Microbiologist

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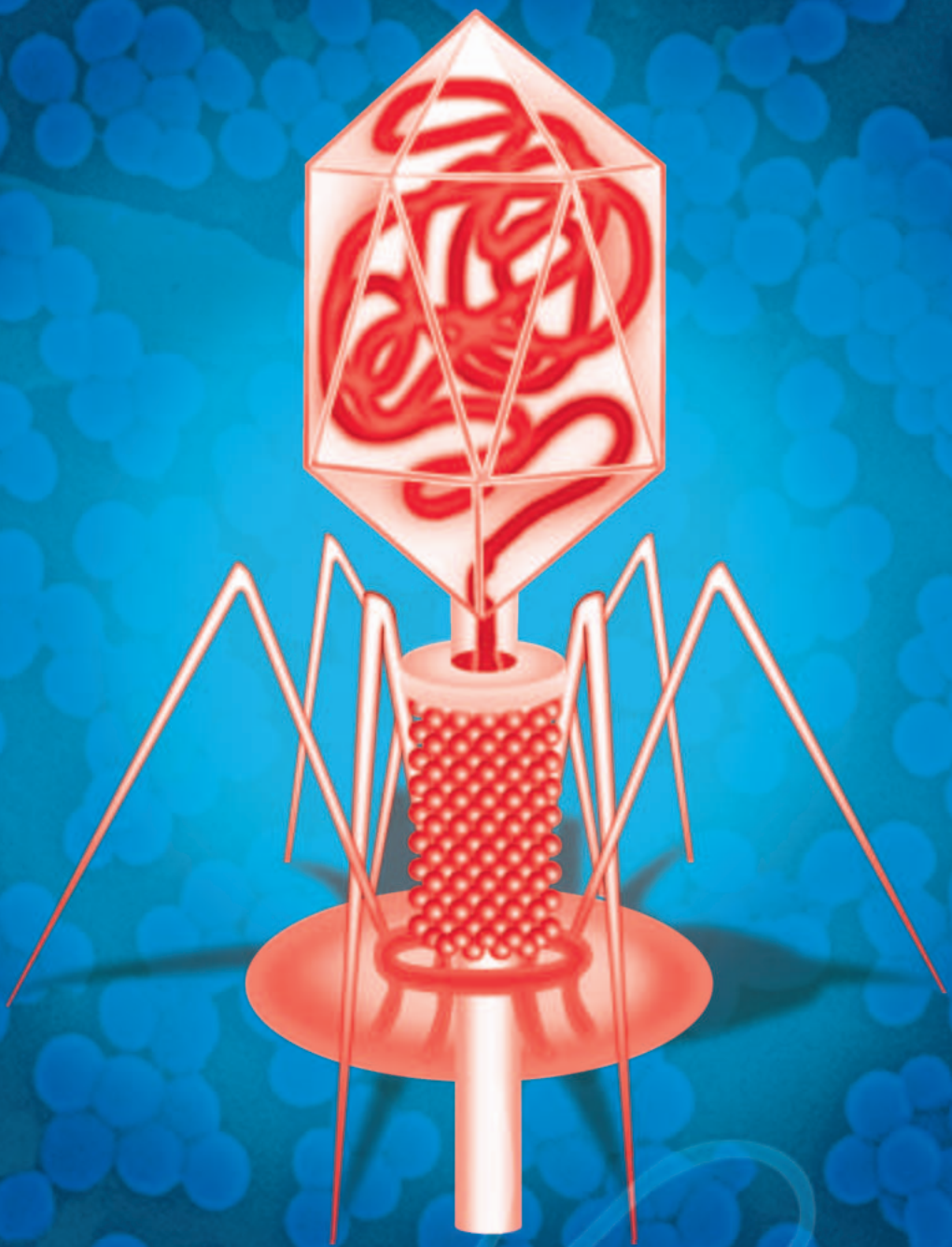

BACTERIOPHAGE THERAPY
old treatment, new focus?

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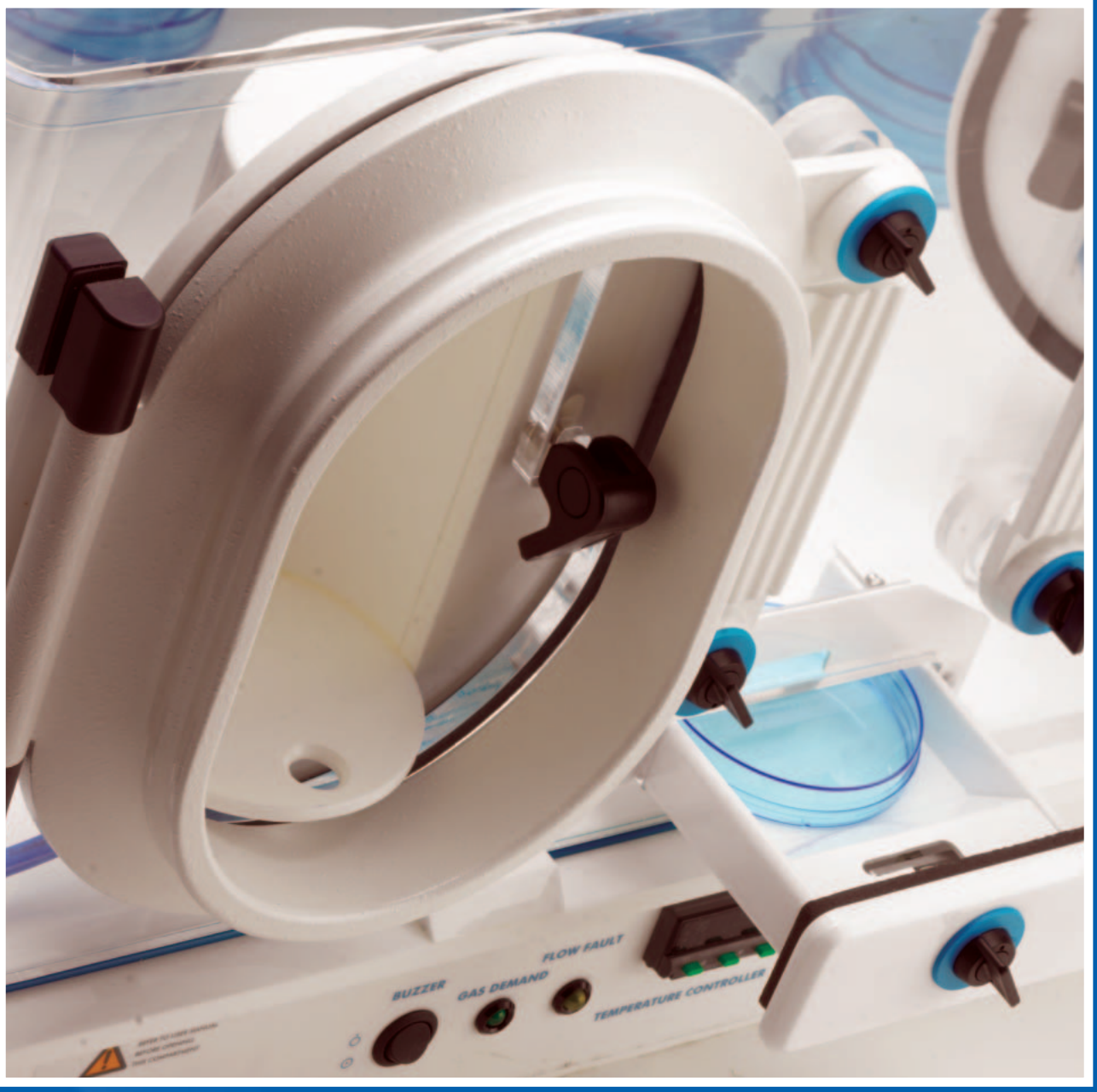
- Antimicrobial resistance in bacterial enteric pathogens
- Mediawatch: university press office
- Summer conference 2009
- Engaging the public in infectious disease
- Charles Darwin and microbes
- Art, cybernetics and normal flora microbiology
- An unwanted guest for dinner
- Careers: sales manager
- Statnote 16: using a regression line for prediction and calibration
- PECS: virtual networking

excellence in microbiology

don whitley scientific



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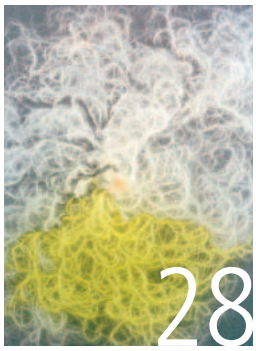
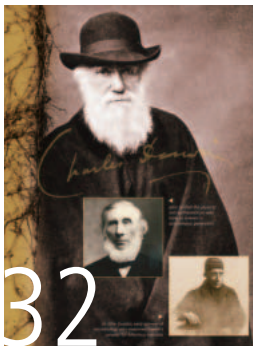


The beauty of bacteria



Charles Darwin and microbes

information

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editorial

Lucy Harper discusses the media coverage of the H1N1 virus and the importance of the effective communication of science

Do you think the media have used hype in their coverage of the recent spread of the H1N1 virus? This is one of the questions the Department of Health are asking the general public in an attempt to assess how effectively the government's messages about this situation are being communicated and whether they are being understood. Other questions include 'Have you visited your GP?' and 'Do you believe thousands have died from it?'* The effective communication of science is important in a world where scientific developments impact on the day-to-day lives of the individual. This importance has been recognised for some time, with the formation of an entire discipline, incorporating qualifications, conferences, organisations and committees dedicated to the subject. But this subject wouldn't be able to thrive were it not for scientists themselves getting involved and acknowledging the importance of the communication of their work.

SfAM supports the communication of science in many ways, from funding public engagement activities, through our Public Engagement/Innovative Project grant, to recognising leading science communicators through the Communications Award. We're always on the look-out for scientists who are keen to talk about their work to a wide and varied audience, from the news media to school children and the general public. After all, when a news story breaks, it's imperative that the public are armed with the correct information so that they can make informed (often important) decisions. Ultimately, the correct information can often only be obtained from scientists — the experts at the bench-top.

One way in which you as members can get involved in communicating science is through publicising your work via the news media. On page 12 you'll find some invaluable advice about communicating your science through your university press office.

SfAM has recently been involved in communicating applied microbiology to the general public through public engagement. We, in collaboration with the Beacons of Public Engagement, held an event in Manchester based around the public's perception of microbiology, and you can read all about the event on page 31.

In this issue of *Microbiologist* we also have a report from the first of our Public Engagement/Innovative Project grants. SfAM's ex-Honorary General Secretary, Dr Anthony Hilton gave a series of Christmas lectures to school children in the North East of England and he reports on the success of these lectures on page 40.

Finally, if you are interested in communicating your work to a wider audience, then get in touch to find out how we can help you publicise your work and ensure that you get the right message across. After all, SfAM is "the voice of applied microbiology".

* all information on the spread of the H1N1 virus from Mexico was correct at the time of going to press

contribute

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

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

Lucy Harper

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

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

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benefits

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

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

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

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

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

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

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

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

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

membership options

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

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

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

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

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

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

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

JOIN US!

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

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

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president's column

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

In these difficult economic times it's good to have a reason to celebrate. That celebration need not necessarily involve cracking open the champagne and dancing until dawn, but may simply be a celebration of achievement, either past or present.

Every year since 1977 the American Society for Microbiology has presented the Procter and Gamble Award, the aim of which is to recognise distinguished achievement in research and development in applied (non-clinical) and environmental microbiology. The award carries a cash prize of $2,000 and the recipient is invited to present a lecture at the ASM General Meeting where they are also an honoured guest at the ASM awards banquet. A glance down the list of previous laureates reveals an array of names representing the glitterati of the world of microbiology.

Since Procter and Gamble is a global company they were conscious that this award tended to recognise American scientists and hence they were very keen to instigate an equivalent award in Europe. I was therefore delighted when last year a colleague at P&G asked if S*f*AM would administer the award in Europe in the same way that ASM does in the USA. The company had looked at different microbiology societies and decided that S*f*AM was the most appropriate. The value of the prize is to be the same ($2,000) and the recipient will be invited to give a lecture at our Spring Meeting in April. The name of the award is the S*f*AM/Procter & Gamble Applied Health Care Microbiology award and will be awarded to distinguished individuals who have used microbiology research to gain a better understanding of human health. By the time you read this article the first award will have been made.

The first, and highly deserved recipient of the award is Professor Sally Bloomfield who qualified initially as a pharmacist, but then went on to develop a career in microbiology, with a specific interest in the prevention of infectious diseases and in microbial quality assurance. For 25 years, Professor Bloomfield was an academic in the Department of Pharmacy, Kings College, London but from 1997 to 2003 joined Unilever Research to develop a programme involved with raising awareness of the importance of hygiene in the domestic setting. During this time she was instrumental in setting up the International Scientific Forum on Home Hygiene.

S*f*AM has, of course, been associated with other awards for outstanding achievement over a number of years. The W. H. Pierce Memorial Prize was instituted in 1984 by Oxoid and is awarded each year to a young scientist (under 40 years of age) who has made an important contribution in the field of applied microbiology. This award is a dual celebration since it acts as a vehicle for highlighting young talent whilst at the same time celebrating the achievements of a distinguished microbiologist of the past. Bill Pierce joined Oxoid Ltd shortly after it was formed at around the time of World War I and was a lifelong member of S*f*AM. He rose to become Chief Bacteriologist and led the development of dehydrated culture media used by all microbiologists in their everyday work.

In a similar vein the Lewis B Perry Memorial lecture is given annually by a distinguished microbiologist in memory of another. Lew Perry spent his entire career with the National Collection of Industrial, Marine and Food Bacteria (NCIMB) where he became a senior scientist. He was a shy, modest man who was extremely generous with his time, willingly teaching younger colleagues the essential skills of microbiology with his trademark attention to detail. Lew Perry, again a lifelong S*f*AM member, died in 2003 and his family have generously offered to fund an award in his memory for a period of 10 years.

Both the W. H. Pierce prize and the Lew Perry lecture are given at the annual summer meeting. At that time also we present the S*f*AM communications award for an individual who has communicated their work most effectively in applied microbiology to the general public. It is also an occasion to recognise young scientists with the student conference prizes for best oral presentation and best poster.

The Denver Russell Memorial lecture was established in 2006 to commemorate the achievements of a microbiologist whom many of us knew personally and had the privilege of calling a colleague. Denver was one of my PhD supervisors and I am proud to have been co-author on just a few of his 450 publications. He was a world authority on biocide usage and its possible association with antibiotic resistance. His knowledge of microbiology was encyclopaedic and he was author or editor of 16 books including the standard undergraduate text *"Pharmaceutical Microbiology"* with his old friend Barry Hugo. The impetus for an award in his name came from the healthcare industry with which he had close working relationship.

Not all of our awards are in memory of individuals. The recently introduced *Environmental Microbiology* lecture is a celebration of the achievements of the journal of that name published by Wiley Blackwell in association with S*f*AM. The inaugural lecture was held in September 2008 and was given by Rita Colwell who is a true thoroughbred in the field of microbiology with a quite astonishing CV.

Her lecture entitled *"Climates, oceans, global warming and Cholera"* was, as you might expect, excellent and a recording placed on the website has been downloaded over 2,000 times. The success of this lecture encouraged us to make this an annual event.

The second *Environmental Microbiology* lecture is to be held this year on 12th October at the Royal Society of Medicine, London and in the last edition of *Microbiologist* you will all have received an invitation to attend. I hope that as many of you as possible will be able to come and listen to what will be another inspiring lecture this time by Professor Edward DeLong of the Massachusetts Institute of Technology. Professor DeLong is recognised for his remarkable achievements in the field of marine microbiology and he is a pioneer in the development and use of metagenomics to address environmental microbiological questions.

I started by saying that the P&G Award presented by the ASM has recognized some truly distinguished microbiologists. It will not therefore surprise you to learn that Rita Colwell and Ed DeLong were both former recipients of this award. I hope you will join me this year in celebrating all these achievements.

Professor Geoff Hanlon
President of the Society

ceo's column

Philip Wheat reports on the latest developments within the Society

In these troubled financial times it is very pleasing to report that membership of S*f*AM is still on the increase. I estimate that our peak membership number for 2009 will be just over 1,500, a large increase compared with this time in 2006 when membership numbers stood at approximately 1,300. It is also pleasing to report that the increase is not only due to new members from the United Kingdom but we now have members from over 80 countries of the world: the Society can truly claim to be an international membership Society.

The increase in membership is due to many different reasons. Firstly, the Society has been a lot more active in the last few years, in terms of promotion and making people aware of all our activities and those with which we are involved. If you would like to help to further promote the Society to colleagues/students in your institution please contact me (pfwheat@sfam.org.uk) and I will be happy to send you our advertising material.

Secondly, in the current challenging financial climate, membership does provide excellent value for money. There are so many benefits — almost too many to discuss in this brief column. We feel we offer terrific value, for instance, standard full and student membership entitles an individual to online access to five peer reviewed journals (*Journal* and *Letters in Applied Microbiology, Environmental Microbiology, Reports in Environmental Microbiology* and *Microbial Biotechnology*). In addition, all members also receive quarterly copies of the *Microbiologist*. This policy of including the society's publications within the membership compares extremely favourably with the policy of other membership societies, where additional charges are made to access scientific publications.

Finally, the society offers a whole plethora of grants for members who are eligible. Once again in recent years more members are successfully applying for the variety of grants on offer (full terms and conditions for all grants can be found at). I must emphasise that grants are available to all eligible members irrespective of whether they are based in the United Kingdom or elsewhere.

During the summer S*f*AM will be exhibiting at a number of conferences. We always appreciate seeing our members and listening to what they have to say about the Society, so don't forget to come visit the S*f*AM stand if you're attending the following meetings: International Food Technology (Anaheim, US, 7th - 9th June) and International Association of Food Protection (Gaylord Resort, US, 12th – 15th July).

Philp Wheat
Chief Executive Officer

Membership Changes

NEW MEMBERS

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

Australia

R. Al Jassim; M. Benghezal; C. Kurekci; H. Rahman

Austria

L. Boddrossy

Columbia

A. Trespalacios

France

D. Meyer

Greece

I. Ntaikou; M. Rissakis

India

R. Kumar

Iran

M. Didari Khamseh Motlagh; A. Makhdoumi-Kakhki; B. Samareh-Abolhasani

Ireland

B. Boots; F. Brennan; J. Brown; S. Cooney; F. Fang

Italy

F Grieco

Mexico

E. Ortiz-Vazquez

New Zealand

P. Andrew

Nigeria

G. Adewumi; O. Okpalanozie

Pakistan

A. Tahir

Singapore

B. Chua

South Africa

J. Lues

Thailand

S. Fuangfong

UAE

S. Singh

UK

S. Ahmed; A. Alnimr; T. Bailey; M. Bell; A. Benson; J. Caddick; E. Campion; H. Ciesielczuk; S. Costello; R. Daniel; M. Dempsey; H. Esom; S. Foley; M. Fowler; N. Gibbins; C. Goswell; N. Green; A. Guha Roy; G. Hayburn; C. Hobday; G. Kane; S. Kava; J. Ma; K. Mallard; L. Marsh; D. McEwan; K. Miller; C. Milne; D. Morrow; E. Nya; G. O'Neill; L. Ouoba; C. Parry; D. Perera; J. Ramsay; A. Salman; V. Shanmuganathan; L. Snyder; H. Tan; E. Theophilou; M. Walker; S. White

USA

H. Barton; Y. Hasegawa; K. Richardson

West Indies

V. Prajapat

UK NEW CORPORATE

E & O Laboratories Ltd; NCIMB Ltd

LOSSES

Dr P W Jones, Full Ordinary Member since 1991, Institute of Animal Health, Berks. UK.

Erratum

The Editors of *Microbiologist* would like to apologise for the following errors which appeared in the article entitled: Zoonoses: past, present and future in the March 2009 issue of Microbiologist. In table 1. the causative agents *Borrelia afzelii* and *Borrelia garninii* should not have been represented as: *B-afzelii* and *B-garinii*, they should have been written: *B. afzelii* and *B. garinii*. Furthermore, the agents *Tinea corporis, Tinea cruris* and *Tinea pedis* should have been written as follows: *T. corporis, T. cruris* and *T. pedis*. In addition, the legend to one image on page 27 should have read: *E. Coli* 0157 (SEM).

Lost members: do you know where these members are?

We currently have some members on our records who have moved away or to another job and have not informed us of their new address. Do you know of the whereabouts of any of the following members?

Name	Last known address
Mr T W G Downey	MedImmune UK Ltd., Liverpool
Dr R A Collett	University College Worcester, UK
Dr Emma Best	Leeds General Infirmary, UK
Professor E Senior	Centre for Advanced Water Technology, Singapore
Professor M H Brown	Unilever Research, Bedfordshire, UK
Mr Kevin Shade	Bio-Products Laboratory, Hertfordshire, UK
Mrs Christine Moody	Norpath Laboratories Ltd., County Durham, UK
Mr Yui Cheung	University of Glasgow, UK
Mr Andrew Lamb	University of Bolton, UK
Miss Nicola Petty	University of Cambridge, UK
Dr P N Hobson	Grampian, UK
Mr R T Parry	Spain
Mr Vicente Gomez-Alvarez	Morrill Science Centre, Amherst, USA
Miss Azra Khan	City Hospital, Birmingham, UK
Dr Joseph Kleinhenz	Cabot Creamery, Vermont, USA
Miss Cassie Pope	Royal Free Hospital, London
Dr Guy Derdelinckx	Kuleuven Malting and Brewing, Belgium

If you can help, then please contact **Julie Wright**, Membership & Finance Co-ordinator.
Email: Julie@sfam.org.uk or telephone: +44 (0) 1234 326661.

membership matters

Congratulations!

Some of our members may be aware that at the end of April, past President of S*f*AM, **Professor Peter Silley**, embarked on his 2009 challenge of riding a push bike from Lands End to John O'Groats. Having successfully completed his challenge, Professor Silley raised a considerable amount of money for two well-deserving charities which are close to his heart. S*f*AM would like to extend our congratulations to Peter for this worthwhile accomplishment.

journalWatch

News about the Society's journals

Journal of Applied Microbiology

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

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

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

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

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

Cloning, expression and characterisation of the serine protease gene from *Chaetomium thermophilum*. A.-N. Li & D.-C. Li, **Vol. 106**, No 2, February 2009.

Letters in Applied Microbiology

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

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

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

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

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

Bifidobacterium and *Lactobacillus* DNA in the human placenta. R. Satokari, T. Grönroos, K. Laitinen, S. Salminen & E. Isolauri, **Vol. 48**, No. 1, January 2009.

Environmental Microbiology

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

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

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

Reverse dissimilatory sulfite reductase as phylogenetic marker for a subgroup of sulfur-oxidizing prokaryotes. Alexander Loy, Stephan Duller, Christian Baranyi, Marc Mußmann, Jörg Ott, Itai Sharon, Oded Béjà, Denis Le Paslier, Christiane Dahl & Michael Wagner, **Vol. 11**, No. 2, February 2009.

Metagenomic and stable isotopic analyses of modern freshwater microbialites in Cuatro Ciénegas, Mexico. Mya Breitbart, Ana Hoare, Anthony Nitti, Janet Siefert, Matthew Haynes, Elizabeth Dinsdale, Robert Edwards, Valeria Souza, Forest Rohwer & David Hollander, **Vol. 11**, No. 1, January 2009.

Swarming motility: a multicellular behaviour conferring antimicrobial resistance. Sandra Lai, Julien Tremblay & Eric Déziel, **Vol. 11**, No. 1, January 2009.

Microbial Biotechnology

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

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

A broad range of themes in Microbial Biotechnology. Craig Daniels & Juan-Luis Ramos, **Vol. 2**, No. 1, January 2009.

Uracil influences quorum sensing and biofilm formation in *Pseudomonas aeruginosa* and fluorouracil is an antagonist. Akihiro Ueda, Can Attila, Marvin Whiteley & Thomas K. Wood, **Vol. 2**, No. 1, January 2009.

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

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

Environmental Microbiology Reports

Highlights of the second issue of Environmental Microbiology Reports:

Oestrogenicity of prenylflavonoids from hops: activation of pro-oestrogens by intestinal bacteria Sam Possemiers & Willy Verstraete, **Vol. 1**, No. 2, April 2009.

Honeybee colony collapse due to *Nosema ceranae* in professional apiaries. Mariano Higes, Raquel Martín-Hernández, Encarna Garrido-Bailón, Amelia V. González-Porto, Pilar García-Palencia, Aranzazu Meana, María J. del Nozal, R. Mayo & José L. Bernal, **Vol. 1**, No. 2, April 2009.

South American native bumblebees (Hymenoptera: Apidae) infected by *Nosema ceranae* (*Microsporidia*), an emerging pathogen of honeybees (*Apis mellifera*). Santiago Plischuk, Raquel Martín-Hernández, Lourdes Prieto, Mariano Lucía, Cristina Botías, Aránzazu Meana, Alberto H. Abrahamovich, Carlos Lange & Mariano Higes, **Vol. 1**, No. 2, April 2009.

Lucy Collister
Wiley-Blackwell

The University Press Office

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.

When I walk into the press office at 8.45am I never know what to expect. I could be met with a tirade of enquiries from the media about that day's superbug story in the first half an hour. What makes this go without a hitch is the able and willing scientist who answers the phone with a positive response.

Your press officers use two different techniques to try to help you get the best media coverage for your research: proactive and reactive response to media enquiries. Proactive work means we're trying to interest the public in the research our scientists are doing at that university, or being funded by that research council, or being presented at that society's conference, for example.

Your research

As a university press officer, typically a researcher or journal will contact me about a forthcoming paper. I will go and meet the researcher to find out more about what they are doing and to decide whether the research is suitable to publicise. If the new findings are newsworthy, if they have an impact on people's lives or if they are intrinsically fascinating, I may decide to issue a press release. If the research is of interest to the university community, I could choose to publicise the new study internally, in a magazine or on the website. There are also lots of new media options, including podcasting, video interviews and even Twitter.

A press officer has to make a judgement call about each story. The research might be wonderful scientifically, with flawless methods that change the way proteins are purified forever, but that does not necessarily make a story newsworthy. A great story has drama, a human aspect and is almost tangible; can you show us images or tell us stories?

Danielle Reeves, Senior Press Officer at Imperial College London, says time is of the essence when it comes to press releases. *"If you have some research that is interesting and newsworthy, that the world needs to know about, the easiest way for us to publicise it is if you have a paper being published. Usually, journalists will only write about something the day it appears in the public domain, so we need to know in advance if we are going to tell them about it. Let your press officer know at an early stage, when your paper has been accepted or even when you submit it for publication."*

***Lucy Goodchild** provides us with an insight into the way your University Press Office can help you publicise your work*

Writing and issuing a press release can be a lengthy process. After the initial meeting with the scientist, I will review my notes and draft a press release, using all the information I can get my hands on. Accuracy is vital – the reputation of the scientist and the university is important to protect – but detail comes down to yet another judgement call.

The purpose of a press release is to sell a story to journalists. We must explain the main findings of your research and say why they are important to the general public in 500 words. Just as there is a certain style to adopt when you write an abstract or a scientific paper, press releases also have their own style. Myc Riggulsford, Managing Director of The Walnut Bureau and science journalist, broadcaster and media training consultant, puts it simply: *"Tell them the most important things first — tell them what you've done. Imagine a sentence that starts 'scientists from the Society for Applied Microbiology announced today that...' — What would you say in the next 25 words? Can you sum up what it is you are trying to say? Work out what the important news is."* This can be difficult for some scientists, especially if they have been working on a study for years; the details a scientist considers important can be very different to those a journalist might be looking for.

The answer? Trust us! *"If you are working with me on a press release and I send it to you for revisions, it would be helpful if you could bear in mind that we are professional experts in this field, just as you are in your field,"* said Danielle. *"If the first draft I send you is somewhat different to the way you characterise your research, bear with me. We will always work together to come to a mutual agreement on a press release, but it would be very helpful if you could keep an open mind and trust us."*

Their research

Press offices can serve as little black books for journalists. Most universities, research councils, societies and charities that have press officers will have a database of experts, which they can search through if a journalist calls asking to speak to an expert. This is where reactive work comes in.

It is common for a press office to be contacted by several media outlets about the same thing, especially if a big story has broken, and it can be a surprise. Scientists who are happy to talk to many journalists and producers about a news story are vital in situations like this. We also receive several different calls and emails about different subjects from different media outlets on any given day. Having access to a large number of scientists who are happy to talk to the media and comment on stories is essential for a press officer to deal with the enquiries effectively.

The first thing you can do as a scientist to help with media enquiries is to sign up to databases. Get in touch with press officers at your university, funding body, professional society and charity to get onto their expert lists and media guides. If we don't know you want to deal with the media, we can't help you!

There are a few main things to remember if you want to be a successful media contact. According to Myc, the first thing is simple: *"Don't use jargon!"* It's all about making your science accessible and interesting. *"Make mental pictures for people. Describe things in a way that lets them see what you're talking about, instead of using theoretical language,"* said Myc. But how can you tell if you're getting it right? *"Imagine yourself bursting through the doors of a primary school and saying 'hey kids, guess what?' You should be understandable."*

Talking to the media can be daunting but don't forget, you are not alone. You are surrounded by press officers. In fact, you will be hard pushed to avoid us. We're not just here to help journalists, we can also support you and offer advice. I never give out a scientist's number without permission each time a journalist calls. I always make sure I know who I'm talking to before I put a producer in touch with a researcher. I love science (especially microbiology) and I could listen to hours of lectures but I also know what the media will want to hear – the two are not always the same. I love to meet scientists and find out about them. And there are lots of press officers just like me waiting to hear from you.

Oh, and last but not least…if you're being interviewed, make sure you mention the name of your university or Society!

Lucy Goodchild
Imperial College London

MED•VET•NET

Workpackage 34 — the prevention and control of *Campylobacter* in broilers

Campylobacter is known to be the world's most common cause of food-borne bacterial infections. Recent studies suggest that up to 60 per cent of campylobacteriosis cases are attributable to the handling and consumption of contaminated poultry meat.

The colonisation of poultry, particularly broilers, by *Campylobacter* is also widely recognised, and poultry meat frequently becomes contaminated with these organisms during processing. Indeed, an EU baseline survey of broilers, completed in January this year, is anticipated to demonstrate that for most EU countries the majority of broiler flocks produced are colonised. Nevertheless, little is known about the sources of *Campylobacter* on poultry farms or the mechanisms of colonisation. Such information, however, is essential for targeted interventions and effective colonisation prevention strategies on farms.

Aiming to breach that information gap by developing and expanding our knowledge of the animal host aspects of *Campylobacter* transmission is Med-Vet-Net's newest research initiative, **Workpackage 34 — Sources, control and prevention of *Campylobacter* in poultry.**

Consolidate, evaluate, disseminate

The Workpackage (WP), which began in September 2008 under the leadership of Professor Diane Newell on behalf of the Veterinary Laboratories Agency, is undertaking a series of tasks designed to consolidate what is presently a highly fragmented information base. At its conclusion in August this year, the WP will also have examined the role of poultry in environmental contamination and developed an understanding of how to best control and prevent the colonisation of the agent in broiler flocks.

The specific objectives of the project are to:

- Collate, manage and archive the European information base on *Campylobacter* in poultry
- Develop and collate knowledge of the molecular basis of colonisation of the avian gut
- Disseminate knowledge on the epidemiology of poultry colonisation and the survival of campylobacters in the poultry environment
- Critically evaluate the indirect poultry-derived routes of *Campylobacter* infections in humans
- Investigate and develop understanding of effective methods of control and prevention

As with all of Med-Vet-Net's projects, WP34 is taking a highly collaborative approach, using an integrated research network of experts from within the network partnership as well as external collaborators who are invited to contribute information and attend meetings.

That exchange of information has been particularly important with colleagues from WP30, who are aiming to develop a combined microbiological and epidemiological approach for investigating host-microbe interactions of *Campylobacter jejuni* — an objective closely related to WP34. Joint meetings between the two WPs are ensuring that any novel information obtained by either project is shared.

Stimulating outcomes

A range of outcomes are anticipated from WP34's activities which are expected to not only enhance Europe's collective knowledge but also generate greater interest, from farmers and vets to researchers and policymakers, in an often overlooked aspect of a global problem.

These outcomes include:

- An integrated European network of expertise on the prevention and control of *Campylobacter* in poultry, with common interests and aims, and a contact list to improve communications and enable collaboration outside of 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.

- An Endnote-based database of publications on *Campylobacter* in poultry from 1980 to date
- A list of genes described to affect colonisation (positively or negatively) and their properties
- Bioinfomatics studies comparing these genes with the core genome (the fraction of genes present in every *Campylobacter* genome) as opposed to the variable genome fraction, identifying gene combinations of non-essential or redundant colonisation factors, identifying, if possible, regulatory networks in which colonisation genes participate, and the protein-protein interactions for colonisation factors that are active in protein complexes
- A report recommending the genes to be investigated in future colonisation studies
- A collation and annotation of *Campylobacter* genes and mechanisms associated with environmental survival as defined using *in vitro* models.
- An international workshop on "Immunity to and vaccination against *Campylobacter jejuni* in chickens" — the first time this topic has been explored in an open forum. The meeting, held in April 2009, attracted a broad audience of scientists and industry representatives.

To learn more about WP 34 and its progress, visit the Med-Vet-Net website at: www.medvetnet.org/wp34.

Med-Vet-Net's final conference

Med-Vet-Net is hosting its fifth and final Annual Scientific Meeting this month. With the theme 'Microbial Communities', this year's conference (3–6 June) will be held in the historically significant and culturally rich Spanish region of San Lorenzo de El Escorial, 45km north-east of Madrid. Organised by Med-Vet-Net's Spanish partners, Complutense University Madrid (UCM) and the Instituto de Salud Carlos III (ISCIII), the meeting at the UNESCO World Heritage Site will attract more than 200 delegates from within and outside the Network.

Headlining the three-day conference are seven keynote speakers from Canada, the UK, Spain, France and the Netherlands who will give presentations on topics ranging from the union of epidemiology and risk assessment to the genomic view of the biodiversity of *Listeria*.

The specific keynote topics and their presenters are:

- Brett Finlay, University of British Columbia, Canada: *Pathogenic E. coli: contribution of the pathogen, host, and microbiota*
- Fernando Baquero, Hospital ´Ramon y Cajal´, Spain: *Ecogenetical Dynamics of Bacterial Pathogens*
- Peter Teunis, National Institute for Public Health and the Environment (RIVM), The Netherlands: *Epidemiology and risk assessment: an unsettled union?*
- Peter Mertens, Biotechnology and Biological Sciences Research Council (BBSRC), UK: *The emergence and spread of Bluetongue virus across Europe: The impact of climate change, insect vectors and vaccination*
- Carmen Buchrieser, Institut Pasteur, France: *Biodiversity and evolution of pathogenic Listeria: a genomics view*
- Patrice Courvalin, Institut Pasteur, France: *Evolution and dissemination of glycopeptide resistance operons*
- JM Sánchez-Vizcaino, Complutense University Madrid (UCM), Spain: *New tools in the prevention and control of emerging diseases*

Tania Cutting
Communications Adviser
Med-Vet-Net

information

A full review of Med-Vet-Net's final Annual Meeting will be in the next issue of *Microbiologist*. More information about the conference can also be found on Med-Vet-Net's website at: **www.medvetnet.org/mvnconf09.**

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

Summer conference 2009

- Including the Lewis B Perry Memorial Lecture — ***Prion zoonoses: past, present and future*** given by John Collinge

CPD ACCREDITATION
22 points awarded

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

Manchester Metropolitan University, Manchester
Monday 6 to Thursday 9 July 2009

Including sessions on:

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

There will be a packed social programme including:

- Drinks reception and lecture on Monday 6 July followed by Quiz Night
- Trade exhibition on Tuesday 7 July with wine and prizes
- Conference dinner at the URBIS Centre on Wednesday 8 July, including the presentation of the S*f*AM Communications Award

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

Programme

Monday 6th July

14.00 onwards **Arrive and Register**

11.00–17.00 **Basic statistics for applied microbiology**
Basil Jarvis **(A short course primarily for PECS members and student members)**

18.00-18.50 **Lewis B Perry memorial lecture: prion zoonoses: past, present and future**
John Collinge

19.00-20.00 **Drinks reception**

20.00 **Evening at leisure**

21.30 **Quiz night — Jury's Inn Hotel**

Tuesday 7th July

Arthropod-borne zoonoses

09.00-09.35 **What is a zoonosis?**
Malcolm Bennett, University of Liverpool, UK

09.35-10.00 **Plague — historical perspectives to modern infection trends**
Nils Chr Stenseth, University of Oslo, Norway

10.10-10.45 **Lyme borreliosis — facts & fantasy**
Sue O'Connell, Health Protection Agency, Southampton

10.45-11.15 **Coffee/posters**

11.15-11.50 **Bartonellosis an increasingly recognised zoonosis**
Bruno Chomel, School of Veterinary Medicine, University of California, USA

11.50-12.25 **Rickettsiosis — the unwanted holiday souvenir**
Speaker to be confirmed

12.25-13.00 **Relapsing fever — forgotten but not gone**
Sally Cutler, University of East London, UK

13.00-14.00 **Lunch**

Wildlife and companion animals

14.00-14.35 **Bats, bites and fury — can we control rabies?**
Tiziana Lembo, University of Glasgow, UK.

14.35-15.10 **Controlling wildlife reservoirs for bovine TB**
Glyn Hewinsonn, Veterinary Laboratories Agency, UK

15.10-15.45 **Zoonoses in UK wildlife and their detection through sentinels**
Anna Meridith, Edinburgh

15.45-16.15 **Tea/posters**

16.15-16.50 **Exotic pets — what are the zoonotic risks?**
F. Meslin, World Health Organisation, Switzerland

16.50-18.00 **Student Session**

16.50-19.00 **Trade Show**

Wednesday 8th July

09.00-09.35 **Controlling wildlife zoonoses without eliminating wildlife**
Marc Artois, Ecole Nationale Veterinaire de, Lyon, France

09.35-10.10 **Nature bites back!**
Marina Morgan

Livestock and foodborne zoonoses

10.10-10.45 **Anthrax — wool-sorters disease in Belgium!**
Pierre Wattiau, VAR-CODA-CERVA, Brussels, Belgium

10.45-11.15 **Coffee/posters**

11.15-11.50 **New challenges and perspectives on brucellosis**
Phil Elzer, LSU, USA

11.50-12.25 **Cryptosporiosis — challenges for control**
Rachel Chalmers

12.25-13.25 **Lunch**

13.25-14.00 **Unravelling the mysteries of Q Fever**
Didier Raoult, Marseilles, France

14.00-14.35 **Farm to fork — emerging trends in foodborne zoonoses**
Tom Humphrey, University of Bristol, UK

14.35-15.00 **Tea/posters**

15.00-16.00 **Student presentations**

16.00-16.30 **W H Pierce memorial prize**

16.30-17.00 **AGM**

19.30-20.00 **Drinks reception, dinner URBIS Centre, Manchester.**

Thursday 9th July

09.00-09.35 **New trends in toxoplasmosis**
Delfien Verhelst, University of Ghent, Belgium

Emerging and re-emerging zoonoses

09.35-10.10 **Modelling zoonotic disease — challenges & successes**
Nigel French Massey Uni, NZ

10.10-10.45 **Drivers of zoonotic disease emergence in general, using Nipah virus as a case study**
Jonathan Epstein, The Consortium for Conservation Medicine, New York and University of Kingston, UK

10.45-11.15 **Coffee/posters**

11.15-11.50 **Predicting pandemics or scare mongering?**
Dilys Morgan, HPA, UK

11.50-12.25 **Emerging/re-emerging viral zoonoses**
Ernie Gould, France

12.25-13.00 **Leishmaniasis and pet travel**
David Hill, National Travel Health Network and Centre

13.00-14.00 **Lunch & Close**

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

BOOKING FORM and INVOICE

SfAM SUMMER CONFERENCE 6 — 9 July 2009

CLOSING DATE FOR REGISTRATIONS: Friday 26 June 2009
EARLY BIRD DISCOUNT of £30.00 is applied to all bookings made before **Friday 5 June 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.

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

FEES BEFORE 5 JUNE 2009	Full Member	Student, Honorary, Associate & Retired Member	Student Non -Member	Non - Member
Full Conference Rate: (inc accommodation)	£450.00 ☐	£200.00 ☐	£450.00 ☐	£650.00 ☐
Conference Rate: (no accommodation)	£145.00 ☐	£100.00 ☐	£145.00 ☐	£300.00 ☐
Conference Day Rate:	£100.00 ☐	£50.00 ☐	£100.00 ☐	£150.00 ☐

FEES BETWEEN 6 JUNE and 26 JUNE 2009	Full Member	Student, Honorary, Associate & Retired Member	Student Non -Member	Non - Member
Full Conference Rate: (inc accommodation)	£480.00 ☐	£230.00 ☐	£480.00 ☐	£680.00 ☐
Conference Rate: (no accommodation)	£175.00 ☐	£130.00 ☐	£175.00 ☐	£330.00 ☐
Conference Day Rate:	£130.00 ☐	£80.00 ☐	£130.00 ☐	£180.00 ☐

Conference Day Rate delegates please tick the day you wish to attend: Mon 6th ☐ Tue 7th ☐ Wed 8th ☐ Thur 9th ☐

CONFERENCE DINNER: £50.00 ☐

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YOUR PAYMENT

● **For all participants: The Society DOES NOT INVOICE for conference fees. Please treat your completed booking form as an invoice. Cheques must be in £ STERLING ONLY and made payable to 'The Society for Applied Microbiology'. Foreign cheques/ drafts MUST be negotiable for the full amount due.** We accept payment **ONLY** by the following credit and debit cards: VISA, Mastercard, Eurocard, Delta, Electron, JCB, Maestro and Solo.

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

SfAM *Environmental Microbiology* Lecture

Deciphering microbial community dynamics, from genomes to biomes

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

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

The *Environmental Microbiology* lecture will be presented by Professor Edward de Long of Massachusetts Institute of Technology (MIT), USA. He will present a lecture entitled *'Deciphering microbial community dynamics, from genomes to biomes'*. Invitations were sent to members in the last issue of the *Microbiologist* giving a deadline for responses of 30th April. Those members who responded to the invitation will be sent further information nearer the date. If you have any queries about attending the lecture please contact Sally Cryer on 01234 761752. For members unable to attend, the lecture will be available online immediately after the event.

Winter meeting 2010

■ Including the Denver Russell Memorial Lecture

A one day meeting on

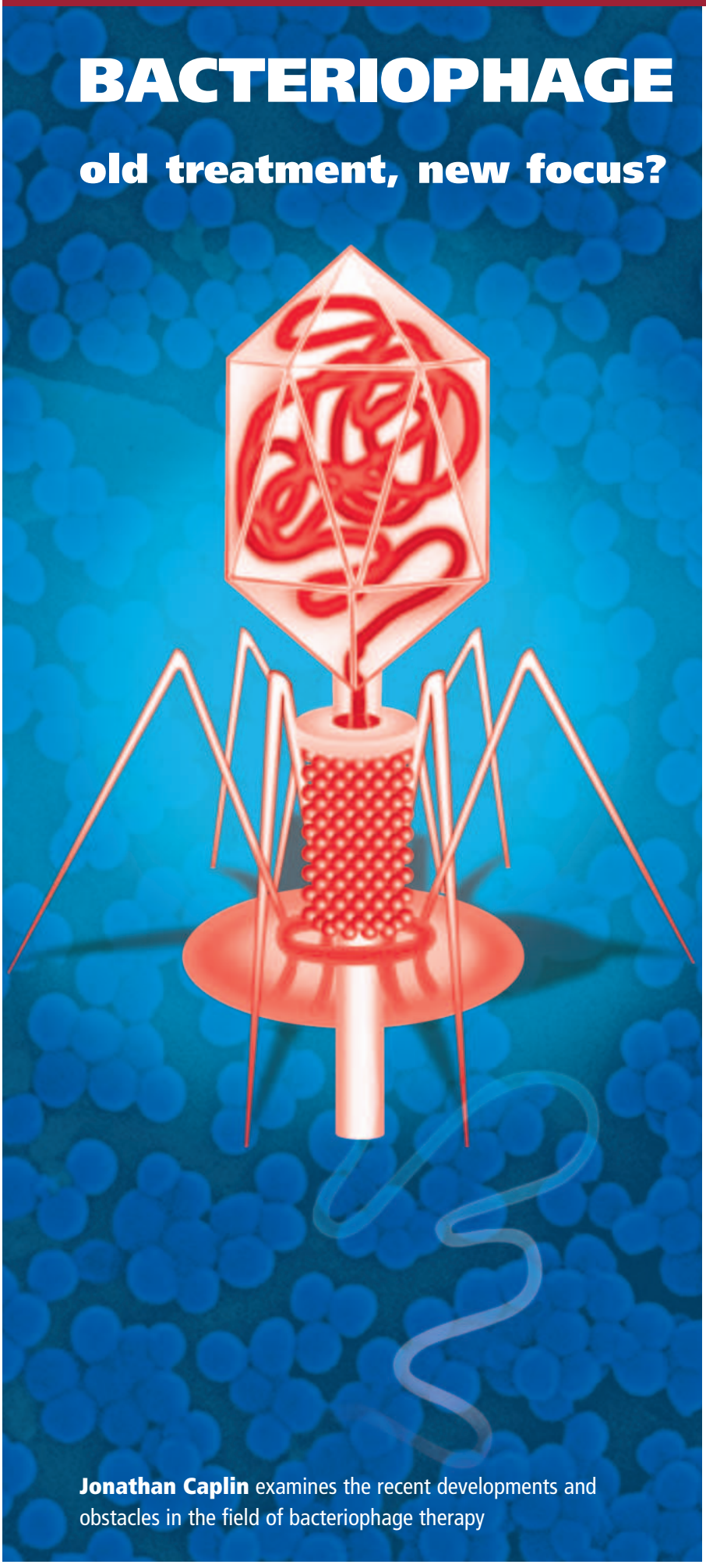
biocides and tuberculosis

Royal Society, London
Monday 11 January 2010

■ For further information please visit the Society website or contact Sally Cryer.
Email: sally@sfam.org.uk. Telephone: 01234 761752

BACTERIOPHAGE THERAPY

old treatment, new focus?



Jonathan Caplin examines the recent developments and obstacles in the field of bacteriophage therapy

The control and management of infections and infectious diseases using antibiotics faces ever-increasing challenges from the development and spread of resistance to such compounds. This has become a major problem worldwide, and there are fears that the clinical management of many infectious diseases will become severely constrained. As a consequence a number of alternative antimicrobial strategies are currently being investigated. One approach to antibacterial therapy which has been used since the beginning of the twentieth century is the application of bacteria-specific lytic viruses or bacteriophages. The history, biology and early therapeutic use of bacteriophages can be found in a number of reviews and textbooks (Kutter & Sulakvelidze, 2005; Hanlon, 2007; Abedon, 2008) and this article will examine some of the recent developments and obstacles in the field of bacteriophage therapy.

Early development of bacteriophage therapy

Much of the pioneering work with bacteriophages and bacteriophage therapy was inconclusive and the therapeutic outcome was sometimes worse than the presenting infection, leading to mistrust and scepticism. The 'Bacteriophage Inquiry' in India in 1927 and an American Medical Association review in 1931 failed to produce conclusive evidence as to the efficacy of the therapy. The development of antibiotics and antimicrobial chemotherapy at around the same time halted further serious investigation, and by the end of the Second World War, bacteriophage therapy was seen as a marginal curiosity.

Research on bacteriophages did not stop with the advent of antibiotics but continued in countries in the former Soviet Republics, where antibiotics were inaccessible. Advances in knowledge, techniques and application increased so that a substantial body of evidence accumulated, and active programmes were initiated to develop and distribute bacteriophage therapeutics throughout the Soviet bloc. The Eliava Institute in Georgia became the premier bacteriophage research establishment, producing a wide range of preparations containing bacteriophage and 'cocktails' of specific bacteriophages. Incidentally, Félix d'Hérelle (who announced his

discovery of bacteriophages in 1917, independently of Frederick Twort who first described their lytic behaviour in 1915) introduced bacteriophages to George Eliava in 1926 on a visit to the then Tbilisi Institute of Bacteriology.

Resurgence of interest in the West

After the collapse of the Soviet Union in 1989, bacteriophage therapy came to the attention of the West once again, this time with supporting evidence of therapeutic efficacy observed in Eastern Europe. Unfortunately, language difficulties and the lack of detailed documentation and double-blind controls in many of the trials, meant that the body of work as a whole was not regarded seriously, and there was insufficient evidence for its acceptance and approval for use as a therapeutic option.

However, the spectre of increasing levels of multi-antibiotic resistance focused minds once again on bacteriophages as therapeutic tools. Basic research into the biology and ecology of bacteriophages, together with advances in molecular biological techniques and purification methods, enabled the development of well characterised, specifically targeted and highly purified bacteriophage mixtures for therapeutic use, and scientists and clinicians in Europe and America began the first clinical trials.

For example, a cocktail of eight bacteriophages (five against *Pseudomonas aeruginosa*, two against *Staphylococcus aureus* and one against *Escherichia coli*) was used to treat infected leg ulcers in Texas, USA (Marza *et al.*, 2006). The bacteriophages were supplied by Intralytix, a US company founded by a former Georgian microbiologist. Following the trial, the Southwest Regional Wound Care Centre in Texas used bacteriophages in conjunction with other methods, to treat antibiotic-resistant infections (www.woundcarecenter.net). The use of bacteriophage T4 against *E. coli* in cases of diarrhoea is being assessed in Bangladesh by Nestlé, the Swiss multinational food corporation. This follows safety testing on human volunteers (Bruttin & Brüssow, 2005). Phase 2a clinical trials were conducted in 2007 at the Royal National Throat, Nose and Ear Hospital in London, on the treatment of chronic inner ear infections caused by *Pseudomonas aeruginosa* using a cocktail of six bacteriophages produced by the UK start-up company Biocontrol. Very positive results have been reported from the trial in terms of clinical and bacteriological efficiency and safety concerns. The therapeutic cocktail is currently being examined in Phase 3 trials. Administration of the bacteriophage cocktail was via an aerosol delivery system which was granted a patent by the European Patent Office in 2008. A similar concept involving the nebulisation of bacteriophages of *Burkholderia cepacia* Complex to treat cystic fibrosis has recently been described (Golshahi *et al.*, 2008).

As well as clinical trials, basic research has been ongoing. At the 17th Biennial International Evergreen Phage Biology Meeting held in Olympia USA in August 2007, bacteriophage therapy sessions covered a range of infections and bacteria such as Otitis Media (ear infection) and *Pseudomonas aeruginosa*, urinary tract infections (UTIs) and *Klebsiella* species, *Burkholderia* species, Group A *Streptococcus*, and *Bacillus anthracis* infections (http://academic.evergreen.edu/ projects/phage/).

Despite all the interest and debate, few clinical evaluations of bacteriophage therapy have been published to date in the West. A possible reason for this lack of advance may be that the data available are at a very early stage with few meaningful and significant results, thus making it difficult to attract funding. Secondly, bacteriophage therapy is somewhat of a grey area to most regulatory agencies and consequently questions of medical ethics and procedural pathways abound. Within existing frameworks it is virtually impossible to start clinical investigations to generate the data required to demonstrate the safety and efficacy of bacteriophage-based therapeutics. It should also be noted that in the former Soviet Republics, the vast majority of bacteriophage preparations were designed for use as non-systemic medications i.e. lavage, sprays, ointments and dressings. Several preparations were for oral or rectal (suppository) delivery, and the few injectable preparations were for intramuscular or intraperitoneal administration. Thus nearly all the work from Eastern Europe involved topical and localised applications, for example 'PhageBioderm' a biodegradable material impregnated with bacteriophage, designed for the treatment of skin infections, and 'PhageBiodent' for periodontal and gingival applications.

Safety concerns

A number of companies focussing on the development of bacteriophage therapy were formed during the 1990s to exploit the niche for intravenous antibacterial therapeutics. However, the combination of clinical trial requirements, problems with unwanted immunological reactions, and intellectual property rights issues led many of these companies to turn to veterinary and agricultural applications, which were perceived as less regulatorily tricky areas. Such a change in direction resulted in the US Food and Drug Administration (FDA) approving a product developed by Intralytix in 2006. The product contained bacteriophages active against *Listeria monocytogenes* and was designed as a disinfectant spray for packaged meats. Subsequently, other products from companies such as Omnilytics and EBI Food Safety have been approved by the US FDA and given approval under the 'generally regarded as safe' (GRAS) system. The approval of these products shows recognition that bacteriophages are considered safe for use on food destined for human consumption. This is a step towards acceptance of bacteriophage therapy in general. The ubiquitous nature of bacteriophages, estimated to be the most numerous organisms on the planet (Abedon, 2008) means that humans are exposed to them from birth, and have been in symbiotic relationship with them for thousands of years. In fact it is impossible not to ingest bacteriophages since they form part of the natural flora of the human oral cavity and gastrointestinal tract, and are present in municipal drinking water and various food items.

There were also concerns regarding the safety of intravenous bacteriophage preparations, a problem not readily encountered in Eastern Europe since there was a preference for topical applications in those countries. Bacteriophages have the potential to elicit strong immune responses mainly due to their protein content, and as a consequence the system can clear the

bacteriophages from the body rapidly. The interaction between bacteriophages and antibodies is of concern because if the bacteriophage elicits an antibody mediated response, further treatment with that particular bacteriophage would be negated. Another concern over the use of intravenous bacteriophage preparations is the chance of the patient developing the Jarisch-Herxheimer reaction - systemic shock resulting from the sudden release of polysaccharide endotoxins into the bloodstream during the lysis of susceptible bacteria. The problem of lytic toxicity has been investigated specifically in a veterinary trial of active bacteriophage therapy, and no significant adverse reactions or effects were noted (Soothill, 2004).

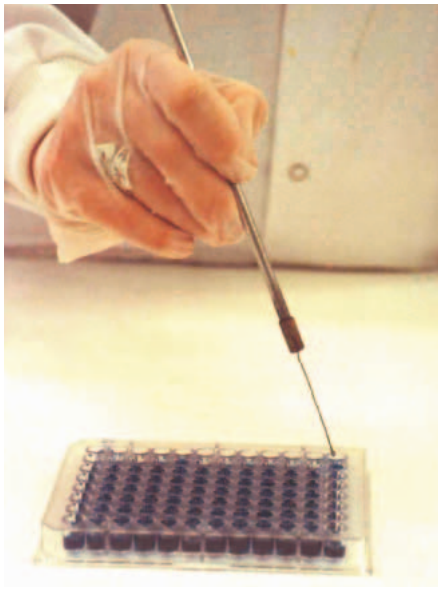
The wealth of data from Eastern Europe suggests that bacteriophage therapy is safe (Sulakvelidze, 2005), and experience at clinics where bacteriophage therapy is provided indicates that the treatment is effective and without negative side-effects. One of the largest bodies of published work in English on bacteriophage therapy comes from the Hirszfeld Institute of Immunology and Experimental Therapy in Wroclaw, Poland. Their experience over the past 30 years in treating nearly 2000 patients suffering from a variety of often life-threatening infections was very positive, with an overall rate of success of 60% to 90% and no reports of serious adverse reactions (Stone, 2002).

Novel approaches to bacteriophage therapy

Until recently, bacteriophage therapies have been based on complete virus particle preparations or purified bacteriophage lytic enzymes such as lysins and holins (Fischetti, 2005). Novel developments include phage display, where an antibacterial peptide or protein is displayed on the surface of a genetically modified bacteriophage. The bacteriophage is designed not to lyse but rather to deliver the attached antibacterial to the target bacteria (Westwater *et al.* 2003).

Bacteriophages have also been used as potential vehicles for the delivery of vaccines. The bacteriophages can carry antigens on their surface or deliver a DNA extension cassette that has been engineered into the bacteriophage genome (Clark & March, 2004). The UK company Phico Therapeutics Ltd. are developing engineered 'improved' bacteriophages for intra-nasal decolonisation of MRSA and for oral therapy against *C. difficile* associated disease (http://www.phicotherapeutics.co.uk/).

The major problem with these approaches is the approval of genetically engineered agents, added to the fact that the bacteriophages themselves are not categorised as therapeutic modalities, thus proving a real challenge to pharmaceutical companies wishing to pursue this route. Two of the major bacteriophage companies, Intralytix and Biocontrol, are only working with 'natural' or unmodified bacteriophages for these very reasons.



PHP innoculation

Obstacles to the acceptance of bacteriophage therapy

Despite the accumulating biological, molecular and clinical evidence supporting the use of bacteriophage therapy, there are a number of hurdles to be overcome before it is accepted and considered a therapeutic option. These problems can be regarded as biological, proteomic and regulatory. For example, little is known about bacteriophage interactions in the gut and other anaerobic environments, and some work has shown that T-even bacteriophage display oxygen-dependent growth on their host strain and inhibition of lysis under anaerobiosis. It is also thought that bile salts and gut carbohydrates may sequester the bivalent metal ions needed by bacteriophages for adsorption and replication (Chibani-Chennoufi *et al.* 2004).

The phenomenon of bacteriophage mutation leading to resistance has also been cited as a concern, and resistance to the therapeutic bacteriophage has been noted in some animal studies. However the use of bacteriophage cocktails has the potential to minimise or prevent the development of resistance.

The conversion of a lytic bacteriophage into a lysogenic lifecycle could be a major problem if the bacteriophage genome integrates with that of its host. Subsequent reactivation could result in the transfer of bacterial virulence factors and the bacteriophage mediated transfer of virulent bacterial genes into other bacteria. In order to avoid this, care must be taken to ensure that the potential therapeutic bacteriophage is predominantly or wholly lytic, and does not carry any toxic genes or housekeeping genes for initiating lysogeny. There are little data on the genomes and proteomes of lytic bacteriophages, so questions of lysogenic, toxic or virulent genes remain unanswered at present.

From a regulatory point of view, bacteriophages are rather ambiguous, and the fact that they are self-replicating biological entities is another complication. To meet current regulatory approval, such a product must comprise of a highly purified, characterised, and validated bacteriophage or mixture of bacteriophages, together with optimised administration protocols, supported by properly controlled efficacy and safety studies. There are no specific frameworks for bacteriophage therapy in the current Medicinal Product Regulation (EC, 2001), so short-term borderline solutions under the responsibility of a medical ethics committee or under the umbrella of the Declaration of Helsinki are possible options. It has been proposed (Verbeken *et al.* 2007) that a long-term solution would be the creation of a specific section for bacteriophage therapy under the Advanced Therapy Medicinal Product Regulation (EC, 2003).

The development of bacteriophage therapeutics is also hindered by issues of intellectual property rights. Bacteriophages have been used as therapeutic agents for almost a century and are thus unpatentable. As a consequence, few pharmaceutical companies would be willing to invest the

sums of money required to develop a product for bacteriophage therapy unless their results are 'protected' by an international patent.

Another potential obstacle for the clinical application of bacteriophages is the perceived 'fear' of viruses. Viruses are seen by many members of the public as 'like bacteria but more dangerous' or as 'enemies of life', and the idea of injecting them into patients or spraying them onto food raises real if unwarranted concerns.

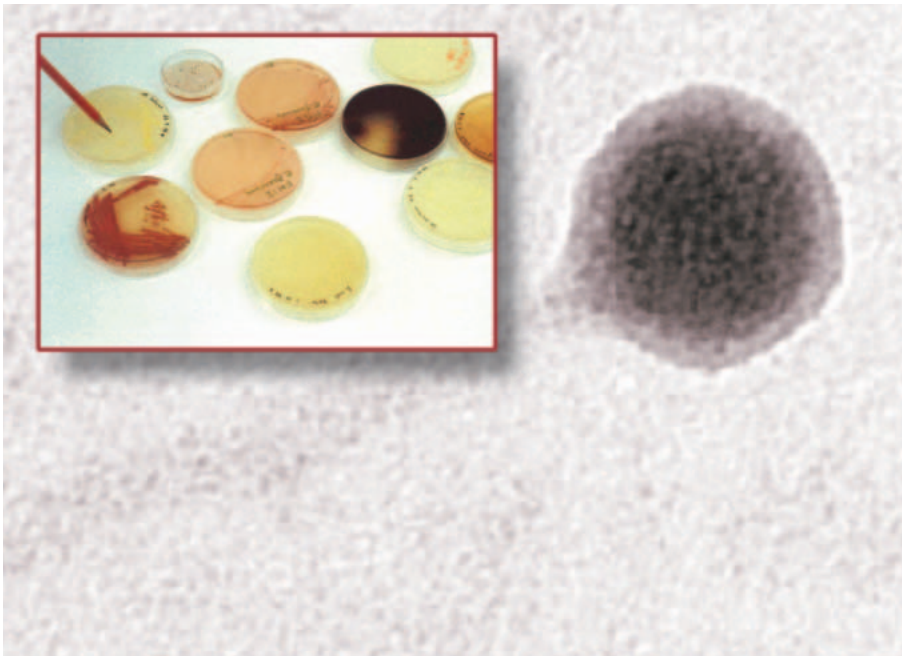
Is there a future for bacteriophage therapy?

If the necessary regulatory and ethical hurdles are overcome and bacteriophages become accepted as therapeutic agents, this could stimulate the development of new approaches and methodologies. It is possible that initially agricultural, veterinary, food hygiene and food safety applications will predominate until sufficient data from clinical and safety trials are available. New pharmacokinetic data, advances in biocompatibility studies and a greater understanding of bacteriophage-bacteria interactions will enable novel targets to be identified and the efficacy of bacteriophage-based therapies to be improved. It is also possible that bacteriophage therapy will be approached like other biological control methods used in agriculture, with the aim to reduce bacterial infections and spread of diseases using a range of modalities and therapeutic agents, as part of an 'integrated pathogen management' plan.

At the start of the twentieth century bacteriophage therapy was seen as the answer to bacterial infections but initial therapeutic problems and the advent of antibiotics pushed it into the background.

Now at the start of the twenty-first century with the ever increasing problem of antibiotic resistance, we have the wealth of clinical experience from colleagues from the former Soviet Republics and the necessary biological knowledge and tools to re-evaluate the therapeutic potential of these 'bacteria eaters'.

Jonathan Caplin
University of Brighton



MAIN PHOTO: Bacteroides ovatus B124 Bacteriophage (Siphoviridae) © D.Diston, University of Brighton, 2009. INSET: Petri plates

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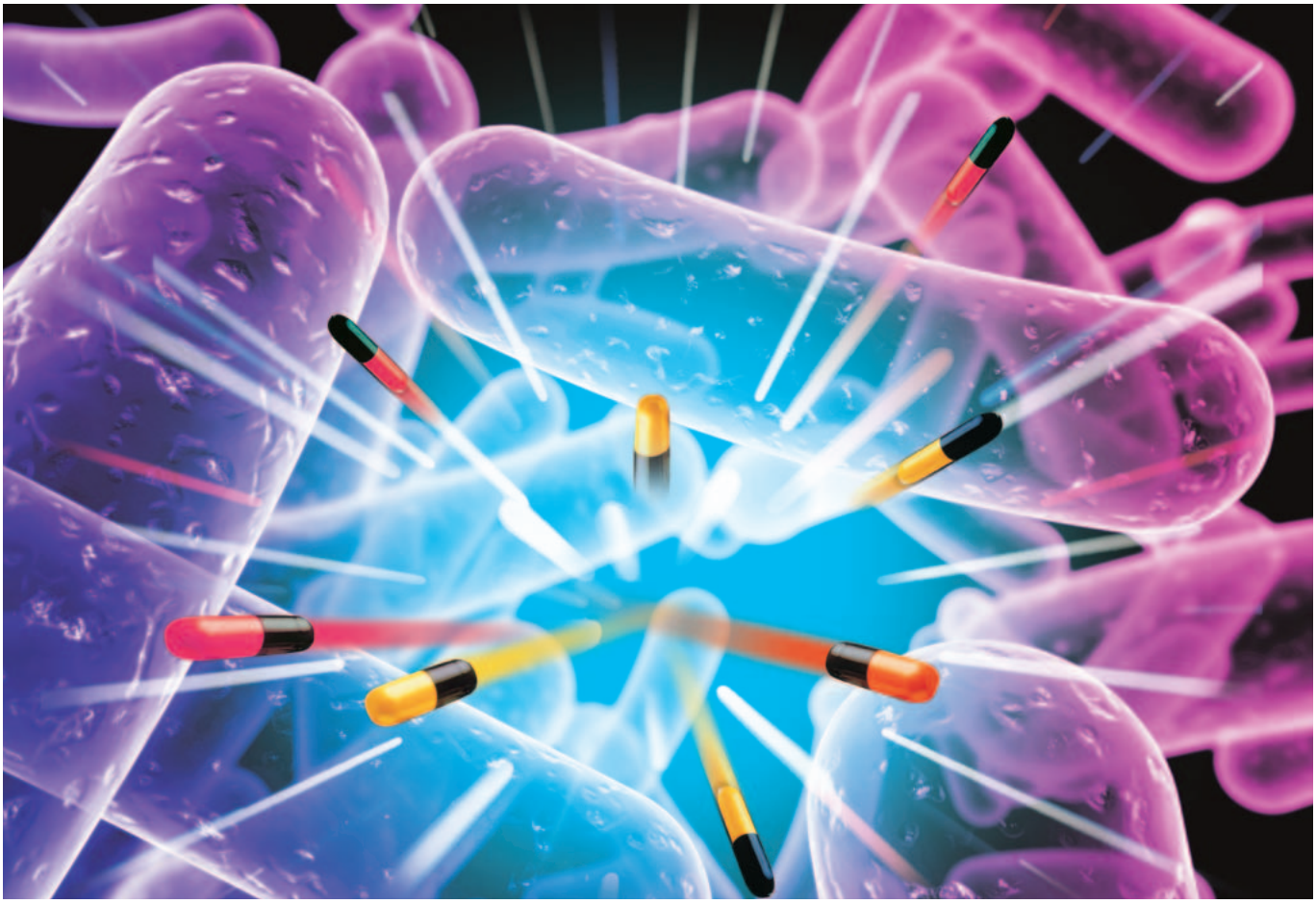
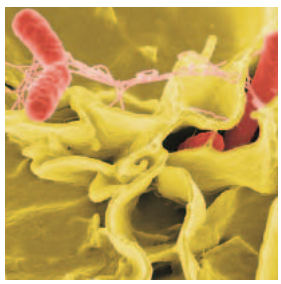


Antimicrobial resistance in bacterial enteric pathogens

John Threlfall provides an overview of resistance in *Salmonella* and *Campylobacter* and discusses the problems associated with the emergence of resistance in such organisms



Salmonella Typhimurium

Antimicrobial resistance was recognised as a potential problem for the treatment of diseases caused by enteric bacteria in both humans and food production animals as long ago as the 1960s.

Following the identification of linked transmissible resistance to several unrelated classes of antimicrobial drugs in *Shigella* from cases of human infection in Japan in 1963, such resistance rapidly became widespread, culminating in the appearance and spread of epidemic clones of *Salmonella* Typhimurium in the United Kingdom (UK) particularly, from 1965. Concern about the origins of resistance in such strains, and the possible role of antibiotics in food animals, particularly calves, contributed significantly to the formation in 1968 of the 'Swann Committee'. This group were tasked with investigating the contribution of antibiotics in food animals to the development of resistance in zoonotic enteric pathogens. The recommendations of the Swann Committee were far reaching and culminated in the withdrawal of

antibiotics as growth promoters in the UK and in due course in the European Union. Nevertheless, resistant strains have continued to proliferate, and at present there are increasing problems not only with a global increase in the occurrence of strains with multiple resistance, but also with the emergence of resistance to antibiotics regarded as 'first-line', or 'critical' by the World Health Organisation (WHO). This article provides an overview of resistance in two key enteric pathogens, namely *Salmonella*, and *Campylobacter* and discusses problems and recent events associated with the emergence of resistance in such organisms.

Salmonella

Salmonella enterica has been regarded as the 'definitive' enteric pathogen in respect of antimicrobial resistance, particularly in relation to its ability to acquire a variety of resistance genes by plasmid acquisition, to develop resistance to certain key antimicrobials by mutation, and also because of the zoonotic reservoirs of many serovars. From a clinical perspective, salmonellosis in humans falls into three broad categories: enteric fever; invasive disease (non-typhoidal); gastroenteritis.

Enteric fever

In developing countries the primary disease presentation is that of enteric fever, caused for the most part by organisms such as *Salmonella* Typhi or *S*. Paratyphi A. Although vaccines are available to combat *S*. Typhi, these are not widely used and there are no vaccines approved for Paratyphi A. Treatment with an appropriate antimicrobial may therefore be essential, and can be life-saving. Chloramphenicol was the undisputed first-line drug used until the 1970s, when its efficacy was seriously undermined by a series of outbreaks in countries as far apart as Mexico, India, and Vietnam. Chloramphenicol-resistant strains which were also resistant to streptomycin, sulphonamides and tetracyclines (CSSuT) became endemic throughout the Indian sub-continent and common in developed countries in travellers returning from such regions. As a result of the emergence and spread of chloramphenicol-resistant strains on an almost global scale, ampicillin was introduced for the first-line treatment of typhoid in the early 1970s, followed by co-trimoxazole in the late 1970s/early 1980s. The efficacy of these antimicrobials was undermined in the late 1980s following the emergence of epidemic strains of *S*. Typhi, not only with resistance to chloramphenicol, streptomycin, sulphonamides and tetracyclines, but also with additional resistance to ampicillin and trimethoprim/sulphamethoxazole (= co-trimoxazole) (=ACSSuTTm). Outbreaks with such strains were initially recognised in Pakistan, but the strains rapidly became widely disseminated. In the 1990s, fluoroquinolones became the drug of choice for the first-line treatment of typhoid fever, initially in adults but subsequently in children. In turn, incidents and outbreaks of infection with strains of *S*. Typhi resistant to quinolone antibiotics, particularly nalidixic acid, coupled with decreased susceptibility to ciprofloxacin (MIC: 0.25 – 1.0 mg/L) became increasingly reported. Of particular note were substantive outbreaks with such strains in Tajikistan and Vietnam in the late 1990s and early 2000s. Clinical failures with infections caused by strains of *S*. Typhi with decreased susceptibility to ciprofloxacin were first reported in the UK in the early 1990s, and have subsequently become increasingly common. In 2006, 68% of typhoid cases in the UK involved strains that exhibited reduced susceptibility to ciprofloxacin and concomitant resistance to nalidixic acid (Threlfall *et al*., 2008). Since 2005, strains of *S*. Typhi with high level fluoroquinolone resistance ('clinical' resistance) have been identified in sporadic cases in India but do not appear to have become widely disseminated. As yet, no strains of typhoid with 'clinical' resistance to ciprofloxacin have been identified in cases of infection in the UK. In cases of treatment failures with fluoroquinolone antibiotics, third generation cephalosporins such as ceftriaxone have been recommended, but in developing countries the cost of such antimicrobials can be prohibitive. Azithromycin, a macrolide antibiotic, has also been evaluated for the treatment of infections caused by multi-resistant typhoid, with encouraging results (Parry, 2004).

In strains of *S*. Typhi with resistance to chloramphenicol, trimethoprim and ampicillin, such resistances have almost invariably been encoded by plasmids of the HI1 incompatibility group. Although ubiquitous in drug-resistant strains of *S*. Typhi, evolutionary diversity within this 'Typhi-specific' compatibility group has recently been observed among HI1 plasmids from multi-resistant strains of *S*.Typhi isolated in Vietnam over a ten-year time period in the 1990s (Wain *et al*., 2003) and more recently worldwide (Phan *et al*., 2009). In strains with decreased susceptibility to ciprofloxacin, such resistance has been chromosomally encoded resulting from a single mutation within the gyrA gene. In contrast, in strains with high level resistance, two mutations in *gyrA* and a further mutation in the *parC* region has been identified.

Infections caused by *S*. Paratyphi A, although not as severe as typhoid fever, may also require antimicrobial intervention before the results of susceptibility tests are available. As with typhoid fever, the first-line drugs are the fluoroquinolones. A substantive increase in strains of *S*. Paratyphi A with decreased susceptibility to ciprofloxacin has been reported in both India and Vietnam since the late 1990s and in the UK, over 70% of isolates from cases of infection in 2006 exhibited decreased susceptibility to ciprofloxacin, an increase of 300% since 2001 (Threlfall *et al*., 2008).

Invasive disease (non-typhoidal)

Certain other serovars — e.g., Cholerae-suis, Dublin, and Virchow – are also invasive. In general such organisms do not exhibit resistance to antimicrobials, although multiple resistance, including clinical resistance to fluoroquinolones, has been reported in fatal cases of infection with *S*. Cholerae-suis in Taiwan. In developing countries a different picture has emerged, with serovars normally regarded as non-invasive. For example, Typhimurium, Wien, Senftenberg, are associated with highly virulent infections with a high degree of morbidity and mortality. In developing countries, particularly in the Indian sub-continent and South-east Asia, serotypes such as Typhimurium, Wien, Johannesburg and Oranienburg have undergone changes both in their epidemiology and their clinical disease. An additional feature of these strains has been the possession of plasmid-mediated multidrug resistance, often exhibiting resistance to seven or more antimicrobials. With the exception of resistance to furazolidone and, since

1990, to nalidixic acid, resistances in these strains have invariably been plasmid-encoded. The most common presentation has been that of severe gastroenteritis often accompanied by septicaemia (up to 40% in some outbreaks) with up to 30% mortality. The main method of transmission is by person-to-person spread either in hospitals or in the community, and antibiotic resistance appears to have developed as a result of the use of antibiotics in human medicine, particularly in those countries where there is little control over the use of antibiotics. A particular property of the majority of these multiresistant strains is the possession of a plasmid of the F_I incompatibility group coding not only for multiple resistance, but also for production of the hydroxamate siderophore aerobactin. This is a known virulence factor for some enteric and urinary tract pathogens. A 30-year retrospective molecular study of this group of plasmids, from strains of *S.* Typhimurium from several countries, has demonstrated that such plasmids have evolved through sequential acquisition of integrons carrying different arrays of antibiotic resistance genes (Carattoli *et al.*, 2001). Nontyphoidal salmonellae with multiple drug resistance have also become the most common cause of bacteremia in tropical Africa, particularly among susceptible children and HIV-infected adults. In a study in Malawi from 1999-2004 a total of 75% of the cases of nontyphoidal salmonella bacteremia were due to *S.* Typhimurium, with most strains exhibiting multidrug resistance to ampicillin, cotrimoxazole, and chloramphenicol (Gordon *et al.*,. 2008). Such strains have now replaced *S.* Typhi as the predominant cause of invasive salmonellosis in tropical Africa.

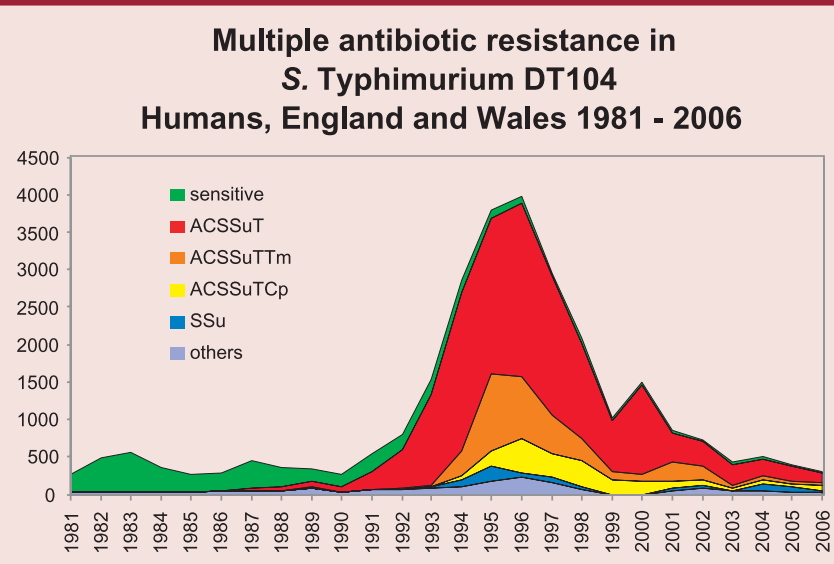
Gastroenteritis

In developed countries salmonella infections are primarily zoonotic in origin and the most common presentation is that of gastroenteritis. When resistance is present, it has often been acquired prior to transmission of the organism through the food chain to humans. The most important serovars in the UK and Europe are Enteritidis and Typhimurium. For all these serovars the main method of spread is through the food chain. In most cases the clinical presentation is that of mild to moderate enteritis. The disease is usually self-limiting and antimicrobial therapy is seldom required.

For the last four decades the history of multiple resistance in *Salmonella enterica* in the UK has been dominated by three major clones of *S.* Typhimurium, namely definitive phage types (DTs) 29, 204/204c/193 and 104 (Figure 1). The most recent epidemic, of multiresistant (MR) *S.* Typhimurium DT 104 (= MR DT 104) with resistance to ampicillin, chloramphenicol, streptomycin/spectinomycin (SSp), sulphonamides and tetracyclines (= ACSSpSuT), started in cattle in the UK the late 1980s (Threlfall, 2000) and is still on-going, albeit with decreasing numbers of isolations (Figure 1). In 1996 infections with MR DT 104 were recognised in cattle and humans in North America, and particularly in the USA the organism has been responsible for many outbreaks in both cattle and humans. In MR DT 104 of R-type ACSSuT resistances are contained in a 16kilobase (kb) region of the 43kb Salmonella Genomic Island SGI-1 comprised of integrons containing respectively the ASu (bla_{CARB-2} and *sul1*) and SSp (*aadA2*) genes (Sp, spectinomycin), with intervening plasmid-derived genes coding for resistance to chloramphenicol / florphenicol (*florR*) and tetracyclines (*tetG*). Although chromosomally-encoded, in recent years SGI-1 has been identified in several different salmonella serovars, including *S.* Agona, *S.* Albany and *S.* Paratyphi B variant Java, which is indicative of horizontal transfer of SGI-1. Such strains have caused infections in humans and cattle and there is speculation of a connection with ornamental fish originating in the Far East.

Both resistance and multiple resistance in salmonellas in European countries are becoming increasingly prevalent and increasingly diverse. A five-year study of antimicrobial resistance in over 130,00 isolates of *Salmonella* from patients in ten European countries from 2000-2004 showed that resistance to nalidixic acid had increased from 14 to 20% over this period, and in *S.* Enteritidis, from 10 to 26% (Meakins *et al.*, 2008). In Spain, the UK and Denmark emergent multi-resistant strains of *S. enterica* serotype [4,5,12:i:-] have been associated with a number of human infections since the mid-1990s (Guerra *et al.*, 2000). In these strains resistance has been mediated by an unusual plasmid containing resistance genes located within a class 1 integron and also the *spvA*, *spvB* and *spvC S.* Typhimurium plasmid virulence genes. CTX-M-9, -15 and -17 to-18 enzymes have recently been identified in different serovars from humans in the UK (Batchelor *et al.*, 2005) and plasmid-mediated CTX-M-like enzymes have been increasingly reported in a range of serovars in food production animals in several European countries, notably *S.* Virchow in Spain, Belgium and France (Carattoli 2009).

Figure 1



The use of third-generation cephalosporins in poultry has been suggested as major contributory factor in this respect. A further recent development in the UK has been the emergence of plasmid-mediated resistance to quinolone antimicrobials in several salmonella serovars, mediated by different qnr genes (Hopkins *et al.*, 2008). Of concern is the frequent association of plasmid-mediated quinolone resistance with resistance to third-generation cephalosporins in such strains. To date plasmid-mediated resistance to quinolone antibiotics in UK salmonella isolates has not been reported in isolates from food-production animals.

Campylobacter is the most commonly isolated pathogen from cases of food-poisoning, not only in the UK but also in many European countries. As with salmonellosis, antibiotics are not recommended for treatment of uncomplicated campylobacteriosis. Nevertheless, should treatment be required, macrolide antibiotics such as erythromycin, and fluoroquinolones are the drugs of choice. In this respect data for human isolates of *C. jejuni* and *C. coli* from Denmark, the UK, the USA, Italy, Finland, the Netherlands, France and Spain have demonstrated increases in the incidence of resistance to fluoroquinolones from <1% to between 10% (UK) and 80% (Spain) in the 1990s and early 2000s (Enberg *et al.*, 2001). More recent data from the UK demonstrated an increase in the incidence of fluoroquinolone-resistant isolates of *C. jejuni* from cases of human infection from 10% in 1993-96 to 21% in 2003. As poultry is generally regarded as a primary reservoir for *Campylobacter*, the use of fluoroquinolones in this food animal was considered an important contributory factor.

Over the last forty years many outbreaks of infection in developed countries, particularly those caused by such drug-resistant non-typhoidal salmonella strains, have been linked to foods of animal origin. In turn this has led to speculation about the role of antimicrobials in animals bred for food in contributing to the development and spread of such strains. In recent years there has been considerable speculation about the contribution of the prophylactic use in food production animals of antimicrobials such as third-generation cephalosporins and quinolones to the development of resistance in a range of organisms, including *Salmonella*. This has culminated in the recent report from the Chief Medical Officer (Chief Medical Officer, 2009), who has stated that in addition to the substantive use of antibiotics in human medicine, antibiotics are also used in large quantities on animals, thereby adding to the threat of resistance. Following on from this observation, he recommended that there should be a ban on the use of certain types of antibiotics (quinolones and cephalosporins) in animals, in order to protect their activity in humans. Whether such a ban can be implemented unilaterally for the UK is debatable, but the comments in the report do highlight increasing concern about the use of certain key therapeutic antimicrobials in livestock.

As antibiotics are not recommended for the treatment of mild to moderate salmonella- or campylobacter-induced enteritis in humans, it may be argued that drug resistance in such organisms is of little consequence for public health. Nevertheless, antibiotics are used for the treatment of gastroenteritis in immunocompromised patients and sometimes for treating particularly vulnerable patients; in such cases treatment with an appropriate antibiotic is often essential and may be life-saving. In this respect the increased occurrence, both nationally and internationally, of strains of *S. enterica* and *Campylobacter* with decreased susceptibility to fluoroquinolones and/or resistance to third-generation cephalosporins is an unwelcome development.

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THE BEAUTY OF BACTERIA

Art, Cybernetics and Normal Flora Microbiology

"Soil Flora" — Anna Dumitriu and Dr Simon Park

Bacteria are beautiful, I know that and you know that, but the majority simply do not. People are exposed to a daily barrage of adverts and newspaper warnings that recommend no less than the total genocide of all bacteria (with the exception of the so called 'friendly' ones of course). The term bacteria is synonymous with dirt and the 'normal' reaction to the suggestion that something is covered with bacteria is one of disgust or fear, rather than a matter-of-fact acceptance or amazement. Through my work I am attempting to look at the deeper importance of bacteria aesthetically, philosophically and scientifically. I am interested in the normal environmental flora and I believe that the wider public need to be given the tools to appreciate the microbiological world. The public would then be capable of making reasoned judgments about what they read in the press. Perhaps this is where art can help.

The normal flora project is an ongoing art project through which I am attempting to engage the public in developing a greater understanding of the microbial world. Many of the artworks I have produced for this are developed collaboratively and project participants take on the role of artist, researcher and scientist in a hands-on way investigating their own eco-systems. The final outcomes emerge (taking the form of digital works, sound works and installations) in a dialogue between myself, other artists, philosophers, scientists, medics and workshop participants or exhibition visitors.

In 2007 I undertook a project with cleaning staff and microbiologists at Eastbourne District General Hospital who collected samples of microbes from their own homes. They worked together with me in the hospital microbiology labs (the microbiologists aiding the cleaning staff), and engaged in the creation of new artworks, including a major sound work for performance. Initially, people are clearly ashamed at any suggestion that their worlds are teeming with microscopic life. Asking someone to reveal their normal flora is an intimate process of negotiation and education. But participation in the project usually leads to a paradigm shift for both participants and audience from that of shame to one of understanding.

An important outcome of the project at Eastbourne hospital was a performance which involved one to one conversations with people waiting in the hospital foyer and café. I invited these people to crochet the microbes found on my own bed with me, whilst we chatted about their perceptions of normal flora microbiology. This crocheted piece has since been exhibited in a number of locations and is now larger than a double bed. The piece is being created collaboratively through conversations with participants who are shown images of the actual bacteria on my bed. These discussions lead to their

"School Flora – Swabbing for Normal Flora" — Anna Dumitriu

interpretations being incorporated into the work, which grows larger every time it is exhibited.

In 2008 I completed 'School Flora', which involved working closely with the science students at Varndean School in Brighton over a period of one year. Working with me (and aided by the medical students I teach) the Varndean students took swabs of microbes from around the school: from the drinking fountain to the books in the library. These samples were then cultured and used as the basis for a series of new artworks inspired by the microbes we found. This was developed collaboratively, between myself, the students and the science teaching staff in the school. The project went far beyond a traditional school art project and stood as a major artwork in its own right. The art installations set out to reveal the unobserved wonders of the microbial eco-system that comprised the school, as well as dispelling myths and misunderstandings.

I have strong views on the structure of art/science collaborative practice and how I believe this should operate. For me it is very important to undertake the practical work myself as much as possible, to understand the techniques and methodologies and for my collaborators to try to do the same from an artistic point of view. I am aiming to assimilate as much practical expertise as I can. I do not believe that it is really possible to engage fully with scientific research without coming from an informed position, so I need to experience processes myself and I constantly strive to increase my knowledge and skills. For me, the process of swabbing for microbes, plating them up, growing them and getting to know them (my constant companions) is a performance, I am taking on a role and attempting to embody that knowledge.

As well as collaborating with microbiologists Dr John Paul (Royal Sussex County Hospital/University of Brighton) and Dr Simon Park (University of Surrey), I am also artist in residence/visiting research fellow in the Centre for Computational Neuroscience and Robotics at The University of Sussex. This is the heart of one of the largest Artificial Life research groups in the world. The primary concern of the group is to understand what constitutes life so they can attempt to model it or even recreate it through artificial life processes (these are generally computational but can include wet processes too). A central question for the group is: can life be considered as an abstract concept not linked to biological processes? Through my ongoing residency, I am able to further research complex living systems, in particular evolutionary and adaptive systems to which bacteria (as the 'simplest' living organisms) are strongly relevant.

Modern Artificial Life research takes the 'bottom up' approach, to try and understand the simplest forms of life before trying to understand more complex organisms. But of course even the simplest organism is highly complex, a complex system of intertwining feedback loops associated with an environment. These ideas stem from the field of Cybernetics, the interdisciplinary study of the structure of complex systems, especially communication processes, control mechanisms and feedback principles.

"Cybernetic Bacteria 1" — Anna Dumitriu and Dr Simon Park

"Chair and Bed Flora" — Anna Dumitriu

Behaviour of bacteria is clearly complex especially in terms of their ability to act co-operatively and communicate. As microbiologists, you recognise that bacteria are constantly sending messages to each other. This research has inspired 'Cybernetic Bacteria', an ongoing transdisciplinary investigation bringing together art, philosophy, computing and microbiology to investigate the relationship of bacterial communication with our own digital communications networks. The project is looking specifically at 'packet data' and bacterial quorum sensing, to reflect the far greater level of complexity in communication taking place at a microscopic level in comparison with human communication technologies such as the Internet.

The first artwork in the series involves a clear plastic tube of liquid agar jelly planted into the earth - allowing the soil bacteria below to grow upwards and become visible to the audience. 100 μL of Homoserine Lactone is added and the bacteria below pass this 'message' on to their neighbours. My performance was in many ways incredibly insignificant, I dropped a small quantity of hormone into a large tube of jelly, and it was all over in a few seconds. But for those few seconds I instigated an action with tiny but incredibly far-reaching effects. I interacted with the bacterial communications network of our planet, and I was connected. The next stage of the project involves a multiple screen artwork showing network traffic taking place in real time (web traffic, mobile technology and Bluetooth), a film of bacterial communication occurring (using *Chromobacterium violaceum* CV026) and an interactive visualisation of the data from both sources generated using artificial life technology (Cellular Automata).

The next exciting chapter for my work will involve working alongside Dr Minna Mannisto (based at The Finnish Forestry Institute METLA), an Arctic Microbiologist investigating the complex ecosystems of the soil dwelling psychrophiles she works with and how they relate to our changing climate.

These kinds of artworks can create a far greater understanding of the complexity and beauty of the microbiological world and enable the wider public to have a much more balanced view when confronted with shocking reports in the news or when observing advertisements that are specifically designed to scare them. In fact the public have a strong distrust of the media messages they are being given and relish the opportunity to investigate further. Art is able to reach out to wide, non-specialist audiences and communicate ideas in news ways. Through this work thousands of people have re-evaluated their understanding of the word bacteria and this looks set to continue as the project broadens to an international scale.

information

■ If you would like to view Anna's work she is currently exhibiting her newly commissioned work on bacterial communication at The Science Gallery in Dublin until Friday 17th July 2009

"Bed Flora – TEM image" — Anna Dumitriu and Dr John Paul

Anna Dumitriu

Outbreak!

Engaging the public in infectious disease

Why were an unexpected collection of members of the general public, scientists, artists, musicians and families gathered in the Mammals Gallery of Manchester Museum on a cold and wet Monday evening in March? Surrounded by mammals of all shapes, sizes and species (of particular relevance was a display of our primate ancestors) and under the watchful eye (well, eye socket) of the skeleton of a whale suspended from the ceiling, we were gathered together to watch the film 'Outbreak' and to find out what non-scientist members of the public want to know about microbiology. We were taking steps to finding out the answers to the following questions: Why does microbiology matter? Why would any non-scientist want to know about microbiology? But most importantly, what can *Sf*AM do to educate, inform and entertain the masses on this fascinating subject?

The evening began with some light refreshment to stop tummies rumbling during the film and to provide some 'brain food' for the participants who had previously been armed with postcards and pens so they could jot down any ideas that sprang to mind. After a bite, we sat beneath the elegant arches of the Gallery and listened to Professor Joanna Verran of Manchester Metropolitan University expertly facilitate a discussion about what microbiology is and how it affects us. We listened intently as Jo brought microbiology to life, describing the different ways in which it is relevant to everybody's lives every day: from infectious disease to food, brewing and the environment. We then heard some ideas from the audience which were as diverse as the audience's backgrounds, ranging from, 'making microbiology sexy' to 'demonstrations of how microbes can be used in the home' — e.g. in home-brewing and baking.

These were all great ideas, but would their thoughts about microbiology (and therefore their ideas) be stimulated by watching the film? Well, after a few more munchies, we sat and watched attentively for the entire duration, through the spread of a novel Ebola-type virus from Africa to a small village in California. How would the governments deal with this deadly disease? Would they identify it, and more importantly, was there a cure? I won't tell you what happens in the end in case you've not seen the film yourself, but rest assured there wasn't a single person who wasn't gripped until the end.

After a quick stretch, Jo continued a stimulating and informed discussion, drawing ideas from the audience who had definitely been inspired through watching the film. Everyone was full of ideas and questions about how microbiology does or could impact on their lives and how *Sf*AM can continue to engage the public in applied microbiology — so much so that we almost stayed beyond our welcome.

After a thought provoking evening, the ideas continue to flood in to the Manchester Beacon of Public Engagement who were instrumental in organising the event. I would like to thank Sam and Erinma in particular. If you have spoken to your non-scientist aunt or uncle, mum or dad, brother or sister about microbiology, then you may have some ideas about ways in which *Sf*AM can help to make microbiology more accessible, and engage the public in applied microbiology. If so, let us know — and watch this space to find out how you can get involved.

Lucy Harper

Historical Perspectives: Charles Darwin and microbes

The fact that Charles Darwin took an interest in the developing science of microbiology is hardly ever mentioned in biographies about his life and work. Here, **Milton Wainwright** explains how Darwin's letters show that he was interested in microbes, even to the point of wondering if his long-suffering stomach problem was caused by a microbial infection

LEFT: Charles Darwin's study at Down House, near London

Since the biographies, and other accounts of Charles Darwin's work, make few, if any, reference to microbes, I have always assumed that Darwin took no interest in microbiology. However, this always seemed unlikely, especially since the publication of *On the Origin of Species* in 1859 coincided with a period which saw major developments in our understanding of the nature and role of 'germs'. Darwin was interested in all aspects of natural history so it would have been strange had he totally ignored this growing science. When I came to do a search of Darwin's letters I soon found that Darwin did, in fact, take a keen interest in the burgeoning science of microbiology, including the more applied aspects of the subject such as the control of potato blight. This interest lead him to interact with some of the lesser known (pre-Pasteur) originators of the germ theory and their work in addition to some of the better known pioneers such as Robert Koch and Eduard Klein. As we shall see, Darwin not only took an interest in the possibility that microbes cause disease but also sought a microbial explanation for the unpleasant stomach disease which plagued him throughout most his life.

Darwin and algae

The first interaction between Darwin and microorganisms comes about while, as a young naturalist, he voyaged on the *Beagle*. While on board, he became interested in some red "dust" which occasionally coated the ship. In his book, *Voyages of the Beagle* he notes that he sent the German microscopist and microbial taxonomist, Professor Christian Ehrenberg five packets of "dust". Ehrenberg, an unjustly neglected pioneer of microbiology, did much to establish a framework for classifying animalcules. On examining Darwin's samples, he found that that "the dust consists in great part of infusoria with siliceous shields". Ehrenberg went on to identify sixty seven different species which; with the exception of two marine species, these proved to be fresh water species.

Darwin's observations on red "dust" have a modern resonance in relation to the recent controversy over so-called red rain. Red rains have long been observed falling all over the world. They can of course be made up of dust, like those which occasionally reach the UK from the Sahara. However, of interest to us is the fact that they are often microbial in nature. The recent red rain event of Kerala in Southern India provides a good example. Wrongly attributed by many to dust, or even more exotically, bat blood, these rains are full of single cell organisms, having an algae-like morphology. It has been suggested that these recent red rain cells have highly unusual properties and may be of extraterrestrial origin; but a more prosaic explanation is that they were picked up from the oceans in some kind of vortex and then deposited over southern India. While on the *Beagle*, Darwin also observed more common algae which he collected near Keeling Atoll, in the Indian Ocean. These he described as little masses of confervae, a few inches square, which consisted of small algae. His interest in algae is also illustrated by a letter he wrote to Joseph Hooker on the 28th April, 1845 when he

John Tyndall the physicist and mathematician who took an interest in spontaneous generation

Sir John Goodsir, early pioneer of microbiology who examined Darwin's samples for infectious microbes

mentioned that he was returning some specimens and drawings of Confervae.

Darwin's interest in potato disease

Darwin's interest in research on the cause of potato disease provides an example of his keen interest in the practical application of biology to human affairs. Between February 1876 and March 1882, Darwin exchanged some 93 letters with a certain James Torbitt concerning support for one of Torbitt's commercial projects, namely the development and distribution of potato plants resistant to the light blight caused by the fungus *Phytopthora infestans*. This was the cause of the repeated crop failures in the UK, Europe, and of course Ireland, during the nineteenth century. Torbitt was an enterprising grocer and wine merchant from Belfast. His project required selecting the small number of plants that survived in a field infested with the blight fungus and using these to produce new seed which would hopefully produce blight-resistant plants. Darwin also lobbied civil servants on Torbitt's behalf in order to secure funding for this important work and also provided some funds of his own.

Darwin's interest in the development of microbiology

Darwin obviously took an interest in the major developments which were taking place in microbiology from the 1860s onwards. In April of 1862, Henry Holland wrote to Darwin to recommend Pasteur's memoir on the subject. Holland was one of Queen Victoria's doctors and a leading expert on fresh water, microscopic algae. In later life, he claimed that he had beaten Robert Koch to the recognition that cholera is caused by bacteria. In a letter, dated 24th July, 1874, Eduard Klein provided Darwin with details of the smallest micrococci which can be distinctly seen under the microscope. Three years later (1st January,1877), Darwin's interest in bacteria was shown to continue when he received, from Ferdinand Cohn, details of Koch's work on bacteria including some of the first microscope photographs of these organisms.

Finally, as late as April 14th 1881, Darwin wrote to Frithiof Holmgren expressing interest in Pasteur's work on the modification of "the germs of the most malignant disease". Holmgren was a Swedish anti-vivisection activist. In his letter, Darwin explained that he had always been against experiments which caused suffering to animals, but he nevertheless emphasised that improvements in medicine could only be achieved by the use of regulated vivisection.

Darwin and Joseph Lister

Joseph Lister is well known as the surgeon usually credited with having introduced antiseptic surgery into medicine. Lister mainly used carbolic acid to disinfect wounds, but he was always on the look out for better, less caustic, alternatives. It is interesting to note then that in a letter dated 7th Oct, 1878, Darwin suggests that *"benzoic acid would be a deadly poison to bacteria and their allies."* He also expresses surprise to Lister that he is considering the use of borax as a disinfectant, as Darwin claims that borax has no effect on the growth of *Drosera* (i.e. the Sundew), although it apparently was readily killed by phosphoric acid. Presumably Darwin's claim that benzoic acid would kill bacteria was similarly based on experiments using *Drosera*, rather than bacteria themselves. Indeed I can find no evidence that Darwin ever actually worked on the isolation and growth of bacteria.

Darwin and spontaneous generation

Unlike that other British Victorian champion of transmutation (or evolution), Robert Chambers, Darwin did not speculate on the origin of life, other than to suggest it might have formed in a *"warm little pond"*, an idea he probably got from his grandfather, Erasmus Darwin. Chambers, the author of the much reviled, but influential book on transmutation, *The Vestiges of the Natural History of Creation* claimed that life could arise spontaneously at any time. He based this belief on what seemed like good science at the time based on the findings of Andrew Crosse and W. H. Weekes. Crosse claimed that he could create life (so-called acari) by passing a low current of electricity through solutions containing silica; Weeks verified this experiment and Chambers used their work in his theory of evolution with the idea that life originally arose spontaneously and continues to do so. Crosse, who lived in a dilapidated mansion in the Quantock Hills in Somerset, was referred to as *"the thunder and lightning man"* and was locally reviled as an atheist; he also probably acted as the model for Mary Shelley's, *Frankenstein*.

As early as 1866, Darwin entered the argument over the continued occurrence of spontaneous generation by stating (in a letter to J.V. Carus, 21st Nov, 1866) that *"As for myself I cannot believe in spontaneous generation."* During the 1870s, Darwin also corresponded with John Tyndall who was working on the spontaneous generation controversy. In his experiments, Tyndall set up a large number of open tubes containing extracts of vegetables and somewhat exotic meats, like venison and pheasant. These, he showed, soon became contaminated with airborne bacteria and fungi. In this way, Tyndall demonstrated what was referred to by Victorian scientists as "panspermia", that is the ability of microbes to live in the air from where they contaminate organic-rich extracts and also, by extension, infect humans and animals (the word panspermia is used today to refer to the transfer of life between planets). Tyndall also observed numerous examples of microbial antagonism, and in particular, the ability of fungi to kill bacteria; unfortunately however, he missed the opportunity to translate these observations into the discovery of antibiotics.

Tyndall sent Darwin one of his unopened tubes which Darwin left exposed to the air. In a letter dated 20th October 1875, Darwin related the news to Tyndall that *"the tube of boiled infusion, dated October the 16th, was clear on the 19th, but on the 20th it was muddy and contained bacteria in living movement"*. Tyndall sent tubes of broth to various people asking them to expose them to the air in their locale; the recipients included, amongst others, Darwin, Mr Francis Darwin (Darwin's son) at High Elms, Sir John Lubbock and Joseph Hooker. Every one of these tubes were reported to be contaminated, no matter where in the country they were exposed, thus giving Tyndall the proof of his idea that airborne microbes were to be found everywhere.

On the 1st of February, 1871, Darwin wrote a letter to Hooker again expressing his interest in the experiments that were then ongoing on

spontaneous generation, when he mentioned B.T Lownes' observations that boiling does not kill certain moulds. This he thought was curious because it flew in the face of Pasteur's experiments. Pasteur claimed that his boiled broth remained sterile and would do so indefinitely, yet Lownes could not repeat this. Five years later, Darwin again took an interest in the spontaneous generation controversy (4th Feb, 1876) when he informed John Tyndall that he was happy that he was not giving up his research into the problem. He suggested that the issue would never be resolved until someone explained how John Burdon Sanderson and others *"succeed in getting bacteria in infusions boiled for a long time"*. Burdon Sanderson, by the way, was one of Britain's leading exponents of the new science of microbiology and although he was totally convinced of the merits of spontaneous generation, his experiments for a while at least tended to give credence to the possibility that life can arise *de novo*.

Did Darwin suffer from a bacterial infection?

It is a well known that after voyaging on the *Beagle*, Darwin suffered throughout his life from a debilitating stomach complaint and many theories have been suggested to provide a *post hoc* diagnose of his illness, most of which centre around the possibility that Darwin suffered from Chagas' disease. In the early 1860s, Darwin sought the advice of one of the leading medical practitioners of the day, Sir John Goodsir. As early as 1842, Goodsir had shown that animalcules could be obtained from the vomit of people suffering from gastric illness. He named the organism *Sarcina goodsir* and went on to suggest that it causes stomach disease. He also claimed that that the organism be eradicated, and the patient cured using hyposulphites. He therefore was arguably the first to demonstrate the presence of a microbe in an internal infection, suggest it caused the disease in question and then provide a cure, this some thirty years before Pasteur took an interest in microorganisms. A great deal was known about *Sarcina ventriculi*, as it became known, by the 1860s when Darwin sent Goodsir a sample of his vomit for analysis. Goodsir tested Darwin's sample for *Sarcina*, but no doubt to Darwin's disappointment, found nothing. The full letter, from Goodsir to Darwin, dated August 21st, 1863 reads as follows:

"I will most certainly examine a slide or a small quantity of fluid with flocculent and tenacious matter sent in a tube or small phial. The spherical bodies are probably the eels of Torula and spores of Penicillium. If Sarcina be present it will be at once detected by its square form and peculiar segmentation. Sarcina and Torula often occur together. Mr (William) Jenner prescribes hydrosulphite of soda. Your medical advisor may try creosote. One drop taken at bedtime and afterwards, two drops in the forenoon and two at bedtime."

We a can infer from this that Darwin used his own microscope to examine the sample before he sent it to Goodsir.

Darwin's interest in extremophiles

During the late Victorian period the Reverend William Henry Dallinger (often collaborating with John Drysdale) did some studies on the ability of bacteria to withstand extremes of heat and cold. Like many naturalists of the period, Dallinger was a man of the cloth, in this case a Wesleyan. His work involved showing how bacterial resistance to extremes of temperature could be achieved by "training" them to grow at high and low temperatures. Darwin would obviously have taken an interest in this work because it showed that organisms are able to adapt to their environment. Dallinger concluded that his experiments demonstrated the inheritance of acquired characteristics and argued with Weismann on this point (letter, July 2nd, 1878). In his letter, Darwin states that he has no doubt that Dallinger's experiments on mutation of lower organism under changed conditions of life will be curious and valuable. He goes on to say that *"the fact of their being accustomed to higher temperatures than to those which they are adapted is very remarkable and explains existence of algae in hot springs"*. A year earlier Darwin wrote to Hooker (Jan 25th 1877) expressing an interest in the ability of bacteria to resist the cold. Like most other scientists of his day, Dallinger conducted experiments on spontaneous generation and became, not surprisingly, an opponent of Henry Charlton Bastian who, even as late as the early years of the twentieth century, claimed he could generate microbes *de novo* in the test tube.

Conclusions

As we have seen, Darwin took a keen interest in many aspects of microbiology including a personal interest in the possible role of *Sarcina* as a cause of his stomach problems. Despite this however, there is no evidence that Darwin ever used the new techniques that were being developed to isolate and grow bacteria and moulds. As a naturalist he had a keen interest in algae, and doubtless used his simple microscope to observe protozoa. Perhaps he felt that the development of the new science of microbiology, which needed practice in the use of some unusual techniques, was best left to younger scientists. As we have seen however, this did not prevent him from taking a keen interest in the development of the new science of microbiology, especially of course where it impinged on anything to do with selection and evolution.

further reading

- Charles Darwin's letters can be accessed by searching Google for "Darwin Correspondence"; unfortunately, most of the letters used here are not yet available in full.
- Torbitt's work on potato blight: Dearce, M. (2008). Correspondence of Charles Darwin on James Torbitt's project to breed blight-resistant potatoes. *Archives of Natural History* **35**, pp208-222.
- William Dallinger's work: Haas, J.W. (2000). The Reverend Dr William Henry Dallinger, *Notes and Records of the Royal Society of London*, **54**, pp53-65.
- John Tyndall's work on microbial antagonism: Wainwright, M. (1985). Re-examination of John Tyndall's studies on microbial antagonism. *Transactions of the British Mycological Society* **85**, pp562-569.
- Sir John Goodsir's work on *Sarcina*: Wainwright, M. (2003). An alternative history of microbiology. *Advances in Applied Microbiology* **52**, pp333-356.

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

Using a regression line for prediction and calibration

Stat Note 17

In Statnote 16 (Hilton & Armstrong, 2009), the use of regression methods to analyse the relationship between two variables X and Y was described. How to fit a regression line to data by the method of least squares was discussed, as well as the methods of testing the *goodness of fit* of the line to the points. Another use of a regression analysis is to use the line to predict a value of Y from a reading of X, e.g., to predict bacterial cell number from optical density (OD) readings. This aspect of regression studies is also called 'calibration', i.e., estimating a quantity that may be difficult to measure from a variable that may be much easier to measure. This Statnote describes the various predictions that can be made using a regression line and discusses their limitations.

Theory

There are two types of prediction problems that can be solved using a regression line and it is important to understand the difference between them. First, there is the prediction of the *population regression line* μ at the point x. Hence, we may wish to make inferences about the height of the *population regression line* at the point X, i.e., the *average value* of Y associated with a value of x. Second, there is prediction of an *individual* new member of the population y_1 for which x_1 has been measured. The second problem is probably the most commonly encountered and is most relevant to calibration studies. In both of these prediction problems, however, the predicted value of Y is actually the same but the standard errors (SE) of the two estimates will be different because in the first instance, a population value or mean is being estimated while in the second, an individual value is being estimated. Formulae for the calculation of the different SE corresponding to these two prediction problems are given by Snedecor & Cochran (1980). In most cases, significantly greater errors will result when estimating an individual rather than a population value.

Background

In some experimental protocols it is necessary to estimate the number of bacterial cells present in a culture broth where time limitations may prevent the use of a standard culture-based colony counting technique. In these circumstances a researcher may employ direct counting techniques using microscopy and a haemocytometer slide, for example, or more frequently use measurements of OD of the culture broth as a prediction of cell number. In this latter situation, a calibration graph must first be derived to reveal the relationship between the cfu per ml of culture broth for a given bacterium and the corresponding OD usually measured at 600nm. When correlation data is used in this way to make predictions of one parameter from measurements of another, it is important to be aware of the limitations in accuracy of such predictions.

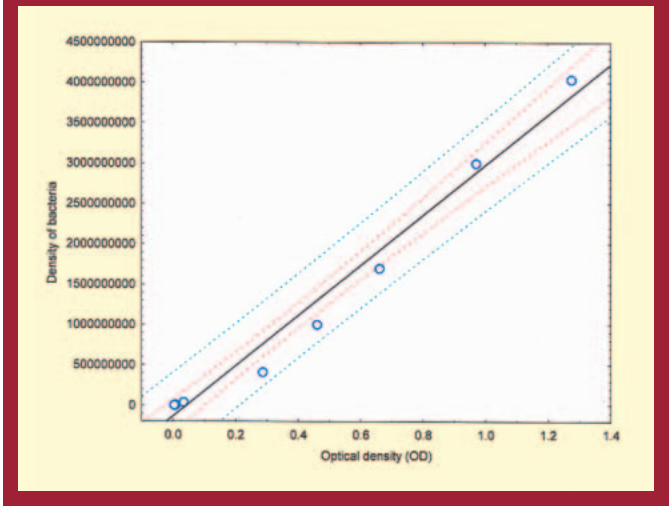
Scenario

A 10ml volume of sterile nutrient broth was inoculated with a culture of *Staphylococcus aureus* and incubated at 37°C for 24 hours. Following incubation the culture was serially diluted down to 10^{-9} by mixing 1ml of culture with 9ml of fresh nutrient broth in a sterile Universal tube. From each of the tubes within the prepared dilution series, 1ml of culture media was transferred into a disposable plastic cuvette and the OD measured at 600nm using a standard spectrophotometer which had been previously blanked against a cuvette containing uninoculated nutrient broth. In a similar manner, from each of the tubes within the first dilution series, further serial dilutions in sterile nutrient broth were prepared as required and 0.1ml of this second dilution series inoculated onto the surface of a nutrient agar plate. The inoculum was spread across the surface of the agar using a sterile spreader and the plates incubated at 37°C for 24 hours. Following incubation the cfu ml^{-1} in each of the tubes within the first dilution series was calculated by counting the colonies at an appropriate dilution. The data were collated and are presented graphically in Figure 1 by plotting the cfu ml^{-1} on the x-axis against the corresponding OD_{600} measurement on the y-axis.

Fitting the regression line

The first stage in a calibration study is to fit a regression line to the data using the method of least squares as

Figure 1. *Regression of bacterial cell numbers (Y) on OD (X) (r2 = 98%, F = 398.04 (P < 0.001),'t' = 19.95 (P < 0.001). The two sets of confidence bands represent confidence intervals for the population regression line (red) and for making individual predictions of y for a new value x (blue).*



described in Statnote 16 (Hilton & Armstrong, 2009). To estimate bacterial cell numbers from OD requires the fitting of the regression of bacterial numbers (Y) on OD (X) and this line (Figure 1) has the equation:

$$Y = -1.333 \times 10^9 + 3.111 \times 10^9 X \qquad (1)$$

All the usual 'goodness of fit' tests (see Statnote 16, Hilton & Armstrong, 2009) suggest that the line is a good fit to the data. Hence, $r^2 = 0.98$, i.e., 98% of the variance in bacterial numbers is accounted for by OD, the value of F = 398.0, suggests a highly significant line is present (P < 0.001) and the 't' test of the slope of the line gave a value of t = 19.95, which suggests the line has a highly significant slope (P < 0.001). All of these tests provide confidence that the regression line is a good fit to the data and therefore is suitable for calibration.

Confidence intervals for a regression line

Two sets of 95% confidence intervals have been fitted to the regression line in Figure 1. The inner confidence bands (in red) are the confidence limits for predictions of the population regression line and hence, we would be 95% confident that the average value of Y for any x would lie within these boundaries. The outer confidence intervals (in blue) are those for predicting an individual y corresponding to a value of x. It is important not to confuse the two types of prediction problem. If, for example, the regression of weight on height was plotted for a sample of 20 year-old men (Snedecor & Cochran, 1980), the purpose might be to predict the *average weight* of such men at a specific height (inner confidence bands) or the *individual weight* of a new male whose height was known (outer confidence bands). As mentioned earlier, the two estimates are the same, but the SE and therefore the confidence bands are different.

Using Figure 1 for prediction and calibration, the predicted value of y for a new value x is:

$$y = Y^* + bx \qquad (2)$$

where Y^* is the mean of the Y values and b the slope of the line. Hence, if OD = 1.1 then estimated Y is 3.28885×10^9 (+95% confidence limits = 3.59498×10^9; -95% confidence limits = 2.982719×10^9).

Interpretation

There are a number of problems that need to be considered when using a regression line for calibration. First, the confidence bands are parabolic in shape and have curved borders that may widen significantly at the limits of the data especially if the line is a relatively poor fit. Hence, estimates of y can have large errors at the limits of the data. As discussed in statnote 16, there is no established 'cut-off' in r^2 below which the line would be regarded as a poor fit for calibration purposes. As the value of r^2 decreases, however, the confidence bands widen and predictions of y become increasingly inaccurate. A regression line is likely to be most useful for calibration if the range of values of the X variable is large, if there is a good representation of the x,y values across the range of X, and if several estimates of y are made at each x. Second, a regression line is sometimes used several times in the course of an investigation to predict a number of new values. In this circumstance, the probability that all of the confidence intervals include the correct value of Y will be less than $P = 0.95$ and a correction of the P values may be necessary using Bonferroni's inequalities (Snedecor & Cochran, 1980). Third, y may have been measured at several fixed values of x but the intention may be to predict x from y and this prediction must be made from the regression of Y on X. This is a significantly more complex calculation and the method together with the appropriate SE is described in Snedecor & Cochran (1980).

The relationship between two variables may have been studied at various times or in different laboratories giving rise to two or more estimates of the relationship between Y and X. In these circumstances, it may be of interest to discover whether the various regression lines are the same. If they are, an investigator may wish to combine the data from different studies and fit a single regression line to the whole of the data. This application of regression studies involves comparing two or more regression lines and will be discussed in the next Statnote.

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careers

Sales Manager

David Warering explains how he has used his life experience to help him on the road to his current career

Where do you see yourself in five years time? This is a typical interview question and must appear in numerous guides to interview technique. My answer to this question is that in fact I have no idea, but I don't necessarily think this is a bad thing. It gives me the opportunity to be anywhere and allows me the freedom to accept or reject opportunities that come my way in the coming months and years. Something I've learned about career decisions is that one has to be able to recognise opportunities for learning and value and draw upon all life experiences. I am out of my comfort zone most of the time, and that is a great place from which to learn.

One year ago I would not have seen myself in my current role as European Sales Manager specialising in Transplantation Diagnostics for the biotechnology giant, Life technologies. Way back then, which seems like a lifetime ago, I was Business Development manager for Europe and Asia Pacific Regions working for Invitrogen's Environmental Diagnostics Division, a rather grand title for my team of one, but nonetheless an important role with a large revenue target and a complex multi-cultural responsibility. Now I am back working in the business of Clinical Diagnostics, which is where I started my science career and I'm loving every minute of the challenge. I am a Sales Manager by title, but really I am a mentor to my team who serve our customers with the latest technology to suit their needs.

How did I get here? Well, to answer this question I must start at the beginning of a strange and winding road. My family has been associated with farming for as long as I can remember. Many of my father's family are farmers and almost all of my siblings have, at one time or another, delivered unpasteurised milk from door to door for a businessman that was a close friend of the family. Many years later I found myself incorporating a hypothesis on population genetics of host association between *Campylobacter jejuni* Sequence type 61 and cattle, with a major vector being the consumption of unpasteurised milk. I have no doubt that my knowledge of milking parlour practice and doorstep delivery had some influence in my confidence to propose such a hypothesis, not to mention the confirmed hypothesis that birds pecking at foil capped milk bottles could also be a vector for transmission of *Campylobacter jejuni*.

Whilst at school I had a job of assisting with the cleaning of the school swimming pool. This offered me free swimming at lunchtimes but also provided the opportunity to learn about chlorination and filtration in swimming pool hygiene. Later in life I found myself in an R&D role developing a protocol to use immunomagnetic separation to detect *Cryptosporidium* oocysts in swimming pool water as well as working in a global business that has a significant interest in detection methods for *Cryptosporidium* and *Giardia* in drinking water.

During the summer break between school and college I worked on a chicken farm collecting eggs and assisting with general farm duties. This was quite a disgusting job for many reasons but good preparation for my later career as Head of the Enteric Microbiology Laboratory at the Public Health Laboratory in Preston analysing 15,000 human faecal samples per year. Chickens defecate a lot and in such cramped conditions it is easy to understand the rapid spread of *Salmonella* enteritidis phage type 4, which was the scourge of the poultry industry in the 1980s and 90s (for younger readers look up "Edwina Curry and eggs" for the start of a national scandal and listen to Professor Tom Humphrey for more details). Later, as a Clinical Scientist I was developing rapid screening methods for *Salmonella* outbreak investigations.

Three of my family were in the armed forces and there was a critical point at the age of 17 when, after a long process I was offered a place in the RAF as an engineering apprentice. I decided not to accept this offer as I was no good at Maths so I am sure this would have been a bad move, but my main reason for this choice was that back at college I was in the first XI at

football and had half the number of lessons that were timetabled at school. Several months later, bored by college, I answered an advert for a Junior Laboratory Technician at the local hospital and my career in science began in earnest.

I had a broad education in pathology sciences — haematology, blood transfusion, biochemistry, histopathology and microbiology, and at the next decision point I decided to specialise in microbiology. On my first day (and, also, by coincidence, my last day) in the microbiology department at Preston, I worked on the fæces bench in the Preston Public Health Laboratory — part of the PHLS network of 53 labs at that time. On the first day I was a junior and on the last day I was Team Leader.

I played soccer for the lab team and after three successive years of comprehensive defeats and relegation, the team resigned from the competitive league and moved to play friendly matches only. At this time I was quite handy at football and I assumed the role of player manager for the team. I carefully selected the opposition and months later we were winning matches. The point of this anecdote is to illustrate that in a different context I was able to shout orders at more senior members of laboratory staff and demonstrate my leadership skills in an environment outside the laboratory. I do believe that this was influential in showing some of those who later became my mentors that I had leadership qualities that they may not have had the opportunity to see quite so clearly in the workplace.

In my first senior and management position and I was fortunate to have the opportunity to setup the *Campylobacter* Reference Unit at Preston. On the back of Dr Sameeh Salama's PhD studies with Professor Eric Bolton, we had the opportunity to use a new Phage typing scheme for *Campylobacter jejuni* in conjunction with the exisiting biotyping scheme developed by Eric Bolton. During this tenure we attempted to put together a resistotyping scheme based on filter paper discs impregnated with various antimicrobials and a trio of discriminatory pre-formed enzymes tests which were dehydrated for stability. We performed a six lab ring trial with reasonable success and the product was eventually manufactured by MAST diagnostics. This was my first attempt at product development, an experience and skill set which I was grateful for when I moved out of the public science arena and into a commercial biotechnology company as Research and Product Development manager.

At the pinnacle of my public science career I was most fortunate to work with Professor Andrew Fox. Our work together during my PhD studies on epidemiology of zoonotic transmission of *Campylobacter* led to collaborative research with Professor Martin Maiden and colleagues at Oxford University (the only chance I would ever get to go to Oxford!), on the first large scale multi-locus sequence analysis studies of well defined sets of isolates. This provided fascinating insights into the likely ecology and host associations with this enigmatic pathogen. We wrote a grant application to the Food Standards Agency for funding to elaborate on these hypotheses and investigate seasonal trends in strain specific ecology and epidemiology. This application was one of the first major business case proposals that I had worked on and allowed me to develop skills in value proposition, budgeting, setting goals and milestones which are crucial in any successful business enterprise.

My name appears on over 40 publications but I won't claim credit for more than two first author papers. My forte is not technical writing but prose and I have an eye for detail that is better suited to editing. I learnt a lot from a single publication working with the eminent Professor Martin Skirrow. I could not believe my luck to be invited to attend an S*f*AM workshop in Reading in 1992 with Eric Bolton, David Hutchinson and Martin Skirrow. The proceedings were published in The Technical Series Manual No 29. Working on this manuscript with Martin Skirrow was painstaking. His perfect use of the English language was enlightening and his eye for the finest grammatical detail and his patience and mentoring through the multitude of draft versions was a valuable learning experience for me and a formative experience. At my second S*f*AM committee meeting and as a newly elected member I happened to look up at the opportune moment of the proceedings and was asked by President Arthur Gilmour if I would consider the opportunity of becoming editor of S*f*AM *News* (the forerunner of *Microbiologist*). With no idea what it entailed but with an interest to learn more, I said confidently — *"Yes, why not"*. I immediately had a deadline to produce the next issue. I signed up for a distance learning course in editing manuscripts and with virtual carte blanche to create a new feel for the magazine, I set about learning the process of soliciting content, writing editorials, agreeing layouts with the designer and editing final copy. I learnt so much about the publication process that I can now interact with marketing colleagues to produce a captivating marketing flyer, technical note or brochure for a new product.

In your career, you have to recognise your strengths and play to them. I don't consider myself a good scientist, writer or salesperson but I do think I am a good mentor. I have also learnt sufficient technical skills in many areas of science and business to be able to apply my teaching and mentoring skills to best effect. My good friend and colleague Dr Andrew Sails is a great scientist and is far better than I could ever be, because he is a scholar. I learnt from him how a real science scholar works with a real passion for scientific understanding, but I think I taught him a thing or two about how to manage in science and to manage people.

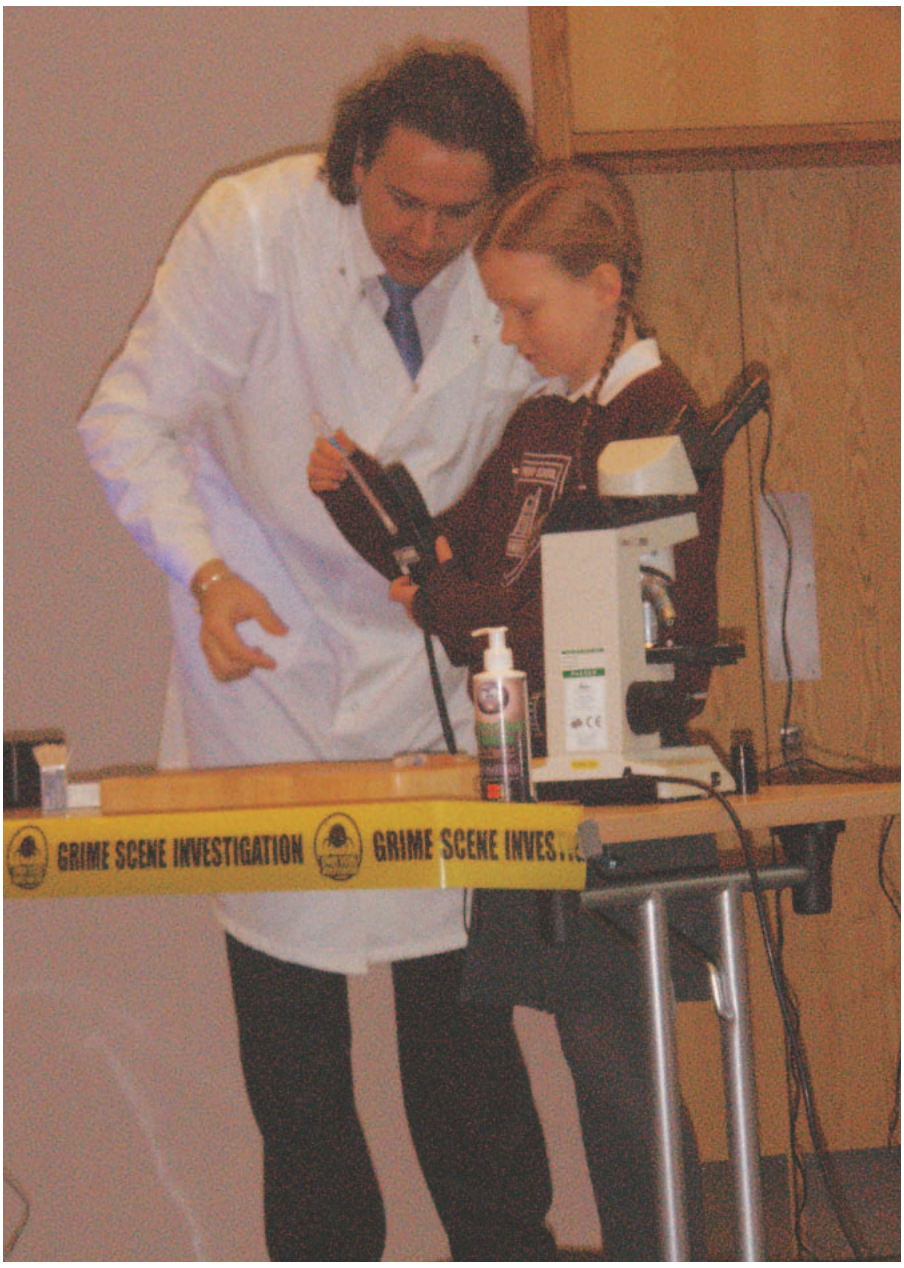
My work is very varied now. I spend time coaching sales people, researching for new market opportunities, writing business cases, planning strategy and negotiating. I have found that in life you always have the opportunity to learn, you just have to recognise what you are learning and use that knowledge for self improvement. I have been fortunate enough to work with many very talented people, some of them scientists, some managers and some just interesting people who can teach you a thing or two.

What will I be doing in five years time? I have no idea but I am training for my next job right now and every day.

David Wareing

An unwanted guest for dinner

Public engagement / innovative project grant report



The Science Learning Centre North East has been engaging teachers and their pupils with their Christmas lecture series for four years. These are demonstration-packed, fun-filled science events for young people targeted at Year 6/7 pupils from local schools.

They take place in industry venues spread across the North East region to provide an opportunity for school children to visit an industrial environment, sometimes for the first time, whilst participating in some exciting science demonstrations. The theme of the lectures changes every year and previous events have involved TV personalities such as Jonathan Hare from BBC TV's Rough Science and Ian Dunne, the science presenter. This year I was approached to deliver a lecture series in microbiology entitled ***An unwanted guest for dinner*** which focused on food hygiene and safety.

The lecture was loosely structured around the '4 Cs' principles of food safety (Cooking, Cleaning, Chilling and Cross-contamination) adopted by the Food Standards Agency and linked in with the 'Germwatch' media campaign. The one-hour lecture session was filled with opportunities for the students to undertake mini-experiments and get involved with demonstrations. The session began by introducing bacteria, particularly their small size, and using a Smartie placed in the palm of the children's hand as a reference they were asked how big their hand would need to grow in order to make a bacterium as big as the Smartie: answers ranged in size from a big as a cat to planet earth. This was followed by the classic demonstration of bacteria isolated from the mouth of a willing volunteer as pioneered by Anton van Leuwenhoek and revealed by a microscope eyepiece video camera attached to an AV projector. If you ever forget why you became a microbiologist you just need look at the wonderment on a child's face when they see for the first time bacteria scurrying over a microscope slide.

The importance of storing chilled food correctly was presented to the students followed by an interactive session using a laser thermometer to probe the temperature of a fridge and a selection of chilled foods. In addition, there was an activity loading a fridge with different food groups to demonstrate the importance of

preventing raw to ready-to-eat food contamination using that essential comedy prop, a rubber chicken! The lecture continued with more information on cooking and with an interactive quiz for students to decide on whether certain meat they were shown was cooked properly.

Cleaning, particularly hand hygiene, was demonstrated using the 'Glitterbug' disclosing cream and UV illumination and a chopping board was sampled using ATP bioluminescence to determine if it had been cleaned properly after use. For each activity a student volunteer was requested (for which there was no shortage) and the reward of a giant microbe to take home made sure it was an experience they would not forget in a hurry. Over the eight lecture sessions approximately 800 pupils attended.

I'd like to thank S*f*AM for their support of this lecture series through the innovative project / public engagement grant, without which it would not have been possible to provide all the hands-on interactive sessions which are so important in maintaining engagement with younger students. This type of activity is invaluable, not only because it increases the visibility of the Society with industry, the public, students and their teachers; it inspires our future generation of microbiologists, inspires achievement and provides aspirational role models for children.

Teachers from the schools whose children saw the lectures had some great things to say:

"It was a very enjoyable afternoon for all of our children and adults as well! I am sure that both schools got a lot out of the hour — our Head was very impressed! We did not expect the goodie-bags but they were much appreciated by all. The pictures that I took will be placed on the web-site that I am developing for school.
Thank you once more for an excellent afternoon"

Robin Carr

"Today I took my class to… listen to the Christmas Lecture by Dr Anthony Hilton 'Grime Investigation' — just thought I'd let you know they found it very interesting, loved the venue and the goody bag! They were very enthusiastic — great to see children enjoying science. The talk was really pitched at the right level for Year 6 to 7 children and Dr Hilton did a graeat job. Many Thanks"

Pauline Taylor

Dr Anthony C Hilton
University of Aston

In the loop pecs

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

PECS NEWS

PECS recently held committee elections and we would like to welcome our newly appointed committee members, Katie Fisher (events officer), Andrew Hall (webmaster), Jo Heaton and James Collins (events team).
The summer conference is fast approaching; join us at the PECS student session which this year focuses on practical networking skills. Remember conference studentships are available for student members who wish to attend the summer conference.
We hope to see you all there!

Congratulations!

Congratulations go to **Andrew Hall** who was awarded his PhD in February at the University of Wales Institute Cardiff. PECS wish Andrew all the best in his future career.

If you know a S*f*AM student or early career scientist who you would like to see congratulated in this section please get in touch and we will publish their achievements on this page. Email: **v.l.mccune@newcastle.ac.uk**

Virtual networking

Networking with fellow researchers outside your department and institution is an integral part of the research process. From an academic perspective it can promote collaboration, generate new ideas and prevent duplication of effort (Graduate Junction, 2008). On a personal level it develops communication skills and confidence and gets you talking about your own research. Generally we network face to face, at conferences, meetings, training workshops and seminars. However, since the conception of chat rooms and social networking sites such as Facebook, MySpace and Twitter, the internet has become an environment in which to network virtually. This has lead to the development of several social / professional networking websites such as LinkedIn (www.linkedin.com), and now there are sites designed specifically for scientists such as nature network (www.network.nature.com), labroots (www.labroots.com), labmeeting (www.labmeeting.com) and Graduate Junction (www.graduatejunction.com).

Graduate Junction is a scientific networking website for graduate researchers worldwide, which aims to promote discussion and collaboration across disciplines and countries. It has a similar format to social networking sites, once registered you create a profile from which you can share your research interests with the global research community. Your profile can contain a summary of your work, keywords to describe your research interests, a list of publications, a description of your research skills and can include a photo and a research blog. To find other researchers who share common interests you simply search by keywords and then get connected by either sending a message or creating a research link. You can also create and join groups to share ideas and discuss particular methods/subjects etc. These groups are also another great way to find researchers with similar interests. General forums are available covering a variety of topics including supervisor relations, surviving your viva, writing your thesis and postgraduate jobs. These are excellent sources of advice and support and cover all aspects of a research degree. The conference diary facility allows you to add and search upcoming conferences, save them into your diary and see who else within the community is also planning to attend.

In addition to networking facilities, Graduate Junction also provides listings of graduate journals. These are online open access journals which are ideal publications for postgraduates to disseminate their research to the wider scientific community. One graduate journal listed is Kaleidoscope, the interdisciplinary postgraduate journal of the institute of advanced study (www.dur.ac.uk/kaleidoscope), which accepts articles and reviews from postgraduates in any discipline.

Online resources like Graduate Junction are invaluable tools which enable early career scientist to build research networks, collaborate nationally and internationally and begin to break the barriers between disciplines, allowing research projects to be considered from a multi-disciplinary perspective. The ability to network is essential to the success of a career in research.

With the number of scientific networking websites increasing I have no doubt that virtual networking will become a routine means for scientists to 'get connected' in the future.

Vicki McCune
PECS Communications Officer, Newcastle University

Research development fund report

Intestinal bacteria and ulcerative colitis

The human colonic microbiota comprises several hundred bacterial species, sub-species and biotypes, and a close metabolic relationship exists between these organisms and the host. Many factors control microbial colonisation of the large intestine, the most significant being diet, environment, digestive function, and the anatomical structure of the digestive tract. Colonic bacteria interact with host physiological processes in a multiplicity of ways, including the metabolism of xenobiotics, mineral absorption and immune system development, as well as the activation and destruction of toxins, genotoxins and mutagens (McBain & Macfarlane, 1998; Macfarlane & Macfarlane, 2004).

The fundamental composition of the colonic microbiota is basically similar in different people, but marked inter-individual variations can be seen at species level (Finegold *et al.*, 1983). The ecological, physiological significance of this is unclear at present, largely because little is really known about the multicellular organisation of the microbiota, or of the metabolic relationships and interactions that exist between various bacterial communities, and how this relates to host health and wellbeing. The normal gut microbiota is a partner in a symbiotic association with the host, particularly those species involved in the final stages of the digestive process, but some organisms, including certain clostridia, enterococci, eubacteria and peptostreptococci have the potential to promote dysbiosis in the large bowel, particularly when the equilibrium of the ecosystem has been disturbed. There is mounting evidence that putatively commensal species in the normal microbiota are linked to the aetiology of inflammatory bowel diseases such as ulcerative colitis (UC) (Cummings *et al.*, 2003).

UC is an acute and chronic relapsing illness that only affects the large gut (Figure 1). The peak age of incidence is between 20 and 40 years, and one of the main symptoms is bloody diarrhoea, which is often associated with mucus, urgency, tenesmus and abdominal pain. The disease begins distally in the rectum, progressing up the bowel over time, towards the right colon. It is thought that 60,000 to 120,000 people

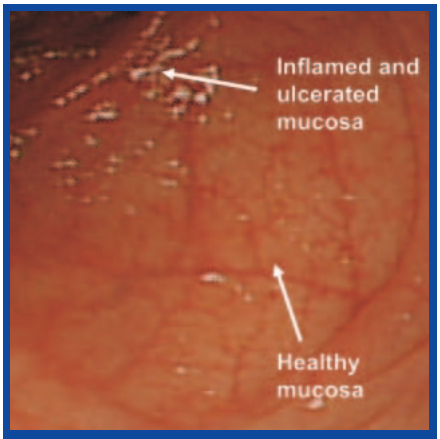


Figure. 1. *Light micrograph taken during endoscopy of the distal colon in a patient with ulcerative colitis, showing the boundary between normal and inflamed mucosae*

are affected by UC in the UK, with 6,000 to 12,000 new cases being diagnosed annually (Shivananda *et al.*, 1996; Loftus, 2004). Studies with animals show that intestinal bacteria are associated with UC initiation and/or maintenance, but there is no firm evidence for a specific transmissible agent in this disease, and Koch's postulates have not been demonstrated. In the search for a singular causative organism, a number of investigators have looked at *Bacteroides* spp. and different subtypes of *E. coli*. However, the various organisms that have been implicated in UC are not found in all patients, although there is some evidence that bacteria growing in mucosal biofilms are involved in the disease. These bacteria could be pathogens that colonise the epithelial surface and invade underlying tissues, or non-pathogenic commensal species occupying adhesion sites on the mucosa and preventing pathogen adhesion (Macfarlane *et al.*, 2009).

Our work on gut biofilms has shown that mucosal surfaces in the large bowel are heavily colonised by complex microbiotas that are structurally distinct from microbial communities found in the gut lumen (Macfarlane *et al.*, 2004). Microscopic analyses demonstrated that many of the bacteria in mucosal biofilms were present in microcolonies (Figure 2). Using culturing techniques, few consistent differences were seen in microbiota composition in rectal biopsies obtained from healthy people and UC patients, which might be explained, in part, by natural inter-individual variation, and the relatively low patient numbers involved in the studies. However, marked variations were recorded in some bacterial groups, but it was difficult to assign a role for specific bacterial species in UC aetiology. Seventy-two bacterial taxa belonging to 18 genera were detected on the rectal mucosa, with 20 species being common to the UC and healthy cohorts, although only differences in bifidobacteria, numbers of which were 30-fold higher in healthy tissues, were statistically significant. Bacteria belonging to the genus *Peptostreptococcus* were only isolated from the UC patients, who also had proportionally more enterobacteria. Previous work had similarly shown that peptostreptococci only occurred on UC rectal mucosae (Matsuda *et al.*, 2000). Some species belonging to this genus are known pathogens (Ezaki *et al.*, 1991), and while they do not seem to be able to colonise the healthy rectal epithelium, these bacteria are commonly found in faeces (Finegold *et al.*, 1983).

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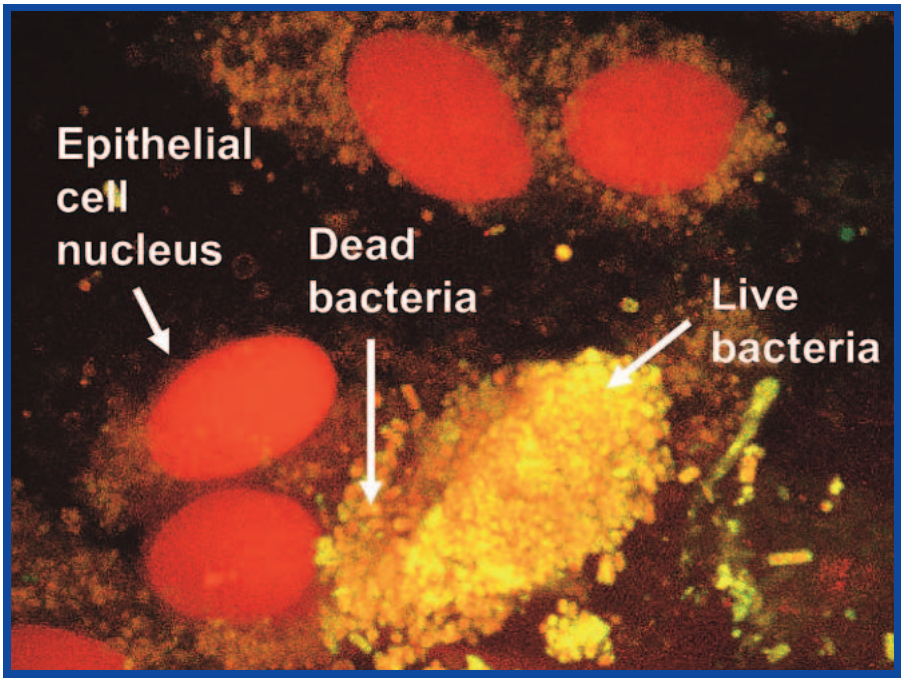

Figure. 2. *Live/dead confocal light micrograph of a bacterial microcolony on the rectal mucosa stained with Baclight™*

Low numbers of bifidobacteria, which are viewed as being beneficial organisms, or the absence of particular bifidobacterial taxa on the mucosa may be of significance in UC, as a number of species have well documented immunomodulatory properties (Famularo *et al.*, 1997). These bacteria are believed to contribute towards host defences through interactions with the gut immune system and via colonisation resistance (Gill *et al.*, 2001; Lu & Walker, 2001). Compared to other mucosal isolates, bifidobacteria and peptostreptococci were shown to be highly immunogenic in UC patients (Furrie *et al.*, 2004), lending further support to the notion that the presence or absence of these organisms could be linked to the disease process.

Since the immune response against normal gut inhabitants is thought to drive inflammatory processes in UC, modulation of bacterial communities on the gut mucosa through the use of probiotics and prebiotics offers prospects for modifying the disease state. With this in mind, a synbiotic combining a probiotic *Bifidobacterium longum* strain isolated from healthy rectal epithelium, and an inulin-based prebiotic (Synergy 1) was used in a pilot study to treat UC patients (Furrie *et al.*, 2005). The investigation was a double-blinded randomised controlled trial, lasting one month, which involved 18 patients with active UC. Results showed that bifidobacterial numbers increased 40-fold on the rectal epithelium, and that mucosal inflammation was reduced in the synbiotic group compared to patients receiving the placebo. Messenger RNA levels for inducible human beta defensins 2, 3 and 4, which are strongly up-regulated in active UC, were significantly reduced in patients receiving the synbiotic. TNFα and IL-1a, which are inflammatory cytokines that drive inflammation and induce defensin expression, were also significantly reduced after treatment. Biopsies taken from the synbiotic patients showed regeneration of epithelial tissue. Overall, the study demonstrated that short-term synbiotic treatment resulted in improvements in the full clinical appearance of chronic inflammation in patients receiving therapy, and indicated that there is potential for developing inexpensive new bifidobacterial-based therapies for use in the treatment of inflammatory bowel disease.

Katie L. Blackett and George T. Macfarlane
University of Dundee

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The Sticky Problem of Biofilms formed by *Staphylococcus aureus*

I am currently entering the fourth year of my BSc with honours Microbiology degree course at Glasgow Caledonian University and was excited when at the end of my third year the opportunity arose to benefit from a 10-week summer studentship in the University's research microbiology lab.

My project aimed to study the sticky problem of biofilm formation in *Staphylococcus aureus* and support the research currently underway in this field.

S. aureus is a Gram-positive bacterium which is a highly successful human pathogen. This organism can cause a range of infections from superficial skin and soft tissue infections to deep-seated infections such as septicaemia and endocarditis. *S. aureus* is one of the most frequently isolated Gram-positive bacteria from hospital associated infections and approximately 45% of *S. aureus* isolates are methicillin-resistant (Biendenbach *et al.*, 2004; Boyce *et al.*, 2005). The number of death certificates mentioning methicillin resistant *S. aureus* (MRSA) stabilised at 1,652 in 2006. This followed a dramatic increase from 51 to 1,649 deaths between 1993 and 2005. There are two dominant clones of MRSA that cause approximately 93% of infections in Scotland. Epidemic MRSA-15 (EMRSA-15) causes 70% of these infections, epidemic MRSA-16 (EMRSA-16) isolates are responsible for approximately 23% of infections and 7% of MRSA infections are caused by sporadically occurring clones (Morrison, 2003). Why the vast proportion of infections is caused by so few clones remains unknown.

Although *S. aureus* is a common commensal organism of the skin surface, inhabiting in particular the epithelia of the nose, it is associated with many types of infection. One of the reasons that *S. aureus* is such a successful pathogen may be due to its ability to form biofilms on almost any abiotic or biotic surface. A biofilm is a structured community of micro-organisms encapsulated within a self-developed polysaccharide matrix. Of all human infections, 65% are thought to be associated with this form of growth. The *ica* operon is currently the best investigated and most widely understood mechanism of biofilm formation, but there is recent evidence that other genes may be involved in the development of these structures. Mack *et al.* (1994) proposed a two-step model for biofilm formation in staphylococci. The two steps can be genetically separated. The first step in biofilm formation is the attachment of cells to a solid surface. The surface can be within the host or it can be an artificial medical device, for example a venous catheter. The second step is secretion of the polysaccharide intracellular adhesin (PIA) causing the cells to stick together and in turn form the biofilm.

In this project, my aim was to see how different environmental factors affect the phenotype of *S. aureus* biofilms. These included supplementing the growth media with different sugars, adding salt and altering the pH of the medium. Six clinical isolates of *S.aureus* were collected from the Scottish MRSA Reference Laboratory (Stobhill Hospital, Glasgow). These consisted of three methicillin sensitive *S. aureus* (MSSA) isolates and three MRSA isolates. The MRSA isolates consisted of two of the most commonly isolated genotypes in Scottish hospitals (an EMRSA-15 and an EMRSA-16) and a sporadic Scottish clone.

After cultivation of each staphylococcal isolate in brain heart infusion (BHI) broth, the number of cells in each culture was adjusted to $1x10^6$cfu/ml. Biofilms were grown in BHI broth in 96-well microtitre plates and biofilm biomass was quantified by crystal violet staining. The absorbance of crystal violet, eluted using 70% ethanol, was measured at 570nm using a microtitre plate reader (BMG LUMIstar* plate reader, BMG, Germany). Characterisation of the strain phenotype was also undertaken using the Congo red agar assay. Congo red agar can give some indication of slime production, which is associated

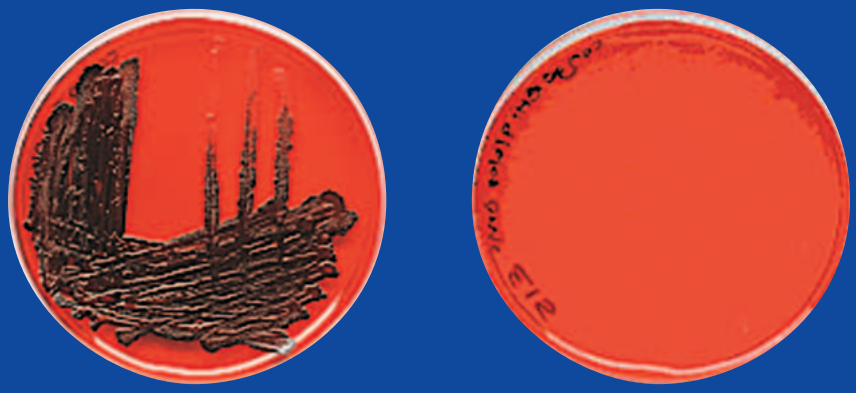

Figure 1. *MRSA isolates grown on Congo red agar plates. The sporadic MRSA clone (left) has black, dry crusty colonies, whereas the EMRSA-15 clone (right) is non-pigmented. Despite the differing phenotypes, both clones were able to form biofilms using the microtitre plate model.*

with biofilm growth, by the appearance of dark, crusty colonies (Figure 1). Using the two techniques, biofilm formation was evaluated in response to each of the environmental stimuli under assessment.

Carriage of selected genes thought to be involved in biofilm formation was also investigated. Genomic DNA was extracted from overnight cultures of each strain using an alkaline lysis and phenol chloroform DNA extraction method. The presence of four biofilm-associated genes (*icaA*, *sasG*, *clpC* and *isaA*) was then evaluated for the six clinical isolates of *S. aureus* by PCR.

After statistical analysis of the results using GraphPad Prism software, it was concluded that adding salt to the growth medium had no effect on any of the strains; neither did altering the pH nor supplementing the medium with lactose. The three remaining sugars produced strain-dependent results; whereby biofilm formation was either unaffected or enhanced. However, the Scottish sporadic isolate in particular produced a significantly enhanced biofilm when mannitol or glucose was added to the growth medium (supplementation of 0.5-5% and 0.5-1% respectively).

Of the MSSA investigated, one strain also demonstrated the ability to form a robust biofilm with mannitol at all concentrations, but no significant enhancement was seen with the addition of glucose. There was no apparent difference between the MSSA and the MRSA strains in their ability to form biofilms under the different conditions tested.

The addition of the various sugars to Congo red agar did alter the strain phenotypes, but the effects noted were strain dependent and did not correlate with the ability of the organisms to form biofilms under comparable growth conditions in the microtitre plate assay.

Consistent with the findings of previous studies, *icaA* was present in all of the staphylococci screened. Similarly, *isaA* and *clpC* were also present. The remaining gene, *sasG*, was present in some of the strains but not others, indicating that it was not essential for biofilm formation.

I would like to take this opportunity to thank S*f*AM for their generous funding which has allowed me to gain valuable work experience. The techniques I have learned in the lab will undoubtedly help me in my final fourth year honours project. I would also like to thank my supervisor, Dr. Sue Lang, who made it possible for me to undertake this studentship.

Finally, thanks to all of lab C122 for all their help, especially Dr. Karen Smith and Cristina Teles. I would fully recommend this scheme to students who wish to gain firsthand experience within a working lab environment and have a passion for research.

Danielle McEwan

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Isolation and characterisation of the periplasmic sensory domain of Tlp7 chemoreceptor of *Campylobacter jejuni*

***Campylobacter jejuni*, which was** first identified as a human diarrhoeal pathogen in 1973, is now the most frequently diagnosed bacterial cause of human gastroenteritis worldwide, surpassing *Escherichia coli* O157:H7, *Salmonella* species and *Shigella* species as causes of enteric infections (Allos, 2001; Altekruse *et al.*, 1999). Apart from gastroenteritis, *Campylobacter* infections have been reported to be associated with other severe conditions, such as meningitis, nephritis, acute arthritis and urinary tract infections (Hu & Kopecko, 2000).

The most recognised serious post-infection complication of *Campylobacter* infection is Guillain-Barré syndrome (GBS), an autoimmune demyelinating disease of the peripheral nervous system (Allos, 2001). *C. jejuni* is zoonotic and is part of the commensal intestinal flora in poultry and domestic animals, which are the main reservoirs for human disease. Human campylobacteriosis is considered to be mainly a food-borne disease. The primary mode of transmission to humans is via consumption of contaminated and undercooked poultry. Less frequent sources include unpasteurised milk, contaminated drinking water and contact with pets and other animals (Blaser, 1997; Ketley, 1997).

Although *C. jejuni* has been recognised as a significant pathogen,

not much is known about the mechanisms of its pathogenesis. The chemotactic behaviour of *C. jejuni* towards the intestinal mucin plays an important role in colonisation, adhesion and subsequent invasion of intestinal epithelial cells (Ketley, 1997; Szymanski *et al.*, 1995). Sequencing of the *C. jejuni* NCTC 11168 genome and comparative genome sequence analysis revealed a number of genes involved in chemotaxis, including ten genes coding for ten putative chemoreceptors, named transducer-like proteins or Tlp receptors. Tlp receptors are classified into several groups according to their structural similarities (Parkhill *et al.*, 2000; Marchant *et al.*, 2002). Group A Tlp receptors have a similar structure to methyl-accepting chemotactic proteins (MCP) of *Escherichia coli*, where the chemotactic signal transduction pathway has been extensively studied. The highly variable periplasmic (chemosensory) domains of group A Tlp receptors are responsible for sensing chemical gradients in the external environment, which is followed by the initiation of a signal transduction cascade allowing the bacteria to relocate to the more favourable environment. It has not yet been established which specific ligand binds to a particular Tlp receptor.

This project aimed to isolate and characterise the *C. jejuni* NCTC 11168 chemosensory domain of the Tlp7 chemoreceptor (Tlp7^{peri}).

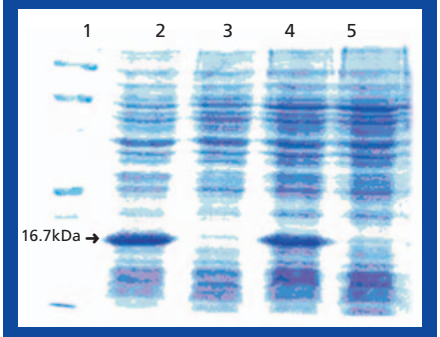
The coding sequence of the Tlp7^{peri} is conserved within *C. jejuni* strains. In some of these strains there is a mutation creating the stop codon after the coding sequence of the periplasmic sensory domain, which means that the Tlp7 receptor is likely to be a pseudogene. Interestingly, in one of *C. jejuni* strains (HB93-13) the Tlp7 receptor is a functional gene as there is no such mutation. This allows us to speculate that the Tlp7 receptor might be functional at least in some *C. jejuni* strains. Characterisation of Tlp7^{peri} will provide a deeper insight into the role of Tlp7 receptor in the chemotactic behaviour of *C. jejuni*. A DNA sequence of Tlp7^{peri} was amplified by PCR from the *C. jejuni* NCTC 11168 strain using specifically designed primers. The *NdeI* and *XhoI* restriction sites were incorporated into the primer termini for future cloning procedures. The 456bp Tlp7 PCR fragments were ligated into the intermediate pGEM-T Easy cloning vector (Promega) by standard molecular cloning techniques. Competent *E.coli* DH5α cells were transformed with the plasmid construct and grown on Luria Broth plates with antibiotic supplementation containing IPTG (isopropyl β-D-thiogalactopyranoside) and X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside). Recombinants were selected by blue/white colony selection. The plasmid was then isolated from the putative recombinants and the presence of the intact Tlp7^{peri} insert was confirmed by PCR, restriction enzyme cleavage and sequencing.

The Tlp7 periplasmic domain encoding DNA fragment was excised from the recombinant pGEM-T Easy cloning vector and cloned into the pET-19b expression vector. The pET-19b expression vector contains a polyhistidine tag, an ampicillin resistance gene selection marker and an IPTG-inducible T7 RNA polymerase promoter, which enables the overexpression of target His-fusion protein. The plasmid construct was then transformed into the *E.coli* expression host strain BL21[DE3].

Expression of the 16.7kDa Tlp7^{peri} -His fusion protein was induced by IPTG which enabled detection of the protein by Western blot analysis using anti-His antibodies (Figure 1a and 1b).

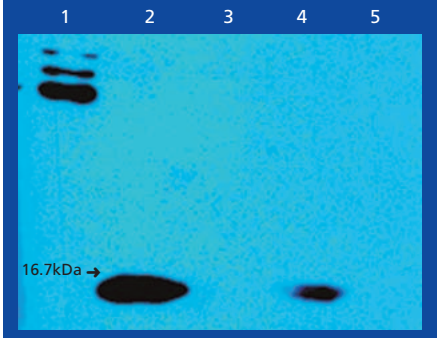
A protein solubility test was performed and showed that large quantities of the target protein were present in the soluble fraction, which will allow the purification of Tlp7^{peri} -His fusion protein by nickel affinity gel chromatography. Due to time limitations the project was paused at this stage.

Figure 1a. *SDS-PAGE gel depicting a small scale protein expression of 16.7kDa Tlp7^{peri} -His fusion protein*



Lane 1: All Blue protein marker (BioRad)
Lane 2: Induced Bl21 (DE3) clone 1
Lane 3: Uninduced Bl21 (DE3) clone 1
Lane 4: Induced Bl21 (DE3) clone 2
Lane 5: Uninduced Bl21 (DE3) clone 2

Figure 1b. Western blot showing the expression of 16.7kDa Tlp7^{peri} -His fusion protein



Lane 1: All Blue protein marker (BioRad)
Lane 2: Induced Bl21 (DE3) clone 1
Lane 3: Uninduced Bl21 (DE3) clone 1
Lane 4: Induced Bl21 (DE3) clone 2
Lane 5: Uninduced Bl21 (DE3) clone 2

The purified Tlp7^{peri} protein will be used in a glycan and amino acid array to determine the binding ligand specific for Tlp7^{peri}. Identification of the specific ligand, which binds to the periplasmic domain, will allow us to elucidate the role of the Tlp7 chemoreceptor in chemotaxis. This may potentially provide an insight into the mechanisms of colonisation in animals and disease production in humans allowing the development of new targets for novel antimicrobials.

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Dina Zarubina

Amplified Fragment Length Polymorphism of *Campylobacter* species

During my study of the microbiology BSc at Queen's University Belfast, the class was informed by Dr Madden of the opportunity to apply for an S*f*AM project over the summer vacation. I was excited when I was offered a 10 week summer placement at the Agri-Food and Biosciences Institute (AFBI) within the Food Microbiology branch. Dr Madden's team has worked with *Campylobacter* species for over 15 years. From the isolates obtained between 1994 and 2002, they have built up a collection of extracted DNA. A variety of different genotyping techniques were applied to the isolates before the method of amplified fragment length polymorphism (AFLP) was seen to best suit the lab's requirements, but this was only applied to isolates obtained after 2002. My aim was to determine whether the quality of the archived DNA samples was adequate for AFLP, since the samples may have degraded during storage. Where the quality was acceptable, DNA from as many *Campylobacter* isolates as possible would be subjected to AFLP typing and all available information added to the existing large bioinformatics database.

AFLP utilises two restriction enzymes to generate fragments of DNA which can then undergo PCR amplification. Hence, the methods I used encompassed many techniques including DNA digestion, PCR, gel electrophoresis, use of the DNA sequencer and bioinformatics. The DNA sequencer sizes fragments providing AFLP profiles which can then be compared using BioNumerics to provide the clustering of subspecies and the degree of relatedness between each profile. For my project, DNA from 514 isolates was available from organisms which had all originally been identified by phenotyping. The isolates were from a variety of sources including clinical, veterinary and food. All of the DNA isolates were initially subjected to quality assessment using the "Thermo NanoDrop 1000." These samples were then subjected to AFLP. Of the 514 samples, 425 provided acceptable DNA profiles. The data of all of the isolates including their sample ID, strain number, source, original speciation, concentration of DNA, country of origin and date received had to be entered manually into the database. This was an extremely tedious and time consuming task but was essential for future reference to these samples and for further analysis to compare these samples with other samples within the database.

Analysis of the sample profiles was by unweighted pair group with mathematical average (UPGMA) cluster analysis. This portrayed four distinct clusters of *Campylobacter* species: *C. jejuni*, *C. coli*, *C. lari* and *C. fetus*. *C. coli* showed the least diversity with isolates clustering at ≥66.9% similarity and *C. jejuni* the greatest diversity with isolates clustering at ≥19.4%. It was evident that many samples had been misidentified by the phenotyping techniques. Of the samples, 18 *C. jejuni* isolates were observed to cluster with *C. coli* and 14 *C. coli* isolates clustered with *C. jejuni*. The majority of the samples clustered within the four main species clusters and some of the outbreak strains showed ≥98% similarity: with the method used, profiles showing ≥90% similarity were regarded as identical.

Another interesting observation was that some of the veterinary samples were labelled *C. upsaliensis*. However, *C. upsaliensis* is resistant to restriction digestion by Bgl*II* hence cannot generate an AFLP profile by the method used. These samples did, however, produce an AFLP profile and clustered with *C. coli*, hence they were definitely not *C. upsaliensis*. Of the isolates which were subject to AFLP, 32 did not cluster with the four major clusters or with each other. Thus, the true identity of these samples is not known and further analysis, such as 16S sequence analysis, will be necessary to identify these isolates.

Overall the data of over 400 isolates are now available within the department's bioinformatics database and will benefit further studies by AFBI staff. The project has highlighted that phenotyping techniques are not always reliable in correctly identifying *Campylobacter* spp.

This opportunity has provided me with the invaluable first hand experience of working within a lab and confirmed my aspirations to continue with research to a PhD. I would like to thank my supervisor Dr Bob Madden for the opportunity he has provided, and also Lynn Moran and Carmel Kelly for their commitment, patience and friendliness in welcoming me to the lab and teaching me the techniques and skills needed to perform AFLP.

I believe that the S*f*AM Students into Work scheme provides an extremely worthwhile and rewarding opportunity to students and I am grateful for the experience that I have gained.

Carole Daly

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The relationship between *H. pylori* colonisation and levels of Leptin and Ghrelin

My visit to Sri Lanka, courtesy of the President's Fund, was at the request of Dr Fernando of the Medical Faculty of the University of Sri Jayawardenapura (USJ) who invited me to give a lecture at the 120th Sri Lanka Medical Association meeting. Whilst in Sri Lanka I took the opportunity to set up a research project on the relationship between colonisation by *Helicobacter pylori* and the levels of the orexigenic hormones leptin and ghrelin.

Increasingly, extra-gastric and extra-intestinal diseases are being linked to colonisation by *H. pylori* and special interest has been focussed on the relationship between body weight regulation, stature and body mass index, after it was reported that the eradication of *H. pylori* was associated with a significant increase in body weight and BMI (Furuta *et al.*, 2002) and a report linked colonisation by *H. pylori* with short stature (Demir *et al.*, 2001). Indeed, it has been suggested that decreasing prevalence of *H. pylori* in the population may be causally related to increasing obesity and therefore gastro-oesophageal reflux disease (GORD) and an increasing incidence of oesophageal adenocarcinoma. Similarly, attention has focused on *H. pylori* as a risk factor for both insulin resistance and cardiovascular disease such as atheroma, stroke, ischaemic heart disease and thrombo-embolic phenomena. On the other hand, some studies have not found an association between colonisation by *H. pylori* and growth retardation or cardiovascular disease.

A complex hormonal interaction exists to control appetite, metabolism and growth. Ghrelin, a recently discovered hormone produced principally by epithelial cells in the fundus of the stomach, has two major biological activities: regulation of energy balance and stimulation of the secretion of growth hormone from the anterior pituitary. It regulates energy balance by increasing hunger through its action upon the hypothalamus and suppression of fat utilisation in adipose tissue (Wren *et al.*, 2001). Plasma concentrations of ghrelin increase prior to a meal and are lowest post-prandially. Plasma ghrelin levels are reduced in obesity. Acylated ghrelin is the active form although the de-acylated form may also have biological relevance.

The release of growth hormone is also regulated by growth hormone releasing hormone (GHRH), which is released from the hypothalamus, and somatostatin (SS) which is an inhibitory factor synthesised by many tissues including D cells in the stomach. Growth hormone has a direct effect upon adipose tissue, increasing triglyceride breakdown, and a direct effect upon the liver to secrete insulin-like growth factor-1 (IGF-1). It is this latter factor that is responsible, in part, for growth enhancing effects by: stimulating the proliferation of chondrocytes and myeloblasts; stimulating protein synthesis in many tissues; increasing fat utilisation and partly controlling blood glucose levels. Plasma levels of growth hormone are highest during deep sleep.

Leptin is also an important factor in regulating body weight and metabolism. It is found predominantly in adipose tissue but also in the stomach and placenta. As triglycerides accumulate in the adipocytes the level of leptin also increases and there is a correlation between body fat and blood leptin levels. Leptin acts upon the hypothalamus to decrease hunger and food consumption and increase energy metabolism (Schneider *et al.*, 2006). It also has effects upon reproduction.

These two hormones are key regulators of the release of growth hormone and metabolic control, and disturbances may represent a plausible biological explanation for short stature and the evolution of atherosclerosis. Both hormones are affected by *H. pylori* colonisation. Serum ghrelin and leptin concentrations are significantly lower in *H. pylori*-infected than in non-infected children (Plonka *et al.*, 2006). Histologically, ghrelin-immunoreactive cells are significantly reduced in *H. pylori* positive individuals. The degree of gastric atrophy associated with *H. pylori* colonisation also appears to be linked to the plasma ghrelin levels. Eradication of *H. pylori* leads to an increase in plasma ghrelin levels. As ghrelin stimulates the release of growth hormone, a deficiency or reduced level of ghrelin may provide an explanation for the reduced height in children colonised by *H. pylori*. The high incidence of *H. pylori* infection in children in some countries seems to contribute to the decreased serum levels of ghrelin and to the decreased appetite and dyspeptic symptoms in these children. On the other hand, some studies have found no relationship between colonisation by *H. pylori* and ghrelin or leptin levels.

Little is known about the relationship between *H. pylori* virulence factors (e.g. CagA/ VacA) and appetite hormones and there is little published data on the effect of different gastric pathologies on these hormones, especially in children. The issue regarding the differences in plasma ghrelin/leptin between *H. pylori* positive and negative patients is still under debate. Also, whether colonisation by *H. pylori* affects ghrelin/leptin dynamics over the long term and its relation to obesity and associated diseases are important questions that remain to be answered. Thus, in developed countries, *H. pylori* negative children may have relatively high concentrations of ghrelin and may reach their full growth potential then grow into overweight adults with obesity and associated cardiovascular problems, whilst in developing countries, where *H. pylori* prevalence is high, they may not achieve full stature and some will develop peptic ulcer disease (PUD) or adenocarcinoma of the stomach.

These few studies confirm that these hormones have not been thoroughly investigated in *H. pylori* infection, particularly in relation to the virulence characteristics of *H. pylori*. This is the rationale for our study which includes Sri Lanka, Italy and Lithuania. We are recruiting both adults and children who are either complaining of dyspepsia or are healthy controls. Their *H. pylori* status will be determined. Patients will undergo gastroscopy, biopsy and PCR for *H. pylori*. All subjects will have serology (HelicoBlot 2), leptin and ghrelin levels determined, their BMI measured and insulin resistance

determined. We shall then be able to correlate the strain type of *H. pylori* with levels of orexigenic hormones, insulin resistance and their BMI.

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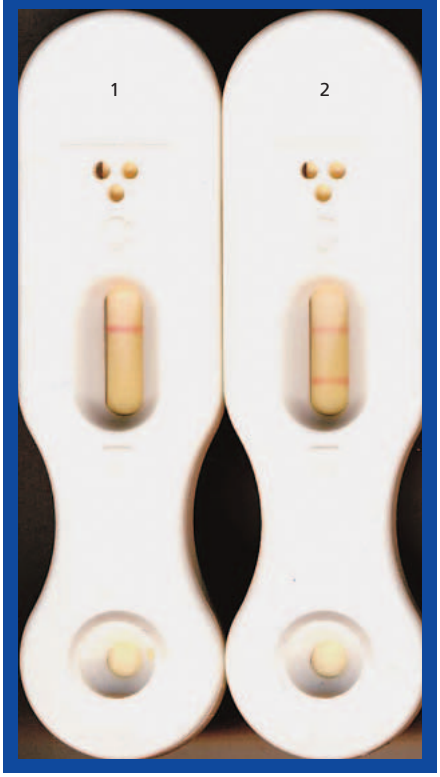
John Holton

Use of monoclonal antibody-based diagnostic assays for the detection of invasive mycoses

The frequency of invasive mycoses by opportunistic fungal pathogens has increased dramatically over the past two decades. This increase is directly related to increasing patient populations at risk for the development of infections, which include the elderly, premature babies, solid-organ and bone marrow transplant recipients, individuals with AIDS, neoplastic disease and those receiving immunosuppressive therapies or cytotoxic drugs. In addition to well-known opportunistic pathogens such as *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*, other species of yeast-like fungi and filamentous fungi have emerged as serious pathogens of humans over recent years. Many of these organisms such as the zygomycete *Rhizopus oryzae* and members of the ascomycete phylum, for example *Paecilomyces* spp., *Scedosporium apiospermum* (the anamorphic, asexual state of the fungus *Pseudallescheria boydii*), *Fusarium solani*, and *Trichoderma longibrachiatum*, are ubiquitous soil-borne organisms. The saprotrophic lifestyles of these fungi and potentially infectious *Aspergillus* spp., enable them to utilise a wide range of substrates including decaying plant material thereby facilitating efficient colonisation of their natural habitats, soils and composts. As soil saprotrophs they play an important role in the recycling of organic matter in terrestrial ecosystems and, while some species such as *F. solani* are plant pathogens also, others such as *Trichoderma* spp display mycoparasitic and plant-growth-promotional activities that have been exploited in the biological control of plant disease and in the enhancement of crop productivity. Despite these positive attributes, soil-borne fungi can have significant negative impacts on human health, causing frequently fatal disseminated infections in the immunocompromised host, with disproportionately high rates of mortality and morbidity.

A. fumigatus, *F. solani* and *S. apiospermum* are agents of 'hyalohyphomycosis', a term used to describe infections caused by hyaline, septate, fungi in infected tissues. *A. fumigatus*, second only to *Candida* spp. as the cause of nosocomial (hospital-acquired) invasive fungal infections, is first and foremost a pulmonary pathogen causing aspergillomas (fungus balls) that occlude the lung cavities. However, the fungus can also invade the host via lung tissue resulting in a disease known as invasive aspergillosis (IA). This disease is now a major direct or contributory cause of death at leukaemia treatment centres and bone marrow and solid organ transplantation centres. *Fusarium* spp. have long been associated with infections of the skin, nail and cornea, but are now becoming increasingly recognised as a cause of invasive fungal infection (fusariosis) in neutropaenic patients and in those undergoing transplantation. Indeed, some hospitals have reported *Fusarium* spp. to be second only to *Aspergillus* spp. as the cause of life-threatening filamentous fungal infections in their transplant patients. *S. apiospermum* is a well known causative agent of mycetoma (tumour-like swellings with draining sinuses), but this species and the related species *Scedosporium prolificans* have also recently emerged as significant invasive pathogens,

Figure 1. Serodiagnosis of invasive aspergillosis using an *Aspergillus*-specific lateral flow device. Samples of serum from a healthy individual (1) and from a patient with invasive aspergillosis (2) were applied to LFDs and the results recorded after 15 minutes. A single internal control line is seen with serum from the healthy individual, while two lines are seen with the invasive aspergillosis patient's serum showing the presence of circulating *Aspergillus* antigen.



particularly of immunocompromised patients, and now account for ~25% of non-*Aspergillus* infections in organ transplant patients. *R. oryzae*, an aseptate fungus, is the most important agent of mucormycosis in patients that have serious underlying conditions such as diabetes mellitus, starvation, burns, or other major trauma.

Opportunistic mycoses represent formidable diagnostic challenges. It is imperative that diagnosis is made without delay, since prognosis worsens significantly in the absence of recognition and timely intervention with antifungal agents. Identification of fungi in histological sections is problematic because of a common appearance (colourless, septate hyphae with a wide range of branching angles). Definitive identification in hyalohyphomycosis

therefore requires isolation of fungi in culture and identification using morphological characteristics such as spore-bearing structures. This takes considerable time and requires taxonomic expertise. Consequently, rapid diagnostic tests that accurately discriminate between the different pathogens are urgently needed. A characteristic of fungi such as *A. fumigatus*, *F. solani*, *R. oryzae* and *Candida* spp., in particular, is angioinvasion and dissemination via the blood. This property presents an opportunity to track the fungi immunologically using techniques that detect characteristic signatures created by their circulating antigens.

One such technique is hybridoma technology. It allows the production of monoclonal antibodies (mAbs) that are specific to individual genera, species or even isolates of fungi and are capable of discriminating between active growth and quiescent spore production. Monoclonal antibodies have been used successfully to detect fungi in soil and composts (Thornton, 2008, Thornton *et al.*, 2002) and in plant material (Thornton & Talbot, 2006) and to develop highly specific and sensitive immunological assays for the quantification of interacting populations of fungi in mixed species soil-based systems (Thornton, 2004). Monoclonal antibodies have also been used to develop user-friendly, diagnostic tests (lateral flow devices (LFDs)) for the rapid detection of plant pathogens and beneficial fungi in soils and in plant materials (Thornton, 2008, Thornton *et al.*, 2004). One such device has been developed for tracking biocontrol strains of *Trichoderma* in the plant rhizosphere (Thornton, 2008). Lateral flow technology is now being used at Exeter University to develop highly specific LFDs for the rapid detection of fungi of medical importance. Monoclonal antibodies specific to *Scedosporium* spp., *Rhizopus* spp., *Fusarium* spp. and the drug-resistant yeast *Candida glabrata* are currently being developed. An LFD specific to *Aspergillus* spp., has already been developed for the rapid serodiagnosis of IA (see accompanying figure). The LFD incorporates a mAb that binds to a protein epitope on an extracellular antigen secreted constitutively during invasive growth of the pathogen. Circulating antigen can be detected in serum or plasma samples from patients at risk from IA and avoids the need for invasive biopsy sampling. Similar devices will be developed for the rapid serodiagnosis of fusariosis, mucormycosis and blood-borne *C. glabrata* infection and will provide diagnostic platforms for the routine testing and management of patients at risk from invasive mycoses.

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Christopher R Thornton

Exploring the microbial flora of Philippine mangroves for enzymes and antimicrobials

The search for natural sources of novel pharmaceutical products is still very much ongoing worldwide. The Philippines, an archipelago of 1700 islands, is one of the most diverse areas in the world, so could be a potential source. Among the places in the country, its mangrove ecosystem is a good candidate to search for novel microorganisms. The Philippines are home to rich mangrove forests. They are still an untapped ecosystem in terms of microbial diversity, especially those that are under the protection of the Department of Environment and Natural Resources.

Mangroves are a dynamic ecosystem. Being a natural sink, microbial interactions in the mangroves are vital, converting plant litter to nutritious food sources for marine organisms. Mangroves, therefore, would naturally harbour diverse types of microflora. Hence, the possibility of finding novel microorganisms and novel products from this environment is not remote. Mangrove lignocellulose supports a vast range of microorganisms. About a thousand species from 585 genera have been cited for fungi alone.

It is distressing to note that much of the mangrove forests in the country have been lost due to overexploitation by coastal dwellers and conversion to settlements, aquaculture, salt beds and industry (Primavera, 2000). From the original estimate of 500,000 hectares, our mangrove forests have decreased to only 120,000 hectares. Hence, being with the Philippine National Collection of Microorganisms, my staff and I took on a major undertaking of bio-prospecting and preserving microbial flora from various mangrove areas in the country before more of this important natural resource is lost forever. With additional funding from the University of the Philippines Center for Integrative and Developmental Studies and the Commission on Higher Education, we were able to conduct the study for two years and to collect samples from 35 mangrove sites in six provinces in four major islands (Luzon, Mindoro, Cebu and Bohol) (Figure 1).

Figure 1. *Collecting samples from mangrove sites in the Philippines*

Decaying leaves, wood (twigs) water and mud samples were collected away from human settlements and bacteria, yeasts and moulds were isolated from them. This was done by serial dilution in 1.5% Sodium Chloride (NaCl) and spread-plating on marine agar supplemented with cycloheximide for bacteria, yeast mannitol agar with 1.5%

NaCl supplemented with Rose Bengal for yeasts and acidified potato dextrose agar with 1.5% NaCl for moulds. Representative colonies, differing in morphological and cultural characteristics, were selected, purified and preserved by L-drying and by deep-freezing at -70°C with glycerol as back-up. Mould isolates were kept on agar slants with sterile mineral oil. A total of 1,234 isolates (929 bacteria, 168 yeasts and 137 moulds) were preserved.

Screening for Enzymes

The isolates were assayed for lipase, amylase, proteinase, cellulase, the lignin-modifying enzymes (LMEs) — laccase, xylanase, polyphenoloxidase — and also the peroxidase types of LMEs. These enzymes were selected for their various agricultural and industrial uses. Cellulase and amylase, which are produced by species of *Trichoderma* and *Aspergillus*, for example, are used as food additives in poultry. Cellulases are used to hydrolyse wood. Some strains of marine bacteria have also been reported to have cellulolytic activities and have been useful in feed formulations (Inagaki, 1998). *Rhizopus* spp., on the other hand, produce lipase that can potentially substitute triclosan in the soap industry. Xylans, the major components of the hemicellulosic fractions of terrestrial plants are tough materials to degrade. A variety of microorganisms have been reported to degrade wood xylans including yeasts e.g. *Cryptococcus albidus*, *Aureobasidium pullulan* and *Trichosporon* spp and bacteria e.g. *Bacillus subtilis* as well as the more common mould species.

Assays for degradative enzymes revealed a good number of strains producing lipase (bacteria: 265 strains of 429 assayed, yeasts: 71 of 100, moulds: 77 of 152), amylase (bacteria: 139 of 421, yeasts: 9 of 100, moulds: 64 of 141), proteinase (bacteria: 218 of 273, yeasts: 41 of 100, moulds: 91 of 144), cellulase (bacteria: 67 of 133, moulds: 40 of 79, yeasts: none of 45) , and the LMEs, xylanase (bacteria: 3 of 133, moulds: 41 of 79, yeasts: none of 45) and polyphenoloxidase (bacteria: 18 of 293, yeasts: 3 of 100, moulds: 5 of 133). Some strains even exhibited multiple enzymatic properties. Figure 2a shows a bacterial isolate exhibiting casein hydrolysis.

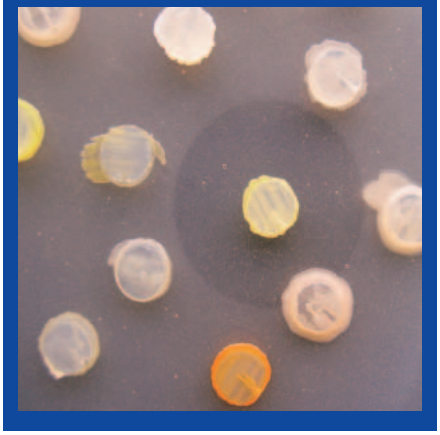
Figure 2a. *Bacterial isolate exhibiting casein hydrolysis*

Screening for antimicrobials

Antimicrobial activity of the purified mangrove bacterial isolates were tested against the human pathogens *Escherichia coli*, *Candida* spp. and *Staphylococcus aureus* using the zone of inhibition assay. Isolates that inhibited *S. aureus* were further assayed against five strains of methicillin-resistant *S. aureus* (MRSA) obtained from the Philippine General Hospital Microbiology Laboratory. The isolates were also tested against important shrimp pathogens in aquaculture, *Vibrio harveyi* and *Vibrio campbellii*.

Microorganisms are a prolific source of structurally diverse bioactive metabolites and have yielded some of the most important products of the pharmaceutical industry. Of the 12,000 antibiotics known in 1995, 55% were produced by filamentous bacteria, 11% from other actinomycetes, 12% from non filamentous bacteria and 22% from filamentous fungi. New antibiotics are needed because of the development of resistant pathogens, the evolution of new diseases, the existence of naturally resistant bacteria (e.g. *Pseudomonas aeruginosa* in cystic fibrosis patients), and the toxicity of some of the current compounds (Strohl, 1997). New bioactive products from microbes are being discovered at an amazing pace: 200-300 per year in the late 1970s, increasing to 500 per year by 1997 (Demain, 1999). Thus, we hope that isolates with promising antimicrobial properties will also be discovered from our diverse mangrove ecosystem. Indeed, a number of the isolates exhibited antimicrobial properties against the human pathogens *S. aureus* (including MRSA), *C. albicans* and *E. coli*. Some isolates also showed vibriostatic activity against the shrimp pathogens *V. harveyi* and *V. campbellii* (Figure 2b).

Figure 2b. *Isolates showing vibriostatic activity against the shrimp pathogens V. harveyi and V. campbellii*



Some of the isolates with promising enzyme and/or antimicrobial properties have been identified. Characterisation and identification of the other isolates is still ongoing.

My sincere gratitude goes to SfAM for the financial support from the President's Fund and to the organisers of the 11th International Culture Collection Congress (ICCC-11) which enabled me, from the other side of the globe, to present part of my work in two poster papers at this event in Goslar, Germany and to participate in the pre-congress training on culture collection quality management in DSMZ in Braunschweig from October 6th-11th, 2007.

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Rosario Gionson-Monsalud

corporate news

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

Microbiological Laboratory Manuals from Campden BRI

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

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

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Oxygen control for microbiology?

Don Whitley Scientific's H35 Hypoxystation was originally developed for cell culture research, however it has emerged that this workstation has applications within microbiology. It's not only cell/tissue culture scientists who might want to have tight control over O_2 and CO_2.

According to Klaus Winzer at the Centre for Biomolecular Sciences at Nottingham University, there is a place for the Hypoxystation in his laboratory. Dr Winzer has ordered a unit specifically for the accurate, precise control of oxygen that the H35 will afford him in his work with microaerophilic and capnophilic bacteria. He also intends to use the H35 to investigate how strictly anaerobic bacteria such as clostridia respond to oxygen exposure.

The main features of the H35 include the ability to control oxygen in 0.1% increments from 0.1% to 20%; control CO_2 in 0.1% increments from 0.1% to 15%; control relative humidity up to 90%; and provide precise temperature control. The data capture option allows information to be downloaded onto a memory stick for further analysis.

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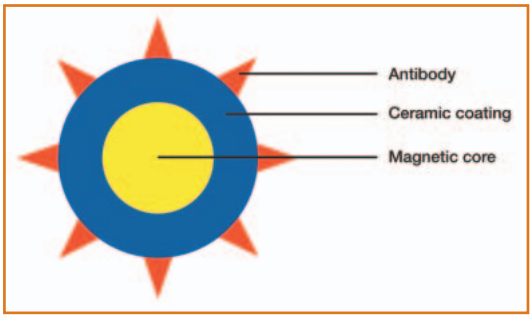

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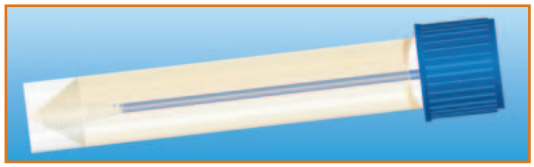

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Each corporate member of the society may publish ***up to*** 200 words on a topic related to their field of activity in each issue of *Microbiologist*. For further information please contact Lucy Harper by email at: **lucy@sfam.org.uk**

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

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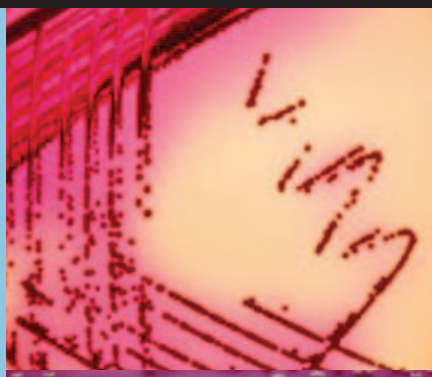

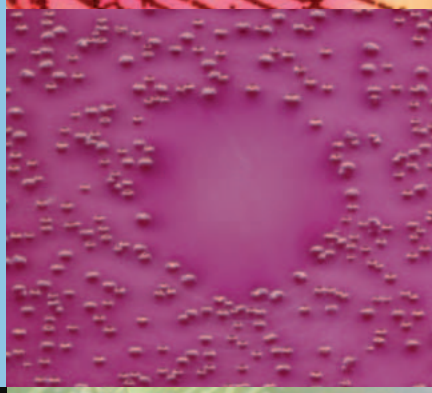

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