protective clothing, covering skin abrasions and always being aware of the risks of leptospirosis.

Professor James Oliver from the University of North Carolina at Charlotte then introduced us to *"Vibrio vulnificus: a Killer Lurking on Our Beaches." V. vulnificus* causes 95% of all seafood-related deaths in the United States and results in the highest rate of hospitalization and highest fatality rate (50%) of any food-borne pathogen. Even wound infections result in a 25% fatality rate which means *V. vulnificus* certainly does deserve the moniker *"a killer lurking on our beaches."* The majority of infections are associated with ingesting raw or undercooked oysters (85%) which lead to primary septicaemia, however secondary tissue lesions also occur following ingestion. The importance of the administration of antibiotics is crucial to treatment and there is a strong correlation between death and the late administration (>72 h) of antibiotic therapy. The organism also has a second portal of entry and is typically acquired when *"removing shrimp shells, stepping on a crab, a finfish puncture, falling on a pier."* The primary lesions rapidly progress to septicaemia, with death occurring on the second day. The organism demonstrates a very distinct seasonality with a summer peak in human infections and a peak in the isolation of the organism from oysters and sea water. This is thought to occur through the viable but non-culturable (VBNC) response which Professor Oliver described as *"a cell which fails to grow on the routine bacteriological media on which it would normally grow and develop into a colony, but which is in fact alive and able to return to a metabolically active and culturable state."* In closing, Professor Oliver hypothesised *"if temperature is so critical to the presence of V. vulnificus, and occurrence of disease, will increases in water temperature lead to increased incidence of infection?"*

He then highlighted a study in Denmark describing the isolation of *V. vulnificus* from seawater during an unusually warm summer in Denmark in 1994, when 11 clinical cases of infection were reported and went on to discuss a second study in Israel describing the possible role of climate change in the emergence of *V. vulnificus* infection. If his hypothesis is correct we may see a dramatic increase in incidence of disease attributed to this organism due to climate change.

The final speaker of this session was Professor Joseph O. Falkinham, III of Virginia Tech who presented *"Surrounded by mycobacteria."* In his presentation Professor Falkinham described the environmental opportunist mycobacteria which are either slow growing such as *M. avium* and *M. intracellulare* or rapid growing such as *M. abscesus* and *M. chelonae*. He then went on to describe some of the risk factors for mycobacterial infection such as reduced immunocompetence through HIV infection, malignancy, or chemotherapy and cystic fibrosis or gastric reflux disease. In particular he highlighted the methods for the diagnosis and treatment of mycobacterial infection and possible sources of environmental opportunistic mycobacteria.

Andrew Sails

Industrial / commercial uses of water

There are many issues relating to the use of water in industrial or commercial settings in terms of microbial loading and the entry of pathogens into the human food chain. This session comprised of talks on a range of subjects: from disease in Koi carp to diseases in humans. Keith Way outlined some issues faced by the aquaculture industry regarding the emergence of fish disease through the use of recycled warm water from industry. If, as the climatologists predict, the climate becomes warmer, these issues may become more general, affecting the industry and therefore the fresh water fish supply in the future. The emergence of antimicrobial resistance as a result of the use of antibiotics within the food chain is a 'hot topic'. Peter Smith from NUI Galway presented data suggesting that there is an increased rate of resistance in the aquatic microbial flora which is related to the use of antibiotics in fish farming. He told us that the issues relating to this were more complex than have been previously considered.

The quality of food in both the food and pharmaceutical industries is a vital consideration. In the food industry the quantitative detection of pathogens is important in terms of food safety control measures and their subsequent evaluation. Norovirus, one of the most frequent causes of food-related gastro-enteritis, most often related to consumption of shell-fish, has until recently been more difficult to quantify than bacterial pathogens. However, development of real-time-PCR methods for the quantification of norovirus, and other viral food-associated pathogens, has allowed the development of an EU standard method — the task of a working group set up by the European Committee for Standardisation. Rachel Ragdale from CEFAS in Weymouth outlined some of the problems encountered by this working group and some of the issues involved in the development of a standard method.

In the pharmaceutical industry it is the presence (or absence) of microbes which is important, rather than the level of microbes in industrial water supplies. David Guy from

Lonza discussed the use of the presence or absence of endotoxin or lipopolysaccharide (LPS) from gram negative bacteria by quality control laboratories to monitor the microbial quality of the water supply.

The final presentation was given by David Coleman from Trinity College Dublin who talked about biofilm formation - an important issue both in the healthcare and food processing environments. David discussed dental chair water systems and the issues around biofilm formation in the pipes integral to the equipment that surrounds somebody when sat in the chair. The audience was comforted by the fact that there have been many developments in the monitoring and design of the dental chair unit (DCU) that will reduce biofilm formation and protect both the patient and the dental workers from cross contamination.

Overall this session brought together a range of different areas of research and gave an excellent overview of the use of water and the microbiological issues involved in a number of diverse industrial and commercial settings.

Carol Phillips

Stormont — the venue for the summer conference dinner

Management of contaminated waters

David Kay began this session by talking about private water supplies. The risks of contamination come from a variety of pollution sources including animal faeces and septic tanks. Other *"drivers of risk"* include the water transport and treatment processes. David went on to discuss the relationship between rainfall and contamination of water supplies. He talked about *"the pathogen study"* where a variety of pathogenic bacteria were measured across sites with different water supply and environmental characteristics. David described The Brighouse Bay study and concluded by raising the challenges and questions for PWS regulation.

Nigel Horan then went on to discuss the disinfection of sewage effluent prior to a receiving watercourse. The procedure varies between water companies - some disinfect all effluents, whereas others restrict disinfection to those effluents discharged to waters that are classified as bathing or recreational waters. Nigel talked about the use of UV treatment and the fact that the cost implications involved provide an opportunity for the use of alternative treatments such as membrane based treatment systems, or land intensive lagoon type treatment. Nigel then discussed the improved quality of receiving water attained through other catchment-based methods. He critically reviewed the process options available for achieving wastewater disinfection and discussed the performance of each option. The additional pressures for water companies, such as the need for energy and carbon reduction are important considerations which may conflict with the requirements to undertake disinfection. Nigel then went on to look at a number of case studies where broader environmental sources of aquatic borne pathogens were tackled and produced the greatest effects for the lowest costs.

Keith Jones then gave a fascinating talk on the *"Impact of sheep dip pesticides on biofilms involved in secondary sewage treatment."* Synthetic pyrethroid pesticides used in sheep dip, kill or inhibit protozoa in farm slurries and soils. This loss of grazing organisms results in an increase in bacterial numbers, including pathogens. The toxicity of synthetic pyrethroids to lower animals has been a major problem after accidental spillage of sheep dip into water courses. Keith reported that if synthetic pyrethroid and / or organophosphate 13 pesticides (still widely used in agriculture), were to reach a waste water treatment works, then biofilms disintegrate, leading to massive increases in numbers of bacteria. Keith discussed the fact that both types of pesticide have this effect and would result in the secondary treatment plant being out of operation until biofilm regeneration was complete.

The next speaker in this interesting session was Lorna Fewtrell, who discussed the topical issue of *"health risks associated with flooding."* Despite a recent concentration of research on the risks of flooding in the UK, there has been little progress in quantifying the microbial risk from flood waters and sediments to a flooded population. Widespread flash flooding across central and western England in 2007 left hundreds of thousands of residents flooded, disabled water and electricity supplies, and triggered tabloid media reports of "killer bugs" in floodwaters. Although sensationalist references to health crises such as cholera were inaccurate in the UK context, the media reports raised awareness of potential microbial health risk from floodwaters and sensible precautions to avoid infection. Lorna discussed the exploratory quantitative microbial risk assessment (QMRA), which has been set up to characterize the pathogenic hazard of flood components, apply dose-response parameters, assess likely routes of exposure for a flooded population, and to estimate the associated risk of gastrointestinal infection and illness from a flood event.

Howard Fallowfield then went on to tell an interested audience about *"Soil acquifer treatment of drinking water to remove cyanobacterial toxins and microorganisms."*

Cyanobacterial hepatotoxins may contribute a risk to public health when present in drinking water supplies. Soil aquifer treatment (SAT) is the movement of water down a hydraulic gradient through soil, whereby cyanobacterial toxins can adsorb to soil particles or be biodegraded. The aim of the study presented here was to examine whether SAT is a viable, low cost treatment option. Howard gave an outline of the study method which used toxins from laboratory-grown

cultures of cyanobacteria, and soils of varying properties from South Australia. Howard explained the results of this study which indicate that SAT is likely to be an effective low-cost treatment option for the removal of cyanobacterial hepatotoxins from drinking water. Complete toxin removal is possible if the hydraulic conditions of the soil and the toxin behaviour are balanced, such that there is flow through the soil, reversible toxin adsorption and microbial degradation. The significance of these results lies in the fact that SAT is potentially a viable treatment option for the removal of cyanobacterial toxins for remote Australian communities and developing nations.

John Lee then concluded this scientific session by presenting an interesting talk entitled *"Myth management and risk assessment for Legionella."* The main methods of diagnosis were displayed and he went on to discuss the contration of Legionnaires disease via aerosols. The growth of Legionella was then discussed in terms of temperature and leakage from hot water systems. John then went on to talk about cases of Legionella in hospitals and the possible causes such as damage to water hose pipes used in the plumbing

Tom Craven receiving his prize as winner of the best student oral presentation

system resulting in slowed speed of water transit. The issue of scalding resulting from measures to prevent legionella (i.e. raising temperatures) was then discussed alongside the implications of the use of thermostatic mixing valves (TMVs). Other methods of water disinfection were then discussed including the use of silver or copper ionisation, hydrogen peroxide and chlorine dioxide. He concluded by discussing the challenges that new technologies such as rainwater collection systems, grey water collection and solar heating pose in the control of legionella contamination.

This session continued with the presentation by the winner of the W. H. Pierce prize, Dr Paul Cotter of University College, Cork, Ireland (see *Microbiologist*, Vol 8, No. 3, pp13), who presented: *"Bacterially produced modified peptides: friend or foe?"* He discussed the use of bacteriocins and lantibiotics as potential antimicrobials. The applications are far-reaching and include food quality and safety, medical and veterinary applications. Paul informed us that use of nisin (the best known lantibiotic) has been approved by the FAO / WHO, EU and US FDA and is used in over 50 countries worldwide.

He went on to describe the potential for lantibiotics to act as templates for the generation of peptides with even greater capabilities. Paul described a nisin-based peptide which has enhanced activity against a variety of gram positive pathogens. He warned of two cases where bacteria-modified peptides actually contributed to the virulence of pathogenic micro-organisms. Looking at a murine model he concluded that these 'foe' peptides could be more widespread and have greater significance than could ever have been imagined.

Lucy Harper

Student presentations

This year's student session saw four excellent presentations on a variety of subjects and was co-chaired by Dr. Mark Fielder and Andrew Hall from the PECS committee. Once again the session demonstrated the excellent research being carried out by the Societies' student members.

The session began with **George Aboagye's** discussion of the occurrence of *Mycobacterium avium* ssp. *paratuberculosis* (Map) in water treatment and distribution systems. His research utilised PCR and DGGE to analyse samples of raw and processed water from Lough Neagh in Northern Ireland, with results suggesting that potable water must be considered as a possible route of exposure of the public to *Map*. The difficulties of culturing and detecting *Map* were also discussed.

David Easterhoff gave a presentation on the survival of *E. coli* in beach sand under conditions of environmental stress. His study characterised over 600 *E. coli* isolates from Lake Michigan and concluded that there is a direct correlation between the ability of environmental isolates to grow under conditions of high osmolarity and survival under desiccation in sand microcosm models. This understanding will assist in the evaluation of public health risk during water monitoring.

The use of Multidimensional Protein Identification Technology (MudPIT) proteomics to identify a molecular mechanism for probiotic efficacy *in vitro* was discussed by **James Collins**. His research has found that changes in the metabolism of *S. Typhimurium* were observed by addition of lactic acid and cell free cell supernatants (CFCS) from *L. plantarum*. These were thought to be due to the reduced pH, but also global regulatory effect on a number of metabolic systems. Further work to investigate the effect on specific pathways was described.

The final presentation was given by **Tom Craven** on his evaluation of *Moringa oleifera* for treatment of drinking water. A survey of 52 drinking water wells in Malawi found widespread contamination with faecal indicators. High levels of turbidity in the water samples were also reported. His presentation described the potential of *Moringa*, a unique water coagulant, to be used in water treatment and the implications for Malawi. Although more research is needed, preliminary results show the potential for reduction of drinking water turbidity and removal of faecal coliforms.

Andrew Hall

Potable Water

The session on potable water was well attended, despite being the last session of a packed programme (and the morning after the conference dinner!). Charles Gerba investigated the health risk of *Salmonella* and enteric viruses from recycled water for use in irrigation. Flood irrigation compared to slow drip irrigation gives rise to higher levels of contamination, with cantaloupe showing higher contamination than bell peppers or lettuce. Microbial survival in soil was demonstrated and the conclusion was that standards need to be applied to recycled irrigation water as there have been produce-related outbreaks of food poisoning linked with this practice.

Jamie Bartram stated that the burden of water sanitation and hygiene (WSH) related illnesses on developing countries was an estimated 98% of the global burden. Safer water could reduce this burden by 15% and reduce child mortality by 28%. He also pointed out that outbreaks of illness as a result of problems with WSH also cause concern in developed countries. The WHO strategy to reduce waterborne disease considers the impact of household filtration, disinfection methods and monitoring of water contamination in the field.

John Watkins discussed the problems of detecting *Cryptosporidium* in drinking water supplies. He highlighted some of the inadequacies of current testing methods and the problems that can be encountered on inter-laboratory trials. He further discussed the practicalities of keeping *Cryptosporidium* out of water supplies. Rainfall is a major contributory factor to *Cryptosporidium* contamination, as are environmental sources. He concluded by identifying control measures that are being developed, such as cross-flow filtration, to minimise the risk to human health.

Gordon Nichols identified poor source maintenance, lack of treatment, biosecurity and animals as potential sources of microbial contamination in private water supplies. There was an increase in the 1990s in the number of outbreaks of *Cryptosporidium* associated with private supplies (this may be due partially to better testing and detection). Heavy rainfall is a contributory factor but water is only a risk factor for *C. parvum*. Most cryptosporidiosis is sporadic rather than outbreak and there is a clear seasonal trend in spring and summer. Reducing the risk from private supplies should concentrate on a risk based, seasonal testing approach with stronger enforcement powers and encouragement to adopt water treatment technologies.

Will Sopwith examined the link between *Campylobacter* and water, unpicking the link with foodborne and environmental infections. Environmental and human isolates of *Campylobacter* were compared in two populations (one rural, one urban/suburban). There was little evidence of direct infection through drinking water exposures, though association between st21 (*C. jejuni*) and private supplies was identified. Causation was difficult to prove epidemiologically but st45 (*C. jejuni*) showed evidence of a seasonal increase in incidence. It is more prevalent in the more rural site, amongst young children, those reporting fishing, those having doorstep delivery of milk and less associated with consumption of chicken than expected. The links between st45, poultry and surface water remain unclear.

Finally, Gilbert Lamothe gave the manufacturers perspective, giving an overview of the microbiology of bottled natural mineral waters. Gilbert began by clarifying what is meant by the terms 'bottled' and 'mineral' and the legal requirements were explained. The process that the water undergoes during bottling was described, including the microbiological and quality issues that may arise – specifically HPC, viruses (including the problem of virus adsorption onto the PET bottle surface) and protozoan parasites.

Louise Fielding

Winter meeting 2009

A one day meeting on

Enterobacteriaceae in foods and rapid methods in microbiology

Royal Society, Carlton House Terrace, London
Wednesday 14 January 2009

CPD ACCREDITATION
6 IBMS CPD Points awarded

For the latest programme please visit us online at **www.sfam.org.uk**

including

The Denver Russell Memorial Lecture

Keeping control: studies in preservation

Delivered by **Stephen Denyer**, School of Pharmacy, University of Cardiff

Programme

Enterobacteriaceae in foods and rapid methods in microbiology

10.00-10.30 **Tea, coffee and registration**

Chair: **Geoff Hanlon**

10.30-11.15 **The Denver Russell Memorial Lecture**
Keeping control: studies in preservation
Stephen Denyer, School of Pharmacy, University of Cardif, UK

11.15-11.45 **Enterobacteriaceae in foods**
Chris Baylis, CCFRA, Chipping Campden, UK

11.45-12.15 **Impact of rapid screening for meticillin-resistant *Staphylococcus aureus* on hospital infection rates.**
Dr P. J. Jenks, Department of Microbiology and Infection Prevention and Control, Derriford Hospital, Plymouth, UK

12.15-13.15 **Lunch**

Session A. Rapid detection and identification in microbiology.

Chair **Andrew Sails**

13.15-13.45 **Microbial DNA sequence profiling of human pathogens by mass spectrometry.**
Cath Arnold, Health Protection Agency, London, UK

13.45-14.15 **New bacteriophage-mediated adenylate kinase assay for high throughput detection of foodborne pathogens.**
Pradip Patel, Alaska Food Diagnostics Ltd, DSTL, UK.

14.15-14.45 **Multi-pathogen microarrays for the diagnosis of infectious disease .**
Nigel Silman, Health Protection Agency, Porton Down, Salisbury, UK

14.45-15.05 **Tea and coffee**

15.05-15.35 **Rapid diagnosis of sepsis.**
Paul Dark, Hope Hospital, Salford, UK

15.35-16.05 **Automation in Clinical Microbiology.**
Martin van der Kaap, Kiestra Laboratory Automation, The Netherlands.

Session B. The Enterobacteriaceae in foods

Chair **Martin Adams**

13.15-13.45 **The ecology of enterobacteriaceae in foods**
Mieke Uyttendaele, University of Ghent, Belgium

13.45-14.15 **Epidemiology of foodborne illness caused by Enterobacteriaceae**
Sarah O'Brien, University of Manchester, UK

14.15-14.45 ***Cronobacter (Enterobacter) sakazakii* and infant formula**
Stephen Forsythe, Nottingham Trent University, UK

14.45-15.05 **Tea and coffee**

15.05-15.35 **Enterobacteriaceae and hygiene monitoring in frozen foods**
Nick Johnson, Unilever, The Netherlands

15.35-16.05 **Pathogenic enterobacteriaceae in foods.**
Tom Cheasty, HPA, London, UK

16.05 **Meeting closes**

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

BOOKING FORM and INVOICE

SfAM WINTER MEETING WEDNESDAY 14 JANUARY 2009

Only ONE person per form please. CLOSING DATE FOR REGISTRATIONS: Wednesday 7 January 2009
EARLY BIRD DISCOUNT of £30.00 is applied to all bookings made before **Friday 19 December 2008**

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

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Student non member	£60 ☐	£90 ☐
Non member	£100 ☐	£130 ☐
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YOUR INTERESTS

Please indicate which of the two afternoon parallel sessions you wish to attend

Session A: Rapid detection and identification in microbiology ☐ **Session B:** The Enterobacteriaceae in foods ☐

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CPD ACCREDITATION
7 IBMS CPD Points awarded

Spring meeting 2009

A one day meeting on

3rd broadening microbiology horizons in biomedial science

Aston University, Birmingham, UK
22nd April 2009

Including talks on:

■ Bioterrorism ■ Emerging respiratory viruses ■ Device related infections

Programme

09.15-10.15 **Coffee, Trade and Exhibition**

10.15-10.20 **Chairman's Welcome**

10.20-11.00 **Procter and Gamble Lecture**
Topic and speaker to be confirmed

11.05-11.35 **Update on ESBLs**
Professor Peter Hawkey, Birmingham, UK

11.35-12.05 **Bioterrorism**
Professor Les Baillie, Cardiff, UK

12.05-12.35 **Emerging respiratory viruses**
Dr Kate Templeton, Edinburgh, UK

12.35-14:00 **Lunch and Trade Exhibition**

14.00-14.30 **Device related infections**
Professor Peter Lambert, Birmingham, UK

14.30-15.00 **Use of copper in the hospital setting**
Dr Tony Worthington, Birmingham, UK

15.00-15.30 **Changing epidemiology of viral hepatitis**
Laura Ryall, Cambridge, UK

15.30-16.00 **Q fever**
Dr Phillipa Moore, Gloucester

16:00 Close and Tea

The programme for this meeting was correct at the time of going to press.
For the latest information please visit: www.sfam.org.uk/spring_meetings.php

Coming soon...

Summer conference 2009

Fur, feather and fever — zoonotic challenges of the 21st century

6 - 9 July 2009, Manchester Metropolitan University

Including sessions on:

■ Arthropod borne zoonoses ■ Wildlife and companion animals
■ Livestock and foodborne zoonoses..■ Emerging and re-emerging zoonoses

BOOKING FORM and INVOICE

SfAM SPRING MEETING WEDNESDAY 22 APRIL 2009

Only ONE person per form please. CLOSING DATE FOR REGISTRATIONS: Wednesday 8 April 2009
EARLY BIRD DISCOUNT of £30.00 is applied to all bookings made before **Wednesday 4 March 2009**

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

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Student non member and IBMS member	£75 ☐	£105 ☐
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Inaugural *Environmental Microbiology* lecture

An elite group of scientists, policy makers and journalists attended the inaugural *Environmental Microbiology* lecture presented by Professor **Rita Colwell** on 8 September 2008 at the Royal Society of Medicine, London

Geoff Hanlon, Ken Timmis and Rita Colwell

Professor Colwell's lecture, entitled *climate, cceans, global warming and cholera* described how her and her team have used remote satellite imaging techniques to predict outbreaks of this serious water-borne disease. Cholera is an ancient water-borne infectious disease, which is an unpredictable and serious problem for developing countries.

The bacterium that causes cholera, *Vibrio cholerae*, has a known association with zooplankton more specifically copepods (water-dwelling crustaceans). Some environmental factors have also been associated with cholera epidemics: these include sea surface temperature (SST), ocean height (OHH), and biomass estimated by measuring chlorophyll

produced by phytoplankton (CHL).

Recently, remote satellite imaging has been used to track this climatologically important information and the data collected now can be used to predict outbreaks of cholera before they occur. Cholera epidemics have been episodic, so the ability to predict them could be one further step towards controlling this serious, water-borne disease by providing rapid response public health measures. In the meantime, a low-tech answer has been developed and successfully employed in Bangladesh: straining drinking water through sari cloth folded over at least four times. The native fabric creates a filter, which strains out the zooplankton associated with cholera and has proved to lower the incidence of the disease by 50%. The climate factors shown to be associated with cholera also play a role in many other infectious diseases. Hence cholera offers a useful model for understanding human health effects related to climate change.

The event began with a drinks reception, where members mingled with high profile scientists, political figures and journalists.

The lecture is available to view online at: http://www.yada-yada.co.uk/podcasts/Blackwell/video /SfAM/ index.html

Microbial populations and coffee

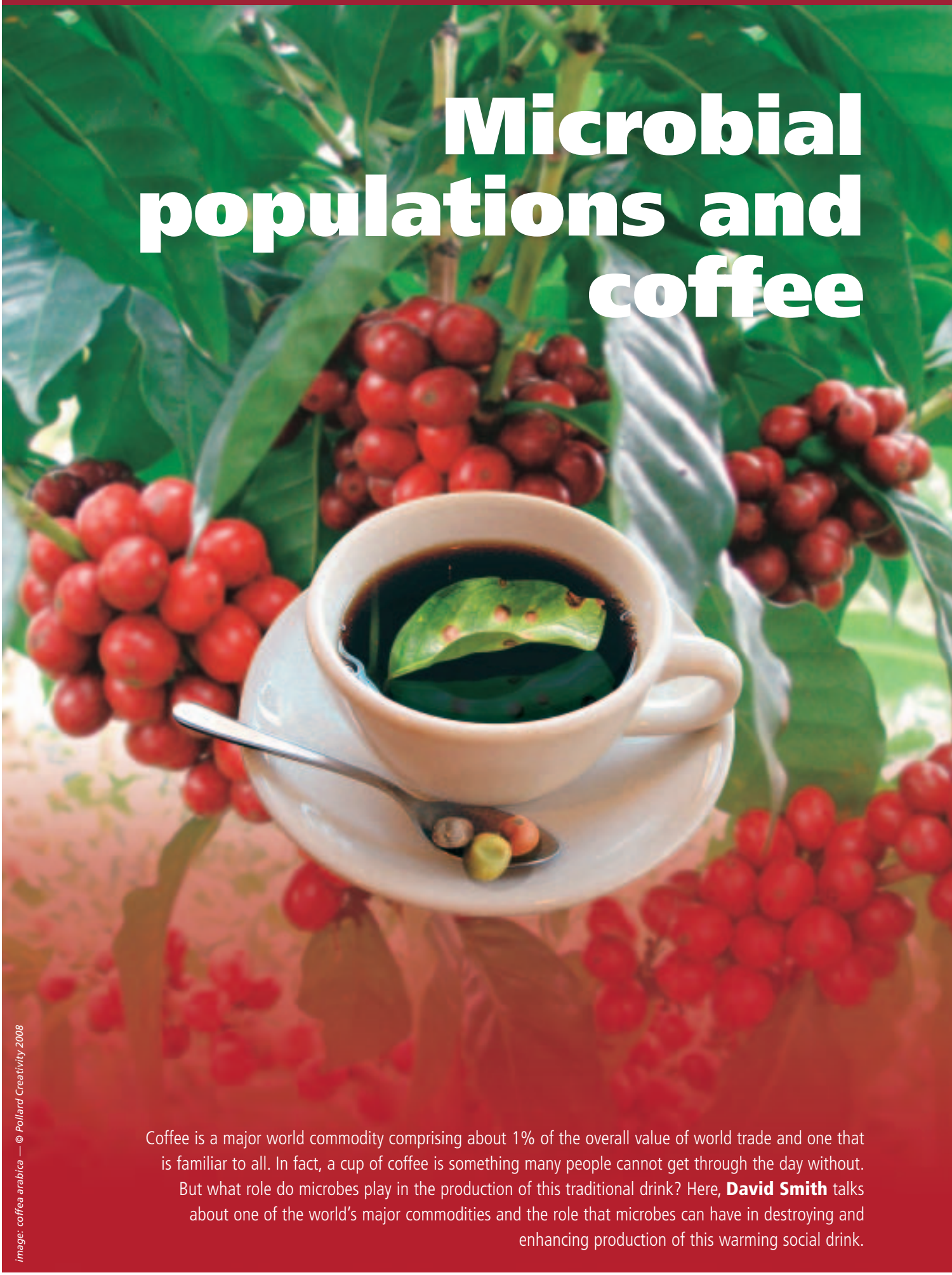

image: coffea arabica — © Pollard Creativity 2008

Coffee is a major world commodity comprising about 1% of the overall value of world trade and one that is familiar to all. In fact, a cup of coffee is something many people cannot get through the day without. But what role do microbes play in the production of this traditional drink? Here, **David Smith** talks about one of the world's major commodities and the role that microbes can have in destroying and enhancing production of this warming social drink.

There are about 100 species of coffee, but for trade purposes *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta) are the only significant ones. The coffee tree can grow to the height of 10 to 15 metres but on plantations continual pruning maintains the trees at 1.5 to 3 metres ensuring a high yield and making harvesting easier. Trees blossom over a six- to eight-week period in countries such as Brazil and Mexico. But in countries located along the equator, such as Kenya and Colombia, a coffee tree can have blossoms, ripening fruit and mature berries on the same branch at the same time. Pickers must go over the trees again and again to pick only perfectly ripe berries. Because harvesting is so labour-intensive, it's one of the most expensive steps in coffee processing. Coffee trees produce for about 20 to 25 years, yielding about 2,000 beans a year, which is about one kilogram of raw coffee per year. With modern cultivation methods the harvest in a good year lies between 3,000 and 4,000kg per hectare.

The present coffee-producing belt around the globe comprises about 70 countries involved in cultivation, and lies between the latitudes of 23 degrees north and 25 degrees south. The ideal growing conditions are an average of 17°C to 23°C as well as abundant precipitation and fertile soil. Brazil is the world's largest coffee producer, providing around 33% of the total world output. Vietnam follows second with about 15%, third lies Colombia with around 10%, and fourth Indonesia with about 5% (2006/7 figures). After that a number of countries follow contributing between 3% and less than 1% to world coffee production. In terms of micro-organisms, the most feared enemy of the coffee plant is leaf rust; a mould that infects the leaf turning it brown, then black and eventually causing it to fall.

Overall, coffee production involves a large number of operations from planting to consumption (Wintgens, 2001), each of which offer micro-organisms various opportunities to invade — and it is not just the disease causing organisms that have an effect...

Growing and harvesting

To provide some background, it is useful to describe the growing and harvesting conditions of a typical coffee plant. The cultivation, care and harvesting of coffee is extremely labour intensive. It begins with the sowing - coffee seeds will only germinate if sown within the eight weeks following the harvest. They are sown one to two cm deep in specially constructed beds, transplanted to special foil planting bags, so called polycovers, or often to peat pots. They are then set 20 to 25 cm apart in large, predominantly shaded beds. Six months later the young plants are 30 to 50 cm tall. At this stage they are transplanted to their final place in the coffee plantation, now at a distance of one to three metres apart. Here, the plants are often protected by trees (e.g. *Inga* spp) which shade the plants from intense sunlight. The newer varieties of coffee tree begin to bear fruit from the third or fourth year and go on to produce an optimal crop for ten years. Older varieties produce their first harvest after five years, but continue to produce maximum crops for 25 years.

Around nine months after the flowers appear; the berries are ripe and can be harvested. Harvesting is mainly carried out by hand. The main harvesting season lasts around four months for Arabica coffee, and for Robusta coffee a little longer. In some countries, however, there is no standard harvesting season because of the significant climatic and geographical differences. In Colombia for example, harvesting continues throughout the year.

For centuries Coffee Leaf Rust Disease has been one of the biggest threats facing the coffee industry worldwide. The disease, which is caused by the fungus *Hemileia vastatrix*, is so destructive that it caused the collapse of the coffee industry in Sri Lanka in the 1860s. The disease spread into Brazil and is now a huge concern in Africa and Asia, where it can cause crop losses in the region of 30-60% per year. Control of the disease has always been problematic due to the ability of the fungus to spread easily and develop new strains which overcome the resistance of the coffee tree. To help tackle the issue, CABI (a Corporate Member of S*f*AM), in collaboration with some of the countries affected, including India, Kenya, Uganda, Rwanda and Zimbabwe, began work on a project: *"Increasing the Resilience of Coffee Production to Leaf Rust and Other Diseases."* The project is supervised by the International Coffee Organisation (ICO) and funded by the Common Fund for Commodities (CFC) with national contributions from the countries involved. The aim of the project is to reduce the economic and environmental costs of controlling the disease for smallholder coffee farmers by reducing the crop and quality losses caused by the disease. This project will assess the situation in each of the countries, with a focus on smallholders, and will identify how practices which work in one country may be adapted to suit others. Already we have found that some countries have a number of Coffee Leaf Rust resistant trees. We hope to utilize these varieties where possible, particularly in troubled areas but the main focus of the project is providing better knowledge to farmers using participatory training methods.

Two other very serious coffee diseases, Coffee Berry Disease and Coffee Wilt Disease, are presently limited to Africa and they are a major reason why coffee production has been declining for a number of years there. If either of them escaped from the continent they would have a major impact on global coffee production.

Stemborers (*Coleoptera: Cerambycidae*) are the one of the most important pests of Arabica coffee in Asia and Africa. The larvae of these organisms bore into the stem causing death or reducing yield. Replacing diseased trees reportedly accounts for an annual loss of about US $8-10 million to the Indian coffee industry. Waller *et al.*, (2007) report 27 different species of stem and branch borers from coffee and these can be controlled using various new technologies in pest management, including the use of resistant varieties and biocontrol agents such as parasitoids, fungal pathogens, nematodes and pheromones. There have been over 380 different fungal taxa isolated from coffee but only between 20-25 are pathogens (see table 1).

Soil fertility plays an important role in growing healthy trees and maintaining good yields as the soil not only provides nutrients only possible through breakdown by microorganisms, it also harbours fungi and bacteria that act against pests and root diseases as well as mycorrhizae and fungi capable of forming symbiotic association with coffee roots. In addition, rhizobial bacteria help fix atmospheric nitrogen. The majority of soil microorganisms cannot yet be grown in culture for study, but those that have been can be made available through culture

collections such as CABI's, who are dedicated to taking scientific information and bench science to the field, providing research and development in invasive species management, commodities and knowledge for development. Consortia of organisms are available as biofertilisers to enhance soil fertility and aid in composting to provide high quality organic matter for crop and commodity health.

Processing

Again, it is useful to provide some background on the processing of coffee beans in order to understand the significance of the role of micro-organisms at this stage of production.

The two coffee beans make up only one third of the coffee berry, the rest consisting of fruit flesh (also known as pulp), skin and husk, all of which must be removed so that only the green beans remain. Two different methods of processing the coffee beans have now been adopted worldwide: wet processing and dry processing. In wet processing the beans are washed and unripe berries removed. The pulp is removed from the ripe berries and the coffee beans are fermented in large containers to dissolve any remaining fruit flesh and to remove the sticky film surrounding the coffee beans, which is not water soluble. This part of the procedure lasts one to two days — opinion is divided about whether the fermentation adds significantly to its cup quality. On completion of the fermentation the coffee beans are washed, spread out on concrete slabs or drying racks and left out in the sun. To ensure that the beans dry evenly they are turned over several times a day for five or six days. Cultivators operating on a medium to large scale make use of drying machines. The coffee is then hulled, sorted and graded.

The dry processing is used for lower quality Arabica and Robusta berries, a simple technique which is less labour intensive but results in a loss of quality. The berries are spread out in the sun on cement or brick slabs in layers five to six cm deep. To ensure that the beans dry evenly, the berries are turned regularly for a period of two to three weeks. On smaller plantations drying mats made of wire netting are often used. Once the beans are completely

Table 1. Coffee diseases (details ex Waller *et al.,* 2007)

Disease	Causative organism	Estimated crop loss	Global cost US$
Coffee leaf rust	*Hemileia vastatrix*	15%	1-3 billion/year
Grey rust (leaf) also known as powdery rust of coffee	*Hemileira coffeicola*	Not a major problem	
Coffee wilt (Fusarium wilt)	*Gibberella xylarioides*	Most serious disease in Africa – Tanzania 70% loss in 2006	
	Fusarium solani		
Coffee berry disease	*Colletotrichum kahawae*	Upto 75%	Kenya 12million in 1966
American leaf spot	*Mycena citricolor*	20-73%	
Brown eye spot	*Cercospora coffeicola*		
Bacterial blight	*Pseudomonas syringae*		
Koleroga	*Coricium koleroga*	Rarely serious	
Pink disease	*Erythricum salmonicolor*	Minor disease	
Anthracnose (Brown blight in India)	*Glomerella cingulata*	Not normally serious	
Leaf blight and die back	*Ascochyta tarda* *Phoma costarricensis* *Ascochyta coffae*		
Blister spot	*Colletotrichum gloeosporoides*		
Other leaf spot fungi	*Cephaleuros virescens*		
Coffee ring spot virus	*CoRSV*	Low incident no significant damage	
Brown blight	*Colletotrichum gloesporoides and C. kahawae*	No yield loss	
Berry blotch	*Cercospora coffeicola*		
Warty berry	*Botrytis cinerea*	Rarely causes significant damage	
Coffee bark disease (Storey's bark disease)	*Gibberella stilboides*	Decline in vigour of tree	
Ceratocystis wilt	*Ceratocystis fimbriata*	Minor disease	
Hymenomycete root rot	*Armillaria ssp*	Sporadic mature coffee tree death	
Brown rot	*Phellinus noxius*		
Black root rot	*Rosellinia spp*		
Santavery root disease	*Fusarium oxysporum f.sp. coffeae*		
Dry root rot	*Fusarium solani*	Not common but lethal	
Phloem necrosis	*Phytomonas leptovasorum*		
Collar rot/ collar canker	*Helicobasidium compactum*		
Stem canker	*Phomopsis coffea*	Minor pathogen	
Collar rot	*Rhizoctonia solani* *Rosellina bunodes* *Botryodiplodia theobromae*		
Root disease	*Phytophthora spp*		
Damping off of seedlings	*Rhizoctonia solani*		
Seedling blight	*Fusarium stilboides*		
Seedling death	*Myrothecium roridum* *Corticium rolfsii* *Aspergillus niger* *Pythium spp*		

dry, hulling begins. In a peeling machine similar to that used during wet processing, the dried fruit flesh (the pulp), the parchment skin and a part of the husk that surrounds the coffee bean are removed. Finally, the beans are cleaned and then sorted according to size by mechanically operating vibrating sieves. The beans are then measured into sacks of a standard size (usually 60 kg). Such processes allow microorganisms to establish themselves and this can cause reduced quality of the final product

In dry processing fungi and bacteria can cause off-flavours for example the production of propionic acid imparts an onion flavour. In addition, other acids are produced for example butyric acid and bacterial growth often gives the product a potato taste. Amongst the most commonly found fungi are in processed coffee *Fusarium*, *Cladosporium*, *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor* and various yeasts. Generally hydrophilic moulds predominate during the wet stages of processing with more xerophilic genera such as *Aspergillus*, *Penicillium* and *Cladosporium* becoming predominant as the coffee dries out. Brazilian coffee is particularly contaminated by *Aspergillus* species including *A. niger*, *A. ochraceus* and *A. flavus*. Silva *et al.*, (2008) investigated the magnitude and diversity of the microbial population associated with dry (natural) processing of coffee (Coffea arabica) on 15 different farms in the Sul de Minas region of Brazil.

The microbial load varied from 3×10^4 to 2.2×10^9cfu/cherry with a median value of 1.6×10^7 cfu/cherry. The Gram-negative bacteria included 17 genera and 26 species, the most common of which were members of the genera *Aeromonas*, *Pseudomonas*, *Enterobacter* and *Serratia*. The Gram-positive bacteria included six *Bacillus* species, and over half of the non-spore formers were *Cellulomonas* with lesser numbers of *Arthrobacter*, *Microbacterium*, *Brochothrix*, *Dermabacter* and *Lactobacillus*. The most common genera of yeast found were *Pichia*, *Candida*, *Arxula* and *Saccharomycopsis*. There were many rarely described yeasts including *Pichia lynferdii* and *Arxula adeninivorans*. *Cladosporium*, *Fusarium* and *Penicillium* were the fungi most frequently found on farms. Only 3% of the isolates were *Aspergillus*. *Beauvaria*, *Monilia*, *Rhizoctonia* and *Arthrobotrys* species. The microbial flora is much more varied and complex than found in wet fermentations. The genera and species identified include members known to have all types of pectinase and cellulase activities.

Ochratoxin is often found in coffee although it is rare to find the fungi producing it, normally *Aspergillus ochraceus* and sometimes *Penicillium verrucosum*, in the coffee at harvest (Baker, 2001; Waller *et al.*, 2007). Furthermore, the presence of these fungi does not mean the presence of the toxin as production depends upon the isolate, temperature and water availability (Waller *et al.*, 2007). Roasting substantially reduces the ochratoxin levels by around 80%. Bucheli *et al*,. (2000) reported increased levels of ochratoxin in sun dried cherries but found the fungal source to be *Aspergillus carbonarious*. The source of the fungi is often the soil and therefore cleanliness in harvesting is important as is preventing the opportunity for contamination in processing and storage. Ochratoxin is a kidney toxin, an immuno-suppressant, a carcinogen and a teratogen but levels in coffee are not usually significant. The EU recommends that it is prudent to keep exposure to below levels in roasted and ground coffee of 5.0 ppb and in soluble coffee of 10 ppb. Levels commonly seen in coffee are around 0.6-0.9 ppb however there have been reports of levels up to 48ppb but still coffee accounts for only a few percentage points of the daily consumption (Baker, 2001).

Storage and transport

Stored appropriately, coffee is not particularly prone to damage. If the moisture content is too high (>13%) *Rhizopus* and *Aspergillus* ssp begin to develop and as moisture content rises yeasts and bacteria become a problem. These infections cause off-flavour and some fungi produce mycotoxins e.g. ochratoxin and aflatoxin from *A. flavus*.

Summary

There are both beneficial and harmful microorganisms associated with coffee growing and production and although the major disease causing organisms and the common contaminants are known well there is still a vast amount of work needed to fully understand the ecosystem function of many organisms. For sustainable production and to find new revenue streams the study of the organisms associated with the crop, commodity, the farm and the plantation is vitally important. The work of Biological Resource Collections (Smith, 2008) linked to specialist research groups can facilitate such processes. Even coffee, a beverage we have been drinking over many centuries can provide us with new and interesting microbiology. For example, 1400 new species of fungi are described annually and according to estimates there may be over 1.4 million more out there, centuries of work for the beleaguered taxonomist. Current culture collections hold many strains from soil, providing a key resource that underpins coffee research and that can input to the development of a knowledge based bioeconomy.

The author would like to thank Dr Peter Baker for updating the figures in the text and his expert opinion.

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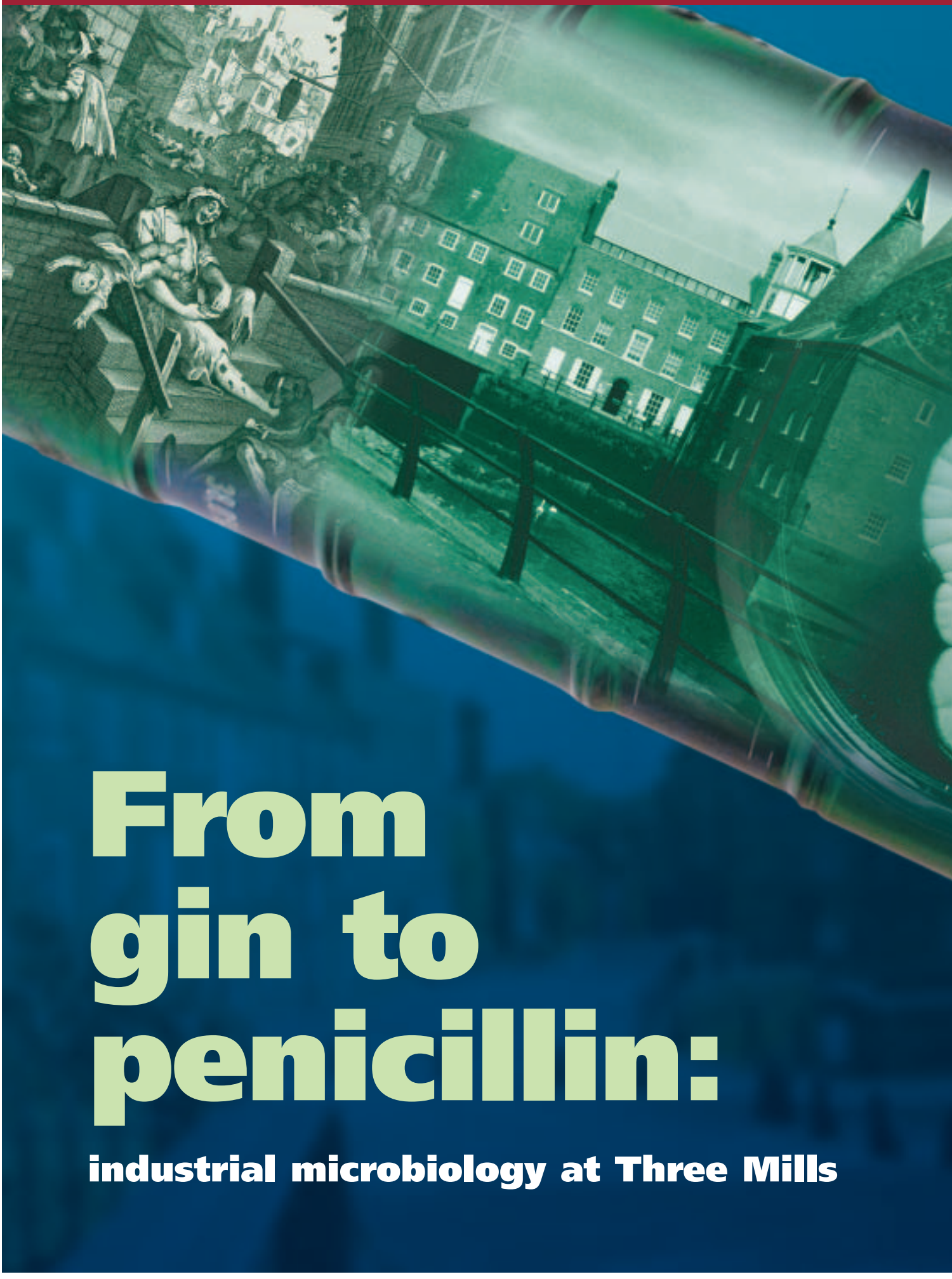
David Smith
CABI Europe UK



From gin to penicillin:

industrial microbiology at Three Mills

The eastern stretches of the District Line from central London offer more than just an opportunity to inspect the building site for the 2012 Olympics or to visit the hallowed turf of West Ham United Football Club. Between Bromley-by-Bow and West Ham stations the line passes an area possibly unique in the history of industrial microbiology. **Martin Adams** explores

There have been mills at Three Mills on the River Lea since the time of the Domesday Book, employing the twin advantages of the tide for motive power in the mills and the river for navigation. For much of their history they were used solely as corn mills but in 1728 they were purchased by the son of a Huguenot immigrant who, with his partners, made them part of an integrated operation that included distilling: the first application of industrial microbiology on the site. This was during the period that later became known as the "gin craze"; a time when spirit production in Great Britain had doubled in the previous 10 years to reach around 4 million gallons per annum (it peaked in 1743 at a level of 8.2 million gallons). There was huge concern about the damage that the massive consumption of gin was doing, exemplified in the engravings Gin Lane (see over) and Beer Street by Hogarth which contrasted the relatively benign and happy lifestyles enjoyed by beer drinkers with the squalor and debauchery associated with the consumption of gin. Several attempts were made to control gin consumption but it wasn't until 1751 that legislation was introduced which finally enjoyed any success, though it is claimed that economic factors which caused the price to increase were at least equally responsible.

To make gin, the grain was ground and mixed with malt (a source of enzymes to convert the starch to fermentable sugars) and water before fermenting with yeast. When fermentation was complete the fermented wash was distilled and the alcoholic distillate was then either compounded into gin by mixing with botanical flavourings and redistilling or sold to gin rectifiers for this purpose. It was at some stages illegal for a distiller both to produce alcohol and rectify it into gin. This was still very much a traditional craft technology; only later in the 18th century were the first successful attempts made to describe the alcoholic fermentation chemically when Joseph Black, the discoverer of carbon dioxide, stated that the sole products of fermentation were ethanol and CO_2. His observations were subject to later refinement by Lavoisier and Gay-Lussac, but it wasn't until the 19th century that the microbiological nature of the process was finally established, even though Van Leeuwenhoek had described yeast in beer in 1680.

They diversified into brewing at Three Mills in 1730 possibly anticipating some restriction of their distilling business and there were several other breweries in the area. Most notable amongst these was Hodgson's Brewery, established at Bow

Three Mills as it is today

Bridge just north of Three Mills in 1751, which was a pioneer in the development of the beer trade to India and the beer style known as India Pale Ale (IPA). The brewery was ideally situated by the River Lea which joined the Thames near what was later to become the East India Dock Basin. George Hodgson was aware of the very cheap freight rates available on ships returning to India almost empty after bringing their high value cargoes of silks and spices to London. He also knew that the porters and stouts then popular at home were less so with the expatriate population of India as they did not retain their condition on the three month sea journey to India and were in any case rather heavy drinks for the hot climate.

In 1780 Hodgson produced what was then described as India Ale; the term Pale was incorporated later by the Burton brewers. It achieved its keeping quality through the application of the hurdle or multiple barrier principle of food preservation where a combination of antimicrobial factors is enlisted to give the required stability. In this case, they used pale malts with high diastatic (starch degrading) power and an initial fermentable extract (wort) of high specific gravity which meant that the final beer contained a high concentration of alcohol, more than 6%. It was more highly carbonated than usual and additional hops were added increasing the antimicrobial isohumulones, giving the beer added stability and a very bitter flavour; so much so that the head brewer at a Burton brewery spat it out when he first tasted it. This sparkling strong ale was much appreciated in India and the beer trade increased from 1,680 barrels in 1775 to 9,000 barrels in 1800; but Hodgson's success was undone by the greed of his son Mark and the aggressive way he protected his market. When faced with competition, he drove down prices by flooding the market and when the threat had passed he increased the price to recoup his losses. This eventually annoyed the East India Company which encouraged brewers from Burton-on-Trent to enter the fray, eventually eclipsing Hodgson's. The company declined and was later sold to another brewer though brewing continued on the site until the 20th century when the brewery was finally demolished in 1933.

Back at Three Mills, the distillery was acquired in 1872 by J & W Nicholson, a gin distillers based in Clerkenwell, but its expertise and facilities later came to prominence during the First World War in an area quite different from gin. Acetone was an essential solvent in the manufacture of cordite, a smokeless explosive. Prior to the First World War a major source of acetone for UK manufacturers had been calcium

Hodgson's Brewery — established at Bow Bridge just north of Three Mills in 1751

acetate imported from Germany, Austria and the United States, two of whom were now reluctant to sell it to us. The ability of some clostridia to produce small amounts of mixed solvents including acetone had been noted in the 19th Century but attracted more serious attention at the beginning of the 20th Century when there were concerns about the future supply of natural rubber. Butanol and isoamyl alcohol produced microbiologically could be used as precursors of butadiene and isoprene from which synthetic rubber could be made. In 1911 the French microbiologist Fernbach isolated a culture that was able to ferment potato starch, to produce butanol, amyl alcohol, acetone and ethanol and this was used in plants established in the UK by Strange and Graham Ltd. With the start of the First World War, the demand for acetone increased hugely and the focus of the fermentation was changed from butanol to the production of acetone, but it could nowhere near meet the demand.

Chaim Weizmann , then at Manchester University, had previously worked for Strange and Graham and had isolated an organism, *Clostridium acetobutylicum* that was able to ferment a wider range of substrates including maize starch, producing only acetone and butanol and in a much better yield than Fernbach's organism: acetone levels were four times higher. He offered this process to the Government which seized on the chance to solve its acute problem. In 1916 the Nicholson distillery was taken over by the Government to act as a pilot plant for the industrial exploitation of the acetone-butanol fermentation. Among the short cuts they tried was to abandon complete asepsis which proved impossible, making the acetone-butanol fermentation a significant forerunner of modern industrial deep fermentations requiring asepsis on a large scale. The process was later transferred to a number of distilleries and the existing Strange and Graham plant at Kings Lynn, although Weizmann noted that the distilleries were "neither very happy about the conversion of their plants, nor particularly helpful". Shortages of imported maize to use in the fermentation gave rise to extreme measures such as the organised national collection of horse chestnuts, but eventually production was moved to Canada and the United States.

The success of the acetone/butanol fermentation had long term consequences in a quite unrelated area. The British Government was indebted to Weizmann who was an ardent Zionist. He declined any personal honour for his contribution but the access it had given him to government circles and the Foreign Secretary in particular eventually contributed to the

Photograph by kind courtesy of the British Museum

Gin Lane — by William Hogarth, published in 1751

so-called Balfour Declaration in which the British Government stated that it favoured *"the establishment in Palestine of a national home for the Jewish people."* Weizmann later became the first President of Israel.

The Distillery finally closed in the 1950s and is now a film studio contributing to our culture at the highest level: it was the site of the first *Big Brother* household.

Immediately across the River Lea from the Three Mills Distillery, on a site now occupied by a Tesco store and its car park, is the site of Kemball Bishop & Co. In 1871 Joseph Kemball established a factory there producing citric and tartaric acids. Calcium citrate, obtained from lemons, was imported from Sicily for citric acid manufacture and tartaric acid was made from argol, a by-product of wine fermentation. Argol is composed principally of potassium hydrogen tartrate and precipitates out from wine during fermentation and storage. It was famously used by Pasteur in his seminal work on molecular asymmetry.

In 1931 Italy restricted exports of calcium citrate and Kemball Bishop made an agreement to licence the citric acid fermentation process from Pfizer in the United States where it had been operating since 1923. This was based on surface culture of the mould *Aspergillus niger* in shallow pans containing a molasses-based medium. When fermentation was complete the surface mat of mycelium was removed and washed and the citric acid extracted from the underlying medium and washings by precipitation as calcium citrate.

Later, Kemball Bishop went on to produce other fermentation products such as itaconic acid and gluconic acid using similar technology, but probably their most heroic period came during the 2nd World War when they turned their hand to penicillin production. Although Fleming had continued to work on penicillin, following its discovery in 1928, well into the 1930s, his work and that of others such as Raistrick petered out as attempts to purify the active principal and retain its potency in the culture broth from *Penicillium notatum* continued to fail. In 1938 it was taken up by the team of Florey, Chain, Abraham and Heatley at Oxford with more success, although development and use were hampered by the small quantities of penicillin available. To meet demand the *Penicillium* was cultured on the surface of liquid medium at several sites in a host of different containers enlisted for the purpose, from biscuit tins to bed pans.

The essential similarity of the fermentation technology employed by Kemball Bishop meant that the company was ideally suited to make an important contribution. Efforts were however hampered by the regular bombing of the area which saw the neighbouring Three Mills Distillery warehouse destroyed along with £1 million worth of alcohol in 1940 (an occasion when some selfless heroes managed to rescue sixteen barrels of brandy from the canal). As a result of their own trials and despite the difficulties, the company was able to start supplying the Oxford group from September 1942, with a regular weekly 200 litres of crude culture filtrate free of charge.

Kemball Bishop was taken over by Pfizer in 1958 and production of organic acids continued at the site until 1971 when it was moved to Ireland.

A more prosaic aspect of industrial microbiology can be found a short walk away at the Abbey Mills Pumping Station. This magnificent "cathedral of cast iron" was built in 1865 as part of Joseph Bazalgette's grand scheme to rid London of its perennial sewage problem, banishing both the foul smells and diseases such as cholera. It contained eight beam engines whose purpose was to raise sewage 36 feet from the lower sewer to the Northern Outfall sewer where it could then continue to run under gravity to Beckton. Once at Beckton it was originally discharged into the Thames on the ebb tide but is now subject to elaborate biological treatment.

Those of an adventurous disposition who visit the sites I've described can continue their excursion by walking along the top of the Northern Outfall Sewer, now described as the Greenway, towards the distant sunrise and Beckton.

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Martin Adams
Meetings Secretary

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

The non-parametric correlation coefficients

Stat Note 15

The most common method of testing the degree of correlation between two variables (X, Y) is to use Pearson's correlation coefficient (r) as described in Statnote 14 (Hilton & Armstrong, 2008). Although Pearson's r is widely used with all types of data, it is essentially a 'parametric' test, i.e., the test is based on the assumption that the individual pairs of values (x,y) are members of the 'bivariate normal distribution' (Snedecor & Cochran, 1980). If the data depart significantly from such a distribution, then a non-parametric correlation coefficient should be used.

This Statnote discusses the most widely used non-parametric correlation coefficients, viz. Spearman's rank correlation (rs), Kendall's tau (t) (Snedecor & Cochran, 1980) and gamma.

The bivariate normal distribution

The correct use of Pearsons' 'r' assumes that the data follow, at least approximately, a bivariate normal distribution. The bivariate normal distribution is a natural extension of the normal distribution (Hilton & Armstrong, 2005 a,b) from one to two variables and has the following properties:

(1) for each value of x, the corresponding values of y are normally distributed; the means of these normal distributions lying on a straight line and the variance being constant for each x,

(2) for each y, the corresponding x values are normally distributed, and

(3) the marginal distributions of x and y are also normally distributed.

Trifolium pratense L

This distribution is defined by five parameters (the *means* and *standard deviations* of the X and Y variables and the *population correlation coefficient p*). The data are likely to approximate to such a distribution if *X* and *Y* are both continuous variables and themselves, normally distributed. Some statisticians have argued that if a test of the null hypothesis that there is no correlation between *X* and *Y* is required, Pearson's *r* may still be used providing one of the variables is normally distributed (Snedecor & Cochran, 1980). However, if one or both variables are small whole numbers, scores based on a limited scale, or percentages, a non-parametric correlation coefficient should be considered as an alternative.

Scenario

As an example of the application of correlation analysis to non-parametric data consider the following experimental scenario.

Background

Many species of bacteria and fungi have been recorded within the rhizosphere and some species may be exclusively rhizosphere organisms. The root surface offers many potential sources of food for soil microorganisms as plant roots exude substances into the soil, the type and amount depending on plant growth conditions. In addition, many of the organisms that accumulate on the root surface are deficient in one or more substances such as carbohydrates, amino acids, thiamine, biotin, organic acids, nucleotides, and various enzymes and many of these substances can only be obtained from the rhizosphere. The degree of root secretion is dependent on the type of plant, e.g., legumes have high rates of root secretion while cruciferous plants tend to exude less. In this scenario, we wished to test the hypothesis that the general abundance of fungi close to the root surface was correlated with the degree of carbohydrate secreted from the root.

Method

Ten plants of the legume *Trifolium pratense* L. obtained from cuttings of wild plants were propagated, each in its own pot, in garden compost for a period of six months. After six months, a 1 gm soil sample was taken from each pot rhizosphere at a depth of 3 cm. These samples were submitted for carbohydrate analysis using gas liquid chromatography; five replicate analyses being made from each sample. In addition, 10 further small soil samples (5 – 15mg) were taken at different depths adjacent to the plant roots using the flattened blade of a sterilized nichrome inoculating needle, and used to crush and disperse the soil aggregates in the bottom of each of 10 sterile Petri dishes. A little sterile water was then added to assist the dispersion. Melted and cooled agar (8-10 ml) was poured into the dish and manipulated before setting so as to secure as complete a dispersion of soil as possible. After suitable incubation, the general abundance of fungal colonies on each dish was expressed on a ranked scale: 0 (none), 1 (few), 2 (frequent), or 3 (abundant). The data were averaged over the 10 samples collected from each plant.

Spearman's rank correlation (r_s)

The total carbohydrate levels in the soil samples are continuous data, measured to four significant figures, and are therefore likely to be normally distributed. However, because the abundance of fungi is expressed on a ranked scale, these data are unlikely to be normally distributed and therefore there may be doubt as to whether the data as a whole conform to the bivariate normal distribution. Pearson's '*r*' may still be used if one variable is normally distributed but a better approach might be to use a non-parametric correlation coefficient.

The calculations involved in making a Spearman's rank

Table 1. Measuring the degree of correlation between the general abundance of fungi in the rhizosphere (average of five plates scored on a four-point scale) (*Y*) and the degree of secretion of carbohydrate (µg mg dry weight of soil) (*X*) using Spearman's rank correlation (r_s).

Plant	X	Y	Rank X	Rank Y	X - Y	D^2
1	51.97	3.4	7	9	-2	4
2	22.23	2.1	3	5	-2	4
3	17.81	1.8	2	2	0	0
4	60.54	2.9	8	6	2	4
5	82.31	3.6	10	10	0	0
6	24.60	1.9	4	3	1	1
7	39.71	3.0	6	7	-1	1
8	61.23	3.2	9	8	1	1
9	14.10	1.1	1	1	0	0
10	29.80	2.0	5	4	1	1

Spearman's correlation coefficient (rs) = 0.903 ($P < 0.01$).

correlation test on an actual data set are shown in Table 1. Essentially, ranks are assigned to the X and Y values separately within each column starting with the lowest value and ending with the highest. If some values within the column are the same (called 'ties'), they get the mean of the ranks that would have been assigned to these values. The ranks are subtracted for each pair of values (D = X - Y), the differences are squared (D2), and the sum of the squared ranks calculated (SD2 = 16). The value of N* is then calculated:

$$N^* = n^3 - n = 990 \quad (1)$$

where 'n' is the number of pairs of observations.

If there are no ties, as in the present example, Spearman's rank correlation (r_s) is given by the equation:

$$r_S = 1 - (6 \times \Sigma D^2 / N^*) \quad (2)$$

It ties are present, the calculation is more complex. In this case, calculate $\Sigma (T^3 - T)$ for each variable where TY and TX are the number of ties in each run of Y and X respectively. Then calculate:

$$SSY = (N^* - TY)/12 \text{ and } SSX = (N^* - TX)/12 \quad (3)$$

and

$$r_S = (SSY + SSX - \Sigma D_2)/ 2 \times \sqrt{(SSY \times SSX)} \quad (4)$$

If the number of pairs <10, then a table of Spearman's rank correlation can be used to obtain a P value (Snedecor & Cochran, 1980). If $N > 10$, then the table of Pearson's r can be used (Fisher & Yates,1963).

In the present case, the value of Spearman's correlation coefficient (r_s) was 0.903 and $P < 0.01$ indicating a significant positive correlation between the general abundance of fungi in the rhizosphere of *Trifolium pratense* and the degree of secretion of carbohydrate from the plant roots.

Kendall's rank correlation (τ)

Kendall's rank correlation (τ) is another non-parametric method of testing the degree of correlation between two variables and like rs can be used as a measure of an ability to appraise or detect a property by scoring. Like r_s, τ varies from +1 (complete concordance) to -1 (complete disagreement) but it is calculated differently (Snedecor & Cochran, 1980). Kendall's rank correlation is closely related to Spearman's r_s and it probably matters little in most applications which method is actually used. One advantage of τ is that it can be extended to study 'partial correlations', a statistical method we will discuss in a future statnote.

'Gamma'

Another non-parametric correlation coefficient sometimes given by statistical software is called 'gamma'. Gamma is probably closer in interpretation to Kendall's τ than Spearman's r_s but is regarded as a preferable test if the data contain many tied values.

Conclusion

If in a correlation test, one or both variables are small whole numbers, scores based on a limited scale, or percentages, a non-parametric correlation coefficient should be considered as an alternative to Pearson's 'r'. Kendall's τ and Spearman's r_s are similar tests but the former should be considered if the analysis is to be extended to include partial correlations. If the data contain many tied values, then gamma should be considered as a suitable test.

A correlation test provides only limited information as to the relationship between two variables. Fitting a regression line to the data using the method known as *'least squares'* provides much more information and the methods of regression and their application in microbiology will be discussed in the next statnote.

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Anthony Hilton


Richard Armstrong

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

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

careers

Being a Medical Writer

Dr Julie Eastgate describes her route into Medical Writing and explains the rewards and pitfalls encountered throughout her career as a Medical Writer

Few people, even in medical and scientific fields, know what a medical writer is or does. As a result there is no clear career path and most seem to 'stumble' into medical writing via all manner of diverse routes. I stumbled into medical writing about five years ago, having spent 13 years in the university environment, working as a researcher/lecturer in Microbiology and Immunology. I had always wanted to do research, but as my academic career progressed I found that the time I had to devote to it was gradually being whittled away. This forced me to consider my career options and decide that, without the challenge of research, academia was no longer for me. In retrospect, my move into medical writing was based on a very sketchy understanding of my skills and how they related to the job: I had once been told that I wrote well; I knew someone who had successfully moved from research into medical writing; and I would not need to uproot my life if I could work as a freelancer! Luckily for me the move worked and I've never looked back.

So what is a medical writer?

Medical writing is described by Wikipedia as *'the activity of writing scientific documentation by a specialized writer (a medical writer) who is generally not one of the scientists or doctors who performed the research.'* The role of the specialist writer is to facilitate effective and clear communication of medical and scientific data to the target audience. In addition, the medical writer needs to ensure that documents comply with any guidelines specifying content and format (http://en.wikipedia.org/wiki/Medical_writing).

There are two main types of medical writing:

Regulatory

This describes the writing of a range of documents associated with clinical trials, with a view to the data subsequently being submitted to the drug licensing authorities. Pharmaceutical companies may have their own medical writers for this purpose or may use a contract research organisation (CRO) that specialises in conducting clinical trial-related activities. Being a regulatory medical writer requires a clear understanding of the guidelines and procedures associated with conducting and reporting clinical trial data (http://www.ich.org/cache/compo/276-254-1.html).

Medical communications

This is the writing of other types of document such as scientific manuscripts, abstracts, slide presentations, meeting reports and transcripts, web articles, patient information, etc. There are many medical communications agencies in the UK that provide such services to the scientific community.

Other aspects of the work include

proof reading and editing of documents, usually to give a unified feel to documents with multiple authors or to help in the presentation of documents authored by non-native English speakers. There is also a great demand for medical writers who can write in other languages, to help with specialist medical and scientific translation.

How do I become a medical writer?

The skills required to be a good medical writer include: an excellent writing ability, to enable you to communicate information in a clear and concise manner that is tailored to your reading audience; the skills to translate complex scientific information into plain English; the ability to learn about new scientific areas rapidly; a scrupulous attention to detail; competence in managing multiple projects; and a commitment to meeting (often tight) deadlines.

The traditional route to becoming a medical writer is to work for a pharmaceutical company, CRO or medical communications agency where you can benefit from in-house training. Most companies require at least a first degree in a medically-relevant life science for those starting in medical writing positions, some will specify the need for a higher degree; editorial experience is also highly regarded.

Alternatively, training is also available in the form of various taught courses. The European Medical Writers Association (EMWA) runs a training programme that can lead to certification either at foundation or advanced level for medical writers. Their training courses cover a range of subject areas and allow participants to opt for a multidisciplinary or more specialised certificate. Other companies offer training and details of these can also be found on the EMWA web site (http://www.emwa.org/), as can details of job opportunities, contact details for medical writing companies operating across Europe and useful information for those interested in becoming a medical writer. There is more information about careers in Medical writing available at http://www.emwa.org/Mum/Career.pdf. Also, the American Medical writers Association hold information for Medical Writers (http://www.amwa.org/default.asp?id=1).

There is also the option to become a freelance medical writer. Realistically this is a fairly risky route for someone with no experience of the field; it probably worked for me as I was initially able to take on writing projects in my spare time and had the option of making my university position part-time as the workload increased. I also found a couple of experienced freelancers who liked my writing style and were prepared to offer help, support and work in the early stages of my writing career. Working as a freelancer has the obvious benefit that you can be flexible about when and where you work, though in reality this often means working long and antisocial hours. The workload can also be very unpredictable; when writing up clinical trials timelines often (usually) slip, so the block of time you set aside is no longer needed and you're left twiddling your thumbs. When the project finally 'lands' you can guarantee that it will coincide with another equally vital piece of work. Knowing a network of other writers who can help out in a crisis is invaluable. At the end of the day, freelancing also means that you are judged on every piece of work and really can't afford to make mistakes.

My experience as a medical writer

The things I enjoy about being a medical writer are numerous:

- The variety in subject matter, documents types and writing styles associated with different projects mean that the work is always interesting and often challenging. Writing up the findings of a new clinical trial or a novel research paper often feels like the 'best of both worlds'— you get to find out about the latest, cutting-edge science without having to work your way through the years of bench and/or clinical research it has taken to get there.

- For many regulatory projects the writer is provided with the basic statistical output and is required to identify key messages from the analyses, before describing them in the final report. As a result the analytical skills I developed during years of research, are still being tested.

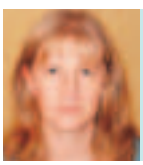
- I find I get great satisfaction from writing documents that are clear and readily understandable! This means tailoring your writing style to a range of audiences e.g. the experts reading a submission for a new drug authorisation or the patient reading the information supplied with their prescription medication. With editing projects there is a great sense of achievement to be had from taking the original document and refining it into the final 'polished' version.

- The satisfaction of having completed a project and, often against the odds, met with the client's deadlines. This has a very different 'feel' when compared with the research environment, where an interesting set of results inevitably leads on to more questions and more experiments...

- The option to travel to attend scientific conferences or client meetings; a real benefit when you usually work alone. However, the travel does sometimes lose its appeal when you add up the amount of time spent in airports and conference centres, and when you realise that the extra couple of days you were planning to add on to the trip to do some sightseeing hasn't happened (again).

- Building a good working relationship with my clients, and having the satisfaction of knowing that they are happy with the work and will return with new projects in the future.

Overall I thoroughly enjoy being a medical writer; the work is interesting, varied and rewarding, and allows me to use many of the scientific skills I developed during my years in academia. Though it is hard work and high-pressure at times, I'm very glad to have stumbled into a career in medical writing.



Dr Julie Eastgate
Eastgate Medical Writing

In the loop pecs

News from the S*f*AM Post-Graduate and Early-Career Scientist Committee

Call for Nominations!

The PECS committee invites nominations for PECS Communications Officer. The post will be available from January 2009 (for one year's term) and is open to all student or early career scientist members of S*f*AM.

Key responsibilities of the role include sourcing articles for the PECS dedicated page in *Microbiologist*, attending PECS committee meetings, representing PECS at the S*f*AM communications sub committee meeting and liaising with both PECS and S*f*AM committee members to develop and implement new communication initiatives. If you are keen to get involved with PECS and think this could be the role for you, then please forward your nomination along with a supporting statement to either:

v.l.mccune@newcastle.ac.uk
gkaboagye@yahoo.com

Vicki McCune
PECS Communications Officer, Newcastle University

get involved

If you would like to get involved with PECS activities please email:

v.l.mccune@newcastle.ac.uk

Roles and responsibilities of a PECS committee member

There are a variety of positions within the Postgraduate and Early Career Scientists committee which are open for nomination each year. These include Chair, Secretary, Communications Officer, Events Officer, Events Team and Webmaster. Each has its own 'job description' and offers the opportunity to develop a range of skills. In this issue of *'in the loop'* we hear from **Jo Heaton** and **Andrew Hall**, who share with us their experiences of being PECS Chair and Secretary.

I have enjoyed acting as Chair of the PECS committee during its first 18 months and contributing to the development of the committee and its dedicated *Microbiologist* page *In the Loop*. I think PECS is fantastic, in that it gives the student members of S*f*AM a real opportunity to get involved with the Society at a higher level, something that is often out of reach. Through PECS I've made some great friends, learnt the value of real teamwork and feel I understand much more about S*f*AM, plus I now know my way around Birmingham!

The duties of Chair are straightforward: liaise with the Secretary to ensure an agenda is prepared for meetings, run the meetings (timekeeping, getting discussions going) and follow-up any actions, if necessary. It helps if you don't mind speaking to a room of (initially) strangers. The activities of PECS are diverse and include organising sessions at the summer conference, contributing articles to *In the Loop*, social events, debating over logos and attendance at S*f*AM sponsored events, such as the Voice of Young Science Media Workshop. I'd encourage all student and early-career scientists to get involved in PECS activities.

Jo Heaton
University of Central Lancashire

It has been my pleasure to help set up the PECS committee and serve as Secretary during the first 18 months of its operation. The committee aims to help student members become more involved with S*f*AM's activities and to assist the society in serving its student members. Our initial focus has been the development of the student activities at the summer conference; we hope you enjoyed the speed networking and

student careers session in Belfast. Serving as secretary has allowed me to become more involved with S*f*AM by representing the PECS at the main committee and various sub-committee meetings. I have also chaired the student presentation session at the last 2 summer conferences. The less glamorous side of the position is the taking and distributing of minutes from the committee meetings.

Being involved with PECS also allows you to build relationships with other postgraduates and more senior members of the scientific community through the Society. It is also an opportunity to get away from the lab and develop your networking and organisational skills in a different context. S*f*AM, through Dr. Anthony Hilton and now Dr. Mark Fielder, support the activities of PECS. However, it is up to the committee members to come up with and drive forward the initiatives for the benefit of the student members and the society as a whole. Look out for a PECS member at your next S*f*AM meeting and get yourself *in the loop*.

Andrew Hall
University of Wales Institute Cardiff

Students into Work Grant reports

information

am I eligible - can I apply?

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

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

Mutation rates of *Campylobacter jejuni*

***Campylobacter* species are** recognised by the World Health Organisation as the most important causative agents of diarrhoea worldwide, with official estimates of up to 400-500 million cases per year.

Campylobacteriosis is transmitted mainly through contaminated food of animal origin; most commonly chickens but to a lesser extent, pigs and cattle are considered to be common vehicles of infection. Up to 80% of chicken broiler flocks in the United Kingdom may be infected with *Campylobacter*, increasing in prevalence of up to 100% in Africa. There have been several studies providing data to support the contention that poultry play an important part in the transmission of *Campylobacter* species to humans.

This study took place at the University of Nottingham, with the aim of determining the mutation rates, to antibiotic resistance, of *Campylobacter* isolated from poultry when cultured at two different temperatures; 42°C, the temperature of the poultry gut, and 37°C, the temperature of the human gut. Our objective was to see if there was a difference between the way *Campylobacter* responded to these two conditions. High mutation rates have been linked to the development of antibiotic resistance, especially multi-drug resistance and we wanted to see if the rates in *Campylobacter* were particularly high and if they showed any response to temperature.

Since their discovery in the 1930s, antibiotics have become arguably the most significant therapeutic advance in medical history, and have saved more human lives than any other drug. There are an increasing number of problems associated with the mass use of antibiotics as therapeutics, most namely the development of antibiotic resistant bacteria. The genetic plasticity of bacterial genomes, combined with short generation times, allow bacteria to rapidly develop and exploit mutations conferring resistance.

Through a diverse range of molecular mechanisms, resistance to every class of antibiotic discovered to date, has been developed by various bacterial species. This has been particularly noted in species of *Campylobacter*, where there has been a substantial increase in the level of antibiotic resistance since the early 1990s.

Strains of *Campylobacter* that are multi-drug resistant are increasingly found. A study in India in 2004 showed that 30.6% of *C. jejuni* and *C. coli* isolates were resistant to multiple antibiotics. The most common combination of resistance was to tetracycline, ciprofloxacin and ampicillin. This level of multi-drug resistance is markedly higher than a similar study performed during 1989-1993, when only 2.2% of strains showed multi-drug resistance.

The increase in prevalence of multi-drug resistant strains may be linked to the use of antibiotics as growth promoters in countries outside the EU and USA. Countries such as India, Taiwan and Thailand still permit this practise and the high frequency of multi-drug resistance in *Campylobacter* is likely to be a major problem in coming years.

The antibiotic used in this study was ciprofloxacin, a compound belonging to the fluoroquinolone class of antibiotics. Fluoroquinolones are a family of broad-spectrum agents commonly used in the treatment of a range of human diseases.

In the past they have also been approved for use in the treatment of enteric and respiratory infections in animals. In 1995 the United States approved the use of the fluoroquinolone enrofloxacin for the treatment of poultry and in 1998 for treatment of cattle. A single mutational event in *C. jejuni* is sufficient to produce high levels of fluoroquinolone resistance. During the 1990s up to 55% of *Campylobacter jejuni* samples isolated, in the USA and Canada, were resistant to ciprofloxacin. As of 2005, enrofloxacin has been withdrawn by the FDA as a treatment for poultry directly due to the development of fluoroquinolone-resistant *Campylobacter* that were subsequently transferred to humans posing a significant hazard to health.

During this study the mutation rates to antibiotic resistance, of various *Campylobacter* strains isolated from poultry, were examined. The strains all showed relatively low mutation rates: 3.9×10^{-11} per cell per generation up to 1.1×10^{-8} per cell per generation. Surprisingly this is well within the range seen for other species of bacteria possessing a full range of DNA repair systems. *Campylobacter jejuni* has a relatively small genome, and it was previously considered that it did not possess a DNA mismatch repair system, or at least one has not so far been identified. The low mutation rates obtained in this study suggest that *Campylobacter* may indeed have some form of post-replicative DNA repair system to cope with point mutations that occur during DNA replication. Despite the 300-fold range in rates, there was a definite clustering of rates in the range 1×10^{-10} to 5×10^{-10} per cell per generation and we did not find any evidence of obvious mutator strains, those strains with highly elevated mutation rates, in the limited sample of 17 strains studied during this project.

A small difference in mutation rates was observed between 42°C and 37°C. Generally the rate was observed to be slightly higher at 42°C than at 37°C. However, the differences were not significant in most cases and may have been caused by a higher level of endogenous oxidative damage occurring at 42°C, or an increase in exogenous mutagens from the growth media at the higher temperature. Unfortunately due to time constraints it was not possible to further investigate this but the project seemed to suggest that growth temperature, under the conditions of our experiments, does not have a significant effect on mutation rates of *Campylobacter* isolates from chickens. I would like to take this opportunity to thank all the staff at the University of Nottingham Food Microbiology department, especially Dr Andrew Timms, for their help during this project.

Craig Derby
Nottingham University

Inter- and intra-species communication between feline oral bacteria

This summer I was fortunate enough to be awarded an S*f*AM Students into Work Award. This allowed me to participate in research that is focused on the microbiology of feline dental plaque in Dr. Alex Rickard's laboratory. Ever since I took my first microbiology class here at Binghamton University, my fascination with microorganism has grown. Indeed, what interests me most is that many microorganisms seem innocuous and yet, if given an opportunity, have the ability to cause debilitating disease. Summer-break research in Dr. Rickard's lab has further elevated my desire to investigate the ability of micro-organisms to become our friends or foes.

My project was to study intra- and inter-species communication between feline dental-plaque bacteria. Intra-species communication between bacteria is a field of study that has received increasing attention over the last decade; especially signaling mediated by extra-cellular low molecular weight signal molecules. Inter-species communication has received less attention and yet most bacterial communities consist of many species (if not hundreds) that are often closely associated in biofilms. This raises the questions: can and how do these different biofilm species communicate with one another? Does communication mediate multi-species biofilm development and changes in community composition? With this in mind, can we exploit inter-bacterial communication to favor innocuous commensal species and select against pathogenic species?

Periodontal disease is one of most common health problems in domestic animals and occurs as a consequence of biofilm development on the teeth of animals. It afflicts approximately 80% of dogs and 70% of cats over three years of age. Periodontal disease begins with the accumulation of plaque, salivary proteins and bacteria on the surfaces of teeth. If not removed, plaque eventually hardens to tartar, which provides a rough surface for even more plaque to accumulate; in turn leading to the inflammation of the gums. As the inflammation of the gums continues, the bone around the roots of the teeth begins to deteriorate. As the bone tissue is destroyed, teeth may become loose and fall out. Animals can also experience infection of vital organs such as liver, kidneys, lungs and heart. Unfortunately, there has been little research performed on periodontal disease in cats and other animals. When considering the discomfort of periodontal disease to pet and owner, as well as the financial burden associated with treatment, a greater knowledge of the microbiology of feline periodontal disease will aid in the development of novel treatment strategies.

Feline dental plaque is an example of a complex multi-species biofilm and, similar to human dental plaque, can contain hundreds of Gram positive, Gram negative, aerobic and anaerobic

species. The vast majority are commensal species. However, as the biofilm community develops, there is potential for pathogenic species to integrate into the community and cause periodontal disease. Research conducted upon bacteria within human dental plaque indicates that oral bacteria can communicate with one-another by at least two distinct mechanisms. One mechanism is through physical communication such as autoaggregation and coaggregation and the other is through chemical signaling via the production of low molecular weight signal molecules such as autoinducer-1 and autoinducer-2. Similar mechanisms of communication may exist in dental plaque from animals. In order to determine if feline oral bacteria are also able to communicate by similar mechanisms, I examined their capacity to coaggregate, autoaggregate, and to produce autoinducer-1 and autoinducer-2.

The ability to aggregate and produce signals is believed to promote multi-species biofilm development. In order to detect signal molecules, filter-sterilized supernatants of exponential and stationary phase batch-cultures of feline oral bacterial species were used to detect autoinducer-1 and -2. This was performed in a 96-well bioluminescence detection system. While work is still underway, my data demonstrated that of 29 morphologically distinct feline oral isolates, greater than 90% produced autoinducer-2 and just one isolate produced low concentrations of autoinducer-1. Such a finding is exciting, as it demonstrates that similar to bacteria from the human oral cavity, autoinducer-2 is produced by species isolated from the feline oral cavity. Is it possible that autoinducer-2 promotes feline oral biofilm development?

With respect to physical communication, by means of aggregation, I studied autoaggregation and coaggregation. Both are believed to promote the ordered succesional integration of species into oral biofilms. Autoaggregation mediates the adhesion of genetically identical bacterial to one-another, whereas coaggregation mediates the specific recognition and adhesion of genetically distinct bacterial to one-another. Both autoaggregation and coaggregation play a role in succession of colonization of human-dental plaque. Preliminary studies of the oral bacteria showed very few coaggregation interactions. However, greater than 25% of the feline dental plaque isolates autoaggregated. Research is being continued to confirm these results and to test the effect of different buffers on autoaggregation and coaggregation.

I found my S*f*AM work placement to be very effective and worth-while. It has provided me with experience, confidence and basic skills to begin working in a research lab in the future. The S*f*AM Students into Work has greatly motivated me to pursue a PhD after completing my undergraduate degree. I would like to thank the Society for Applied Microbiology for giving me this great opportunity and also to my fellow laboratory coworkers for their help and guidance.

Toyosi Muse
Binghampton University, USA

Genotyping *Campylobacter* spp. of Human Origin

Having recently graduated in Food Science at the Queen's University of Belfast, I did not hesitate in accepting the opportunity to work on a microbiology project in the Food Microbiology Branch of the Agri-Food and Biosciences Institute (AFBI). I have been awarded a postgraduate studentship by the Department of Agriculture and Rural Development (DARD) to undertake research commencing in October, and considered the summer experience offered a valuable opportunity to enhance my practical skills and gain hands on experience of working in a laboratory setting.

Human isolates of *Campylobacter* spp. from Hammersmith Hospital in London, isolated over a period of six months, were investigated. Prior to typing, DNA was extracted from pure cultures by use of the phenol chloroform method. The clinical isolates were typed using the amplified fragment length polymorphism (AFLP) protocols described by Kokotovic *et al.*, (1999). This technique is based on the selective polymerase chain reaction (PCR) amplification of the restriction fragments produced by the digest of genomic DNA of the bacterium. The technique is a multi-step procedure and can be broadly divided into three stages:

- digestion of the bacterial DNA by the use of two restriction enzymes or endonucleases (*Bgl* II and *Csp* 61) and subsequent ligation of adaptors to the restriction fragments produced by these enzymes
- direct selective PCR amplification of these fragments with two primers (BGL2F-0 and CSP61-A) that have corresponding adaptor and restriction site specific sequences
- field inversion gel electrophoresis for the separation of the amplicons produced and visualisation of the band pattern to ensure the success of the previous two steps.

The samples were run on an ABI 3100 sequencer and the resulting AFLP profiles were imported into BioNumerics 4.50 software and analysed using the Pearson product-moment correlation co-efficient and clustered by the UPGMA method - the clusters being expressed as dendrograms. These clusters are calculated on the basis of the similarity of each of the samples DNA fragment sizes. A calculated similarity of 90% or greater between samples is viewed to indicate that the samples are identical.

Of the 28 samples, 26 were identified as *Campylobacter jejuni* and two as *Campylobacter coli*. These are the two most common species of

the *Campylobacter* genus which cause human illness or campylobacteriosis (Jay, 2000). Samples were initially identified using phenotypic methods, such as biochemical tests of Gram staining, the oxidase and catalase test and the hippurate hydrolysis test, in conjunction with a multiplex PCR assay. From the resulting dendrogram, two significant clusters were obtained; cluster one containing 28 isolates (*Campylobacter jejuni*) and cluster two containing two isolates (*Campylobacter coli*). The similarity between these two clusters was 9.1%. Within the cluster of *Campylobacter jejuni*, genetic diversity was observable as there were five main groups. Group 1 (n=4) clustered at 82.7% similarity, Group 2 (n=3) at 89.5%, Group 3 (n=3) at 88.4%, Group 4 (n=4) at 70.6% and Group 5 (n=4) at 76.5%.

Within these clusters, it was found that some isolates were effectively identical, with over 93% similarity shown; perhaps indicating that the cases had a common source. The remaining isolates clustered at over 60% similarity. The *Campylobacter coli* isolates (n=2) clustered at 77.7% similarity. In total 39% of the isolates showed similarity over 90% with at least on other isolate.

As the Bionumerics software used had a vast array of AFLP profiles stored on its database, it was possible to compare the Hammersmith Hospital isolates with those from other geographical areas. This analysis produced some interesting results. When compared with clinical isolates from Northern Ireland, a significant number of the Hammersmith Hospital isolates showed over 80% similarity, with 10 isolates showing over 90% similarity, thus indicating a reoccurrence of these strains as a cause of human illness. Only seven of the isolates were not associated in either a 80% or 90% cluster. Similar results were also shown through a comparison with clinical isolates from the Republic of Ireland, with 16 Hammersmith Hospital isolates showing similarity of over 70% with those isolated in Dublin; two of which showing over 90% similarity. Comparison of the isolates with other countries, however, such as Spain, Sweden and the Czech Republic did not show as significant similarities between isolates.

The BioNumerics database also holds information on isolates obtained from food sources. Comparison of the London isolates with those from poultry also produced some interesting similarities. Of the 28 isolates from Hammersmith Hospital, 21 isolates showed over 80% similarity with poultry isolates, six of these isolates showing over 90% similarity. This information highlights the role of food, poultry in particular, in transmitting the organism to humans, thus causing illness in the form of food-borne gastroenteritis.

I consider the placement extremely beneficial in providing an invaluable experience and insight into scientific research; an experience that has provided me with the opportunity to develop a wide range of skills such as time management, planning and working as part of a team, as well as providing me with knowledge of practical laboratory techniques and giving me an opportunity to develop practical skills. It has prepared me for undertaking of the more in-depth research which I am about to embark on.

I would like to thank Dr. Robert Madden for supervising this project, Mrs Carmel Kelly and Mrs Lynn Moran for their guidance, knowledge and advice throughout. I would also like to thank the Society for Applied Microbiology for enabling me to carry out this research and providing me with this valuable experience.

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Sarah Broderick
Agri-Food & Biosciences Institute, Belfast

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Development of a qualitative exposure assessment for *Salmonella* in grade A shell eggs on the island of Ireland

In the 1980s the incidence of salmonellosis in the human population in Europe rose markedly due to the emergence of *Salmonella* Enteritidis PT4. This organism caused a systemic infection in laying hens with the result that grade A shell eggs were laid with the organism present in the egg contents. Subsequently foods prepared from eggs which were not fully cooked could act as vehicles for human infection. Consequently measures were introduced to obviate this threat to human health. In the UK, the Lion Quality code of practice was re-introduced in 1998 and now covers approximately 85% of eggs produced.

This scheme requires the vaccination of commercial layer flocks against *Salmonella* Enteritidis, in addition to controls for welfare, hygiene and bio-security. This scheme is widely adopted in Northern Ireland (NI). In contrast, in the Republic of Ireland (RoI) vaccination of flocks is not permitted. Egg production is regulated by law requiring routine monitoring of feeds and flocks for *Salmonella*. Any flocks found to be infected with *S*. Enteritidis or *Salmonella* Typhimurium must be slaughtered under this legislation. Also, since 1999, eggs produced under the voluntary Bord Bia (Irish Food Marketing Board) Egg Quality Assurance scheme have been subject to further controls governing aspects of hygiene, flock welfare, packaging of eggs and environmental protection.

*Safe*food, The Food Safety Promotion Board, funded a project to investigate the efficacy of the two regimes, with the author as project leader. The project aims were to determine the current prevalence of salmonellas in eggs produced under the NI and RoI control regimes, and then to develop both a qualitative and a quantitative risk assessment for salmonellas in eggs produced on the island of Ireland. The Food Microbiology Branch, Food Science Department, Queen's University of Belfast (QUB) analysed the Northern Ireland eggs whilst Republic of Ireland eggs were analysed at the Centre for Food Safety, School of Agriculture, Food Science and Veterinary Medicine, University College Dublin (UCD).

The statistical design of the egg survey, based on UK data, required that 2500 boxes of six eggs, in both the Republic of Ireland and Northern Ireland, were to be obtained and analysed for the presence of salmonellas using methodology based on BS EN 12824:1998. The analysis involved separating the egg contents from the shells and analysing these components separately. In total 5,018 samples, comprising 30,108 eggs, were analysed with 2503 samples produced in Northern Ireland and 2515 in the Republic of Ireland. External quality assurance samples were obtained during the project and both labs correctly identified all positive samples.

Only two samples were positive for *Salmonella*, and in both cases only the shells were contaminated. One serovar was isolated from each positive sample and these were identified as *Salmonella* Infantis and *Salmonella* Montevideo. No significance difference ($p>0.05$) between the frequency of isolation of salmonellae in samples from Northern Ireland and the Republic of Ireland was found; hence the data were used to estimate of the prevalence on the island of Ireland as a whole. This data was used as a starting point in the development of the exposure assessment for eggs produced on the island of Ireland.

The results of the survey thus indicated a negligible incidence of *Salmonella* contamination of eggs produced on the island of Ireland, at the time of sampling. Most available evidence suggests that internal egg contamination typically involves small initial numbers of *S*. Enteritidis. Studies of eggs produced by naturally infected birds have demonstrated contamination levels of less than 10 CFU per egg after 7 days and it has been suggested that numbers introduced at time of lay could be as low as only one or two cells, hence the former data may represent numbers occurring after an initial growth phase seen in eggs inoculated less than 24 hours after lay. Based on this data the level of *Salmonella* in eggs, when present, was judged to be low. The combination these two parameters resulted in a low risk from *Salmonella* spp. in eggs produced in Ireland, at time of sampling.

Both qualitative and quantitative models are being completed at the time of writing and the author was able to present the current results at the 53rd International Congress of Meat Science and Technology (ICoMST) held in Beijing, August 5 to 9, 2007. Professor Tom McMeekin, University of Tasmania, gave a keynote address detailing the quantifiable benefits of predictive microbiology and explaining how microbiological risk assessment could benefit the meat industry. He subsequently co-chaired a discussion of the poster presentations and our study was selected to open the discussion forum therefore I had the opportunity to explain to the conference delegates the rationale for our study and the benefits that *safe*food, our funding body, expected to accrue from it.

During the congress I was able to meet meat microbiologists from across the globe and discuss this study, and related work, with them. The dynamism of staff and students from the Chinese universities came across strongly in these meetings.

I am grateful to S*f*AM for helping me to make our work known internationally, and allowing me to have face-to-face discussions with fellow microbiologists from over 40 countries. I am also grateful to my colleagues who are currently completing the project; Dr Laura Murchie (QUB), Dr Paul Whyte (UCD) and Mr Bin Xia (UCD), and finally Dr Louise Kelly of Strathclyde University who is supervising the development of the models.

R Madden
Agri-Food & Biosciences Institute, Belfast

Statistical analysis and data handling

Statistical analysis and data handling skills are an important research tool. A good understanding of statistics is necessary for successful experimental design and results in increased confidence in the data generated. Ph.D students are often offered statistical training, either at University or by the research councils. However, these courses are often rather general and led by social scientists, which can lead to confusion and misinterpretation of methods.

Statistics for Industry specialise in the provision of tailored and accessible statistical courses for applications throughout industry and research. I received a President's Fund Award to enable me to attend a 'Statistics for Microbiologists' course, held from 22-24 February in Harrogate.

The course began with discussion of how each delegate used statistics at work, and what they hoped to achieve from the course: from increasing confidence in quality control measures, to the best way to conduct a ring trial and how accurate a plate count really is. The course leader, Gordon Smith, structured the course material so that each need was covered over three days.

The first session started immediately after dinner. The different types of error were discussed and the need to distinguish between these in order to use the correct statistical test. The group were then introduced to different types of distribution, with the examples of MPN, spiral plate and pour plate counts used to give a clear understanding. The session ended with a tutorial problem to work through, putting the new information to the test.

Day two aimed to answer the question 'what is the true mean?' Confidence intervals, sample size and degrees of freedom were explained, and their relevance to the mean demonstrated with another tutorial problem. With this background knowledge firmly established, the group were introduced to S4I's Crunch program, a series of Excel macros for each statistical test. With coloured cells for data entry, Crunch was an immediate hit with those who found their usual stats software incomprehensible, and there was a promise of a free copy to take back to the lab! Outlier tests and T-tests were covered before the group were split into teams and asked to demonstrate their new knowledge by using a computer model to analyse the performance of different yeasts in brewing. It was considered rather unfair when the team with a microbiologist from a well-known brewery won hands down!

The remaining sessions covered regression and correlation, quality control, repeatability and reproducibility, the distribution of microorganisms in solids, Cusum analysis and the ubiquitous ANOVA. The combination of lecture, tutorial and group work ensured that everyone understood each aspect of the course, with course members able to share their experience and discuss experimental examples requiring each type of analysis.

Evening sessions allowed each delegate to bring their statistical problems to the table, with advice from Gordon and input from other course members. Naturally, the conversation also turned to microbiological issues and the more experienced microbiologists were happy to share their knowledge.

"The course was a great success, providing in-depth statistical training targeted directly at the problems I had encountered in data analysis. Not only that, but I found it really useful to speak to industrial microbiologists and gain an understanding of their work at the bench. Having microbiological examples made the use of the tests really clear and I've since carried out a number of experiments confident that I am generating testable data that I know exactly how to work with! Thanks very much SfAM."

Statistics for Industry offer a number of targeted courses, including analysing health and safety statistics, process control and experimental design. Courses are residential but in-house training and consultancy is also available. Further information is available at and any enquiries can be directed to, Michelle Hughes at s4i@aol.com.

I would also like to thank Michelle at S4I for offering the course at a reduced 'student' rate.

Jo Heaton
Lancaster University

The effect of low-dose macrolides on *Pseudomonas aeruginosa* and *Vibrio fischeri*

Macrolide antibiotics have many attractive properties for patient treatment as they are well tolerated, can be taken orally and display good tissue penetration. Their spectrum of activity makes them particularly suitable for the treatment of respiratory and soft tissue disease and for infections caused by susceptible intracellular bacteria (Hoyt & Robbins, 2001). Macrolides have been used for more than 45 years as the drug of choice for upper and lower respiratory tract infection. Erythromycin is a relatively broad spectrum macrolide antibiotic used to treat most Gram positive infections and is effective against some Gram negative infections. It has also been used as an alternative therapy in penicillin-allergic patients and is usually prescribed at an initial high dose of up to 1g/L on the first day of treatment, dropping to 500mg/L for the rest of the antibiotic course (Equil *et al.*, 2006; Hoyt & Robbins, 2001).

Studies have shown that macrolide concentrations below the minimum inhibitory concentration (sub-MIC) are an effective treatment for panbronchiolitis and chronic sinusitis (Suzuki & Ikeda, 2002). These antibiotics may exert secondary effects in addition to their well established binding to the 50S ribosomal sub-unit, which interferes with elongation of peptide chain. Secondary effects are thought to include modifying the host inflammatory response and interference

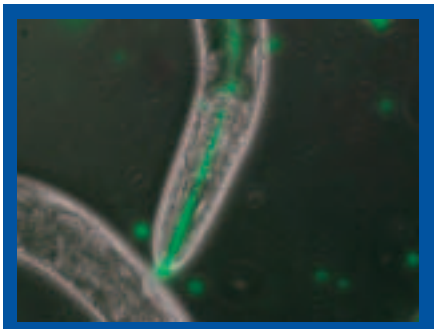

Figure 1. *C. elegans fed with GFP labelled P. aeruginosa.*

with bacterial signalling systems that regulate virulence factor production (Amsden, 2005; Hoyt & Robbins, 2001).

Macrolide antibiotics are known to reduce inflammation associated with a variety of airway diseases (Williams, 2001) and increasing evidence suggests that macrolides may play a role in modulation of the immune response (Hoyt & Robbins, 2001: Chu, 1999). Effective modulation of the inflammatory response by macrolide antibiotics has been shown in airway diseases such as asthma, diffuse panbronchiolitis and a variety of bacterial and viral airway infections caused by *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Haemophilus influenzae* and influenzae virus (Garu, 2001).

The aim of this study was to examine the effect of sub-MIC macrolides on bacterial quorum sensing systems including bioluminescence in *V. fischeri* and virulence in *P. aeruginosa*. Initial work used *V. fischeri* bioluminescence as a paradigm for quorum sensing, and examined the effects of macrolides on this system. It was found that concentrations as low as 1/500th of the MIC of erythromycin reduced the bioluminescence of *V. fischeri*, but the growth of the organism remained unaffected. This suggested that low level erythromycin may exert a specific effect on the quorum sensing regulation of bioluminescence. To investigate this further, the transcription of the *lux* D gene was analysed. Reverse Transcription-PCR (RT-PCR) was used to examine the transcription of the *lux*D gene in *V. fischeri*, which is under the control of the lux quorum sensing system. This was compared to the transcription of part of the *lux*R gene, coding for a constitutively produced regulator protein. The transcription of both of these genes was compared to the 16s rRNA gene which is transcribed as long as the bacterial cells are metabolising. Results showed that low dose erythromycin specifically inhibited transcripts from the quorum regulated *lux*D gene, but did not alter transcription from the other 2 genes.

The study also examined the effect of sub-MIC macrolide antibiotics on bioluminescent constructs of *P. aeruginosa*, where the lux genes were constitutively expressed. A plasmid was constructed using the lux gene cassette from *E. coli* pLITE 27 (Parveen *et al.*, 2001) inserted into a broad host range vector (pBBR 1MCS-5). The *lux* genes in this newly constructed plasmid were regulated by a constitutive *lac*Z promoter. The plasmid was used to transform strains of *P. aeruginosa* including clinical isolates from cystic fibrosis patients obtained from the Public Health Laboratory in Bristol.

The effect of low dose erythromycin on *P. aeruginosa* strains was examined by recording bioluminescence and optical density, either manually or with an automated plate reader. The results showed that sub-MIC erythromycin did not affect growth or constitutively regulated bioluminescence of *P. aeruginosa*.

To investigate the *in vivo* effects of sub-lethal erythromycin, a host-pathogen infection model was set up, using the nematode worm *Caenorhabditis elegans* (Figure 1 – Lizeth's worm pics) as a host for infection with self-bioluminescent reporter strains of *P. aeruginosa*. This enabled real-time *in-situ* monitoring of bacteria inside the host. The *C. elegans/ P. aeruginosa* pathogenisis model is well established for elucidating *in vivo* bacterial virulence and previous work has shown that when ingested by the nematode, bacterial virulence factors play an important role in killing *C. elegans* over a twenty four hour period. (Tan *et al.*, 99 Kurtz and Ewbank etc).

To observe the effects of sub-MIC macrolides, *C. elegans* worms were placed on a lawn of bacteria with and without sub-MIC antibiotics and checked for survival after 24 hours. When the *C. elegans* were fed on lawns of clinical *P. aeruginosa*, they were killed within a period of 24 hours. Control experiments, where worms were fed with *E. coli* DH5α and *P. aeruginosa* PAO1, showed no significant killing. When the same experiment was repeated with clinical *P. aeruginosa* grown on agar plates containing sub-MIC erythromycin, 95% of the *C. elegans* survived. This model was then modified further by feeding the worms with a bioluminescent construct of the clinical *P. aeruginosa* isolate, which produced light as long as the bacteria were metabolically active. When the experiment was repeated with the lux+ *P. aeruginosa* clinical isolate, the results were consistent with the first experiment. The infected worms were removed from the agar and washed thoroughly to remove any surface bacteria and then their bioluminescence was measured. The level of bioluminescence was similar from live and dead worms, indicating that the clinical *P. aeruginosa* inside *C. elegans* were viable, as they were producing bioluminescence, but did not produce lethal effects on the host in the presence of sub-MIC erythromycin.

The use of a host-pathogen infection model, in conjunction with bioluminescence technology, has enabled real-time, *in vivo* monitoring of bacterial virulence modulation and I would like to thank S*f*AM for giving me the opportunity to present part of this work at the 47th Interscience Conference of Antimicrobial Agents and Chemotherapy in Chicago, USA.

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Gill Smith
University of the West of England

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A new edition of CCFRA's Manual of microbiological methods for the food and drink industry (CCFRA Guideline No. 43), used widely by food industry microbiology laboratories, is now available. This, the fifth edition, contains 10 new methods, while many of the existing 58 methods have been updated to take account of recent developments and feedback from their practical use.

Chris Baylis, who edits the manual, explains: *"The methods themselves, which cover the detection, confirmation and enumeration of a range of established and emerging pathogens and spoilage organisms, are fully consistent with CLAS (Campden Laboratory Accreditation Scheme) and will also be of use to companies seeking UKAS (United Kingdom Accreditation Service) accreditation. Flow diagrams provide an at-a-glance overview of each method, which is presented in a standard format which specifies the scope, principle, media and reagents, apparatus and procedure, and results interpretation. References point to valuable additional information such as validation studies and British Standards."*

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VLA launches phenotyping technology access initiative

Scientists at the Veterinary Laboratory Agency, UK, lead by Professor Martin Woodward launched an initiative to allow access to Biolog Phenotype MicroArray technology last month.

The event, in association with Technopath, the UK/ Ireland distributor for Biolog, was attended by scientists from the UK and Ireland. It featured presentations by users of the technology including Prof Shea Fanning (UCD), Prof Jon Hobman (University of Nottingham), Prof John Greenman (University of West of England), Dr Gail Preston (University of Oxford), Dr Julian Marchesi (Cardiff University) and members of the VLA research staff.

Phenotype MicroArray enables microbial cell phenotyping across nearly 2,000 kinetic phenotypic assays including cellular nutritional pathways for C, N, P, and S metabolism (800 tests), pH growth range and regulation of pH control (100 tests), sensitivity to NaCl and various other ions (100 tests), and sensitivity to 240 inhibitors of biological pathways (1,000 tests). It has resulted in an international community of users and over 90 publications.

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For information on the VLA initiative, please contact Prof. Martin Woodward at m.j.woodward@vla.defra.gsi.gov.uk
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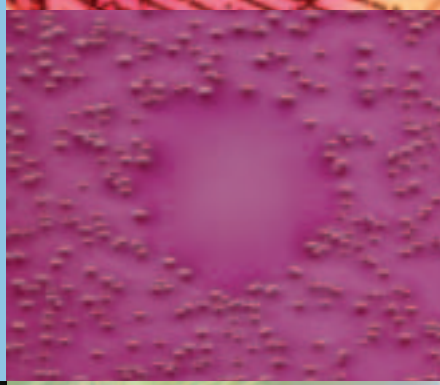

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