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Microbiologist

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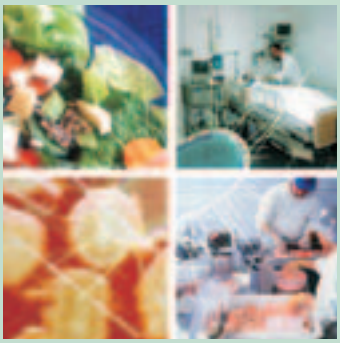
REGULARS

MEETINGS



FEATURES

WRITE FOR US!

The editor is always looking for enthusiastic writers who wish to contribute articles to *Microbiologist* on their chosen microbiological subject.

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

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Website: the society website 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.

www.sfam.org.uk

Editorial

BY NOW I EXPECT MOST OF you will be making preparations for the forthcoming yuletide festivities. I expect you'll all be buying your Christmas trees, stocking up the fridge and freezer with food, and wading through the shops dragging carrier-bags full of presents. This year, while I was looking for presents for my friends and family, I found an interesting gift idea - cuddly microbe toys. There are many to choose from: *Orthomyxovirus*, *Rhinovirus*, *Shigella*, *Streptococcus pneumoniae*, *Bordetella pertussis* to name but a few. On finding these cuddly microbes it struck me that you can literally give your friends and relatives the 'flu this Christmas!

As well as the giving of gifts, food is a big part of Christmas celebrations, and as Microbiologists, I'm sure the safety of food is at the forefront of your minds. In the UK there are many local council websites with guides for the general public on how to make sure you cook your turkey properly, and there is usually at least one article in the media offering advice on the preparation and cooking of festive food. This is probably just a precaution at a time when joviality may take over from common sense. But regardless of the time of year, we must always take care when handling and preparing food. In the USA approximately 76 million people are affected by a foodborne illness annually, with 2 million being affected in the UK. It is not only poor hygiene in the home that is responsible for food-borne disease. The growth of produce and preparation of food can also hold some danger. As we all know, Environmental Health Officers have quite a job assessing the kitchens of restaurants and cafes to ensure they maintain standards of hygiene. This leads me nicely on to the subject of our forthcoming Winter meeting: **Food and Health**, with sessions on Hospital Acquired Infection and Food Safety. The Food Safety session *'Simmering issues in Food Safety'* has been put together with Environmental Health Officers in mind and there are discounted rates and CPD points for members of the CIEH and IBMS. This meeting is nearly fully booked and numbers are limited so see page 23 for a booking form and book your place now!

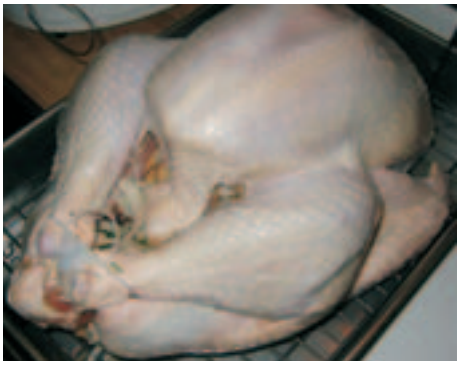
Another source of food-borne illness is the contamination of fresh produce with pathogenic bacteria. There have been a

number of outbreaks of *Salmonella* caused by consumption of Tomatoes in the USA, and I'm sure you will all have heard about the well-publicised *E. Coli* O157:H7 outbreak from contaminated Spinach. The Microbiology of fresh produce is the subject of our feature article in this issue of *Microbiologist*. Turn to page 28 to read more. This is also the theme for our Summer Conference 2007, the full programme for which is now available on page 27 and online at www.sfam.org.uk/sumconf.php.

Looking to the more immediate future, we return to this festive time of year. Whilst researching the theme of Christmas traditions, I learnt that in Austria one of the traditional dishes at Christmas is fried Carp. Although not fried, the subject of fish forms the basis of our second feature article: fermented fish and their products. Read more on page 32.

It is at this time of year that our thoughts turn to reflection, and our President takes this opportunity to reflect upon the successes the Society has accomplished during the first half of her reign (page 8).

Finally, it just leaves me to wish you all a Merry Christmas and a Happy and Healthy New Year.



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MEDIAwatch

Voice of Young Science Workshop, Sense About Science

Microbiology is often a media hot topic, with items such as avian flu, MRSA and *E. coli* 0157 outbreaks hitting the headlines. As such, it is important that scientists know how to liaise with the press, to ensure that research is reported accurately and to avoid any potentially embarrassing situations! This is a skill that many young researchers have little or no instruction in.

The Voice of Young Science (VoYS) media workshop programme, run by Sense About Science, introduces early-stage researchers to the world of science communication and includes practical guidance on how to get involved in public debate. Four SfAM members, Clare Benskin, Dursun Cinar, Joanna Heaton and Niamh Murphy attended a workshop on 23 June 2006, thanks to funding from SfAM.

The workshop was divided into three separate sessions, each addressing a different area of science communication. We began with Science in the Media, a participant discussion on the role of science in the media, and the changes in the perception of science and scientists by the public. The session was chaired by Shereen El-Feki from Al Jazeera News, with speakers Professor David McAlpine from UCL and Dr Azra Ghani from the London School of Hygiene and Tropical Medicine. Problems surrounding the reporting of science were discussed, with examples of 'bad science' in the press

Once we were all firmly of the opinion that exaggerated reporting and misrepresentation were the norm, a panel of (brave!) journalists were brought in to tackle our concerns. Mark Henderson (*The Times*), Alok Jha (*The Guardian*), Fiona McCrae (*Daily Mail*) and Tom Fielden (BBC Radio 4 *Today* Programme) gave their views on how they try to balance real science and the need for entertainment. The session ended with a lively question and answer session debating how journalists and scientists can help each other. We established that the press do not aim to misrepresent science, and that scientists are as much to blame for 'bad science' reporting as the media.

Finally the big question was addressed: exactly when should you present your research to the press? Dr Claire Bithell from the Science Media Centre gave practical advice on how to provide comment to the press and Professor Michael Mabe from Elsevier spoke about the role of peer review. The discussion continued well after the official end of the workshop at a nearby bar.

As scientists, many of us tend to see only the misinterpretations of scientific research that are presented by the media, such as the hyped headlines and scaremongering documentaries. Personally, I initially felt that journalists made little effort to research articles prior to publication, and that quite often a great story was more important than a factual one. The VoYS workshop showed me just how wrong I was, and has opened my eyes to how scientists themselves can give rise to these misrepresentations. The science correspondents made it clear that the pressure on them to create a dramatic story from little more than a few lines of a press release was huge but their integrity and desire to put across the real science was evident. I now find that I respect the research that has been done, rather than pick out information that is missing.

Scientists have to understand that journalists often have no scientific background and we need to be clear in what we tell them. It is very easy for us to go off-message when trying to explain research that we are involved in, especially if we are trying to explain the relevance of that research. Journalists rely on contacts within the scientific community to give comment and perhaps background information on items in the news. Sense About Science aim to link journalists and scientists, ensuring that journalists have access to 'good science' from experts in a particular field. You can (and should!) join the Evidence Bank at http://www.senseaboutscience.org.uk/index.php/site/about/8/

The VoYS workshop was a valuable opportunity to speak directly to journalists and to establish exactly what they want from us as scientists. We were left with increased confidence in the media and in our ability to present our own research, to add to our passionate interest in microbiology. Many thanks to Sense About Science and SfAM.

Clare Benskin said *"I certainly feel more confident about talking to the media now. We've been given some great advice and had the chance to speak to journalists in an informal setting"* Jo Heaton added, *"I really hope that I do get the chance to work within science communication. I'd like to put some of the information from today to the test!"*

Joanna Heaton
University of Lancaster

MICROBIOLOGY IN THE NEWS

If you spot a story in the media which you think should feature in this column, then send it to the Editor at: lucy@sfam.org.uk.

SfAM 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.

LifeSciences2007

A joint meeting of the Biochemical Society, the British Pharmacological Society and The Physiological Society

8–12 July 2007, Glasgow, UK

THEMES:

● Cancer ● Cardiovascular Bioscience ● Central Nervous System
● Education ● Exercise ● GPCR ● Imaging ● Inflammation
● Ion Channels ● Metabolism ● Signalling

ABSTRACT SUBMISSION DEADLINE:
26 February 2007

EARLY REGISTRATION DEADLINE:
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Biochemical Society

BPS BRITISH PHARMACOLOGICAL SOCIETY
Today's science, tomorrow's medicines

The Physiological Society

Dr Margaret Patterson stakes stock of what the Society has achieved in the last 18 months

THEY SAY THAT TIME FLIES when you are enjoying yourself. Therefore, I think it is a good sign that I was very surprised to calculate that I am already halfway through my term of office as President. The first 18 months have been very enjoyable and I continually realise how fortunate I am to be President of the Society in a time when it goes from strength to strength. This is due in no small part to the excellent leadership of Past Presidents.

Perhaps this is an appropriate time to take stock of what has been achieved over the last year and a half, and to consider our future plans. The 75th Anniversary of the Society has made 2006 extra special and it was good to see so many "old" friends and previous Committee members at the summer conference. I am also pleased to announce the publication of *"The Society for Applied Microbiology — A Short History"* by Professor Max Sussman, to commemorate our Anniversary. We have a number of free copies to give away to SfAM members – see page 13 for details.

Soon after I became President we had a Committee away day where we reviewed the Society's activities and discussed the way we would like to see it develop in the future. One thing we were all agreed upon was to retain our mission statement for SfAM to be recognised as *'the voice of Applied Microbiology.'* We discussed strategies to help achieve this aim and I am pleased to say that one year later we are starting to see positive results. Our external affairs activities have increased and we are talking with various international microbiological societies to seek mutually beneficial opportunities — where we can be complementary without being in competition. For example, we are exploring the possibility of collaborative meetings with organisations such as the International Association for Food Protection. We feel this could provide membership benefits, such as reduced rates at international conferences and a wider audience for our own events. Giving benefits to members is still a high priority for SfAM, and the Finance sub-Committee have allocated increased funds for grants. This will allow us to give more awards for 'Students into Work', which has become very popular as well as studentships to attend conferences and the President's Fund. Do take time to check the web site for the terms and conditions of the awards and consider making an application if you need all or part funding to attend a conference. For example. I am constantly amazed at how many of our members have never applied to the President's Fund, even after many years of membership (see page 44).

Our new office in Bedford Heights

We also have regular involvement with the Biosciences Federation and Phil Wheat has been invited on to its European Liaison Group, which is chaired by Professor Nigel Poole. Our membership sub-committee have also been active and we are seeing an increasing diversity of people joining. A good example is the number of microbiologists from within the Biomedical Science community attending SfAM meetings and joining the Society. The number of corporate members has also increased to 17 compared to 9, three years ago.

Our core journals, *JAM* and *LAM*, along with *Environmental Microbiology*, continue to flourish with improved impact factors and a more streamlined paper submission and referring process. The feedback we have received at international conferences indicates the journals are well respected and the fact that there is online submission and no paper charges make them particularly popular in the USA.

As well as these external activities, there have been a number of changes closer to home. The lease of the Blore Tower has now expired and the Office has moved to a different part of Bedford to a modern office facility. Our phone numbers remain unchanged but please take note of our new address (page 9). Also, we are making progress with SfAM becoming a Company limited by guarantee as well as a registered Charity but, as with all legal issues, it does seem to be taking a lot longer than we initially envisaged.

There is still plenty of work to do and challenges ahead as we look to the future and I am very pleased to say that all our Officers and Committee work hard on behalf of the Society to help us meet these challenges. Without such support, the job of President and those of the Office staff would be much more difficult. However, all members can play their part and we welcome any ideas from our membership. So if you have any comments or suggestions about how you would like to see SfAM develop, please don't hesitate to contact the Office or me directly.

With very best wishes for 2007.

Dr Margaret Patterson
President of the Society

Philip Wheat reports on the latest developments within the Society

As I mentioned in my last column, the Society has now moved into new office accommodation. We moved out of the Blore Tower (which had been home to the Society Office since 1997) between the 22 and 24 August. Like any move it was not without its trials and tribulations! There was a situation on the first day where one third of the office was packed into a removal van and we still did not have the keys for the new office facility. Fortunately, the problems were resolved and in fact by the last day of the move the office was fully operational in the new facility. We have now been in the new office for several weeks and whilst there are still boxes to unpack it already feels as though we have occupied the facility for a long time! For your records the new address is:

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

We have managed to keep all other contact details i.e telephone, fax and email the same.

One of my many roles as CEO is to raise the profile and awareness of the Society both in terms of the general public and the scientific community. This can obviously be achieved in a variety of ways. One of these is to attend appropriate scientific meetings as either a delegate or as an exhibitor. As I have previously reported, the Society has attended several international meetings which have been successful in recruiting new members. In addition, we also exhibited in September at the *Microbe 2006* symposium which was held at Ranmoor House, University of Sheffield. The conference was attended by over 300 delegates who were predominantly Biomedical Scientists (BMS) working in Clinical Microbiology laboratories. Once again we had significant interest from the delegates in the activities of the Society and we recruited a number as members.

The Officer's and myself see BMS as a group of people who may be interested in the activities of the Society and may consider becoming members. Therefore, I am pleased to announce that the Society are organising a one day meeting on 11 April 2007 at Manchester Metropolitan University entitled *'Broadening Microbiology Horizons'* which is targeting BMS as the audience. The topics to be covered will consist predominantly of the latest developments and update lectures covering many aspects of microbiology relevant to the work undertaken in the Clinical Microbiology laboratory. Further details of the meeting can be found on page 24 of this issue of *Microbiologist*. A delegate booking form will appear shortly on the website.

Another initiative which promotes the Society is to present the benefits of membership to potential members in their own workplaces. One such meeting was held in the Microbiology Department of Bedford Hospital at a lunch time in October. If there are any members who would like to hold such a meeting in their own institution, myself or Lucy Harper would be delighted to undertake the presentation. Please contact the office for further information.

We already have had a very good response to the forthcoming Winter Meeting (11 January, Royal Society, London — see page 21). Please ensure that if you are proposing to attend you book as soon as possible, as places are limited.

All that remains for me to do is wish you all a happy and peaceful Christmas and a prosperous New Year.

Philip Wheat
Chief Executive Officer

MED•VET•NET

Teresa Belcher reports on the challenges of emerging and re-emerging viral zoonoses

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 16 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. **Med-Vet-Net** officially commenced on 1 September 2004, and is funded to the value of €14.4 million for five years.

Among domestic animals, swine appear to be particularly suited for transmitting viral infections to man

VIRUSES ARE NOT USUALLY considered an important cause of food-borne zoonoses. Nonetheless, the changing epidemiology of several viruses as well as new concepts in animal health and food chain make it necessary to investigate possible risks associated with virus infected animals.

Humans and animals are infected by a wide variety of viruses belonging to the same families and genera. However, within the same genus, viral strains infecting humans are generally different from those infecting animal species. In some cases, host restriction is so strict that strains pathogenic for animals do not replicate at all in man or *vice versa*. On the whole, evolution appears to direct differentiation of viruses towards forms more and more distant from their shared ancestor to improve adaptation to the different host cells and tissues.

Nonetheless, in a minority of cases viruses maintain or regain the ability to break the species barriers and can move efficiently between animals and humans. This creates a risk for novel routes of transmission, reservoirs and generation of new and possibly more aggressive forms of an otherwise known pathogen.

The change in habits of modern populations including increased travel, large-scale animal farming and international food export is producing conditions for the emergence or re-emergence of unconventional human pathogens transmitted via zoonotic routes and/or foods.

Food producing animals as a potential source of viral zoonoses

Among domestic animals, swine appear to be particularly suited for transmitting viral infections to man, among which hepatitis E (HEV) might become a candidate for an emerging food-borne zoonotic disease.

Med-Vet-Net has a research Workpackage (WP 31) that aims to examine 'food producing animals as a potential source of emerging viral zoonoses'. Known as ZOOVIR-NET, this project will first survey and compare the methods, reference materials, samples and data already available in the different countries and laboratories of the network. The results will provide both guidelines and a database aimed at making available to all members harmonized and updated protocols. The construction of a strain and genomic database, which will be created with assistance from the **Med-Vet-Net** Communications Unit, will allow development of sensitive tests including PCR and *in situ* hybridization for detecting viruses in animals, foods and

milk, and cell cultures.

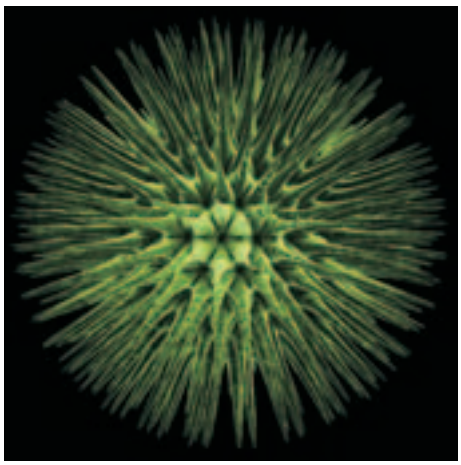
The Workpackage aims at evaluating the potential zoonotic role and/or foodborne transmission of emerging viruses such as porcine hepatitis E virus (SHEV), *Anellovirus* and *Encephalomyocarditis Virus* (EMCV), as well as tickborne encephalitis virus (TBEV).

Researchers will establish cell culture

methods for HEV, EMCV and *Anellovirus* replication and/or expression of viral proteins and virus-like particles, and the antigens generated will be beneficial to the production of immune sera and monoclonal antibodies and the development of immunological assays for viral antigen detection and characterization.

Established and newly developed methods will be adopted to evaluate prevalence of emerging viruses in swine herds, and serological ELISA (enzyme-linked immunosorbent assay) tests will be adapted to viral detection in animals to investigate the presence and carriage of TBEV along the milk production chain and to assess milk-borne transmission of this pathogen. Detection and characterization of HEV, in particular the datasets on genome detection and typing, will be shared and integrated with knowledge from other EU-supported networks.

The Workpackage 31 team are facing questions as to whether swine are an important reservoir for zoonotic viral infections, what is the evolutionary potential of these viruses, and whether and to what extent the food chain involves a virus risk. The Workpackage team is focused to conduct a coordinated effort to provide tools for epidemiological assessments, viral detection and typing including *in vitro* cultivation



This tick species is known to be a vector for Tick-borne encephalitis virus, caused by a member of the Tick-borne encephalitis virus complex, Flaviviridae, and Q fever, which is caused by the bacteria Coxiella burnetii (Image courtesy of CDC) ▼

Further Information

■ For more information about **Met-Vet-Net**, visit our website at http://www.medvetnet.org/ or contact Teresa Belcher at the SfAM offices in Bedford on: **+44 (0)1234 271020**

Teresa Belcher
Med-Vet-Net Communications Director

Emerging and Neglected Zoonoses – Collaborate to capture research opportunities

There are a range of zoonotic pathogens and diseases that receive relatively little attention and funding, and what is today's minority interest can become a major public health problem tomorrow. This threat is recognized by the European Commission and it is likely that Framework Programme 7 (FP7) will acknowledge this and there may well be a call within FP7 for research into some neglected zoonoses.

A 'special interest group' – ***Emerging and Neglected Zoonoses***, has been formed within **Med-Vet-Net** to create a number of directories of people who are interested in several neglected zoonoses. These directories will contain not only interested people within **Med-Vet-Net** but also the details of relevant partners from outside our formal network. It is anticipated that by identifying interested parties, this will maximize the chance of being successful in FP7 calls, or any other research bids.

The group aims to start by highlighting the following diseases: bartonellosis; borreliosis; rickettsiosis; anaplasmosis and erlichiosis; Q-fever; chlamydiosis; MRSA; *Streptococcus suis*; Flaviviruses; hepatitis E virus and borna disease. The list above may well change following discussions, and it is important to emphasize that this is not the end of the process.

If you want to know more, or feel that your own favourite neglected disease is being neglected by us please visit the **Med-Vet-Net** website at http://www.medvetnet.org/cms/templates/doc.php?id=80 or contact Kumar Sivam email: k.sivam@vla.defra.gsi.gov.uk, Tel: +44 (0)7795 060716

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

Mrs J Green; Mr A Pavic

Ireland

Miss D Caly; Mr F Douillard; Dr G Gardiner

Japan

Dr K Goto; Dr Y Konagaya

Korea

Dr S Kim

Mexico

Dr M Sanchez Mendoza

New Zealand

Dr G E Greening

Nigeria

Mrs C I Chikwendu; Mr G O Oyetibo

Philppines

Mr P Chua; Dr R Monsalud

South Korea

Prof S Oh

Taiwan

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USA

Dr C Campbell; Ms W M Crawford; Mrs G Duran; Dr J A McGarvey; Dr Omar Oyarzabal; Mr D Ramirez; Dr M Samadpour; Dr J Stratton; Ms A Tuck; Dr M Weibel

CORPORATE

Pall Life Sciences, UK

New look website

We will soon be launching a new-look website. The new site will have everything our current site has with more to offer our members and non-members alike. On the new website there will be a members only area, where you can change your membership information and gain access to exclusive SfAM documents. As a member you will also be able to download the latest issue of *Microbiologist* and the archive of this topical magazine. Even non-members will have access to a full issue of the magazine.

You will be informed of the launch of the site by email, but to make sure you keep in touch with the latest developments, you should regularly visit **www.sfam.org.uk.**

Our Journals: The top five most downloaded articles

Journal of Applied Microbiology

Novel antiviral agents: a medicinal plant perspective
S.A.A. Jassim, M.A. Naji
Volume **95**, Issue 3, pps 412-427

Antimicrobial agents from plants: antibacterial activity of plant volatile oils
H. J. D. Dorman, S. G. Deans
Volume **88**, Issue 2, pps 308-316

A history of influenza
C.W. Potter
Volume **91**, Issue 4, pps 572-579

Antimicrobial activity of essential oils and other plant extracts
K. A. Hammer, C. F. Carson, T. V. Riley
Volume **86**, Issue 6, pps 985-990

Probiotics and their fermented food products are beneficial for health
S. Parvez, K.A. Malik, S. Ah Kang, H.-Y. Kim
Volume 100, Issue 6, pps 1171-1185

Letters in Applied Microbiology

Campylobacter jejuni
W.J. Snelling, M. Matsuda, J.E. Moore, J.S.G. Dooley
Volume **41**, Issue 4, pps 297-302

Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity
A. Nostro, M.P. Germanò, V. D'Angelo, A. Marino, M.A. Cannatelli
Volume **30**, Issue 5, pps 379-384

Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7
S.A. Burt, R.D. Reinders
Volume **36**, Issue 3, pps 162-167

Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris* pathovars
S. Satish, K. A. Raveesha, G. R. Janardhana
Volume **28**, Issue 2, pps 145-147

Under the Microscope: *Arcobacter*
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Grime Scene Investigation

Our general secretary, Dr Anthony Hilton, and postgraduate student members; Jess Rollason, Tarja Karpanen and Laura Wheeldon, have featured in a TV series called *Grime Scene Investigation* which ran on BBC3. The series started on Tuesday 3 October 2006 and ran for eight episodes. However, we have recently learnt that the series has been so popular that they've all been asked to take part in a second series. Watch this space and URL below for details and tune in if you can.

For more information about the programme and to see Anthony and the 'Antoinettes', please visit the link below:

Photographs by kind courtesy of BBC3 Television

http://www.bbc.co.uk/bbcthree/tv/grime_scene/index.shtml

New Office Premises

As our CEO and President mentioned, the Society have moved to new office premises. We are now located in the building which used to house Texas Instruments and I ask you to make change the contact information in your address books. Our new address is:

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

All other contact information remains the same.

The History of the Society

To mark our 75th anniversary, we have published a book of the History of the Society, from well before the first gathering of Dairy Microbiologists in 1931, up to our 75th anniversary conference this year. The book, written by Past President Max Sussman, is published by Blackwell Publishing Ltd. A number of copies are available to members, so if you would like to receive a copy, please contact the Office and the book will be distributed on a first-come first served basis.

Call for nominations for W H Pierce Prize Award

Do you know a young microbiologist (under 40 years of age) who has made a substantial contribution to microbiology? If so, why not nominate them for this prestigious and substantial award which is worth £2,000. The award was instituted in 1984 by Oxoid to commemorate the life and works of the late W H (Bill) pierce, former chief bacteriologist at Oxoid Ltd and a long-time member of the Society. The prize is presented annually at the summer conference. Full Members wishing to make a nomination for the 2007 prize should write in confidence to the Hon General Secretary, Dr Anthony Hilton, at the Society Office in Bedford, including a full cv of the person nominated and a letter of support. Please note there are no official forms for this award.

Closing date for nominations is 27 April 2007.

Please note that application is through nomination by Full Members of *Sf*AM only.

New Committee Members

We are delighted to welcome three new committee members, Professor Carol Phillips, Professor Joanna Verran and Dr Mark Fielder

Dr Mark Fielder

Dr Mark Fielder trained as a biomedical scientist in the microbiology department at the Princess Royal Hospital (PRH) in Sussex. From the department at PRH he moved to King's College, London where he completed an honours degree in Microbiology in two years and followed this up with a PhD in microbiology and immunology. Following the completion of a PhD at King's, he was awarded the 1995 Tadion Rideal Prize for outstanding doctoral work in molecular science. He then joined the Infectious Diseases department at St George's Hospital Medical School as a Wellcome Trust-funded postdoctoral scientist. He subsequently moved to Kingston University as a lecturer, where he has established a microbiology research group within the school of Life Sciences. Here his work is of an applied nature focussing on two pathogens (staphylococci and mycoplasma) of human and animal importance.

Mark has 20 years experience in applied microbiology and excellent networking and communication skills. As an academic he is experienced in communicating science, scientific concepts and research to a wide audience both academic and non-academic. He is keen to contribute his time and efforts to assist in the influence of the public's perception of science through involvement with S*f*AM.

Professor Joanna Verran

Joanna obtained her first degree in Bacteriology and Virology at the University of Manchester. Her PhD was on the effect of a novel sucrose substitute on cariogenic properties of *Streptococcus mutans*. She began lecturing at the then Manchester Polytechnic directly, and has continued to teach microbiology at all levels of undergraduate provision. She is particularly interested in novel approaches to teaching and learning, and is currently convener for the SGM Education and Training Group. Her research developed from plaque on teeth to plaque on dentures, and thence to the attachment and accumulation of microorganisms on inert surfaces. There are several postgraduates in her laboratories working on various aspects of this cell-substratum interaction, with applications in oral, food and environmental microbiology. She is currently Professor of Microbiology in the School of Biology, Chemistry and Health Science at Manchester Metropolitan University.

Professor Carol Phillips

Carol completed her BSc and PhD in Microbiology at Cardiff University, followed by postdoctoral research at Sussex University and the Cancer Research Institute.

She worked in a NHS pathology department for several years before joining The University of Northampton in 1990 as a Lecturer, then Senior Lecturer, Reader and now as Associate Dean for Research in the School of Health. She leads a research group, whose work focuses on foodborne bacteria and their survival in foods and the environment. She has published many papers in academic journals, given conference presentations and is on the editorial board of *International Journal of Food Science and Technology*. She also acts as a reviewer for several journals. She is an active member of the Institute of Biology (IoB) and the Institute of Food Science and Technology (IFST), presently sitting on the Finance Committee of the IoB and the Council of the IFST. Previously, she has organised conferences for IFST and the Biochemical Society and also a number of conferences at the University. Professor Phillips has been a member of the Society for several years and received a number of grants. She would therefore like to reciprocate by becoming more active within S*f*AM and very much appreciates this opportunity to serve the society as a member of Committee.

Stay in touch!

We strive to keep in touch all our members and hopefully you will have been receiving your monthly e-bulletin to your email address. The aim is to keep you up to date with S*f*AM events and members news. To ensure that you are kept up to date, if you are not receiving these bulletins then please contact membership co-ordinator, Julie Wright on +44(0)1234 326661 or email her on julie@sfam.org.uk and let her know your up to date email address. Also, if you have any news that you think our membership should be aware of, then please send it to Communications Officer, Lucy Harper (lucy@sfam.org.uk).

BIOSCIENCES FEDERATION

Biosciences Federation – a message from the Chief Executive

THE BSF HAS GROWN IN strength during the summer months. This is because we have made two important appointments to help with the policy work. First, we have recruited Dr Caroline Wallace. She will have particular responsibility for our Animals Science Group and our European Liaison Group. Caroline has a PhD in molecular biology and has worked with us for the last two years in a contract/consultancy role. The second appointee is Dr Richard Bateman. He has resigned from a senior position at The Natural History Museum to become our Head of Policy in a part time capacity. With his background in systematics and plant science he will increase the width of our "in house" skill base.

These important appointments have become possible because of increased membership and, importantly, a substantial voluntary increase in the subscription paid by several Member Organisations. As a consequence of these appointments we will be even more effective than hitherto in reacting to Government and other enquiries and initiatives. More significantly, we will be able to be more proactive. That is, we can start to identify initiatives as they are born and influence their gestation, and also give birth to some ourselves. In this context, the BSF will look to you, the Member Organisation and the individual, to help with horizon scanning and the identification of areas where we should take the lead.

Have you seen our response to the Cooksey enquiry? If you haven't, it is on our web site and it gives you some idea about what we are doing for you. I am sure you know that Cooksey is concerned with putting the funding for NHS Research and Development under the same umbrella organisation as MRC grant awards. Following our submission, the BSF was invited to a meeting with the Cooksey team to discuss four questions. In summary, these can be distilled down to two points. They were about translation (the conversion of world class science to medicines and improved clinical practice) and the incentives to offer scientists, Departments and Universities in order to achieve this goal. We sat at tables of eight and took it in turns to give our answers to the questions. Interestingly, the answers reflected a broad swathe of agreement that both translation and incentives were not only desirable but essential. However we did not tackle what I believe to be a central concern for the BSF. That is, under which *modus vivendi* will the new joint fund operate? Will it be that of the MRC or that of the NHS? We are absolutely clear about this question: it has to be that of the MRC. We should only give grants for potentially excellent world class research. If areas need strengthening we should not pretend that the science is excellent in order to make an award. If the country needs to strengthen an area then earmarked funds should be used for this explicit purpose. The marriage of funds for world class research and capacity building generally reduces the integrity of awards for both.

By the time that you read this we will have submitted our views about new RAE metrics to the Department for Education and Skills. The BSF strongly holds the view that a metrics only approach to the RAE after 2008 is wholly undesirable. The Federation takes the view that metrics should be there to guide and inform panels but we cannot imagine a suitable series of stand alone algorithms for dealing with all the complexities and different emphases across the biosciences. We also hold the view that metrics should not only be about inputs (for example, grant income) but also about outputs (for example, citations). However the key element is that metrics are assessed by people and not software.

How do we undertake these policy reviews? From this summer we have developed a closer relationship with S*f*AM in order that we might work together more effectively on key policy issues for the biosciences. As an issue comes to the fore, we write to all Member Organisations and ask them if they want to nominate someone to be a member of an *ad hoc* task force to work on our response. Therefore if this sort of work interests you at all — and you have something to say (!) — you should let S*f*AM know.

And finally, are you a postdoc or graduate student looking for a job? If you are, you should find a new page on our web site helpful. This page provides links with very many of the sites that you might want to look at for job advertisements. If you think that there are important links missing please inform Dr Emma Southern (esouthern.bsf@physoc.org).

Richard Dyer,
Chief Executive, Biosciences Federation

An Innovation at the Summer Conference - the Postgraduate Student Session – 'Making Good use of your Supervisor'

Kate Exley and **David McCleery** report

IT WAS FITTING THAT during our 75th anniversary year, *Sf*AM introduced a new student initiative at the Summer Conference. A workshop-style 'student session' was organised for postgraduate research students to provide support for them in their studies. The topic covered was 'making good use of your supervisor.' This session was led and facilitated by Dr Kate Exley* and 20 students at all stages of their PhDs participated. This article provides an overview of the workshop and the topics discussed. It also outlines the comments made by the students about the student:supervisor relationship and the supervisory process itself. We hope it will be of interest to students and supervisors alike.

Postgraduate training has been a hot topic for the past five or so years. The need for postgraduate students to develop transferable skills required by employers was highlighted in the 'SET for Success' review by Sir Gareth Roberts in 2001. This resulted in extra funding being made available to universities to develop additional research and transferable skills training.

Support for the personal and professional development of students is not a new thing for *Sf*AM. Students are essential for the future of Applied Microbiology and the Society. Indeed, student sessions have been very important and popular elements of Summer Conferences for many years and the Society also offers a number of student grants and awards.

In looking to new ways to respond to the needs of our membership, the idea of running a workshop-style session for research students was proposed by the membership committee. This was a viewed as an unique opportunity to bring together students to not only share their experiences (and often frustrations) of a postgraduate research project with their peers, but also to discuss this with others also working in the area of Applied Microbiology. The potential for interchange between students based in different Departments, Universities and countries was an additional benefit.

This led to *Sf*AM approaching Kate, a Consultant in HE and Senior Staff Development Officer for The University of Leeds, who kindly agreed to run the student session for us. She developed an interactive session that aimed to offer research students the chance to review and discuss their approaches to being supervised and pool strategies for getting really effective support from their supervisors.

The 'Student Session'

The outline programme for the session included the following topics:

- The nature of the UK PhD in 2006
- Supervisory roles, styles and practices
- Student and supervisor expectations — any gaps?
- How can supervisors help you?
- How can you make yourself easy to help?

To set the scene, key issues arising from the updated QAA code of practice (2004) on research degree provision were reviewed. This highlighted the concerns, shared by both the UK Government and many funders of research degrees, that some students do not reach their full potential and could leave their research studies early if the supervisory arrangements were ineffective. Many of those attending were surprised to hear that only 57% of full time students completed their PhDs within five years (Hefce, 2005) and that the comparable completion rates were significantly poorer for those studying part-time. The central importance of the Student: Supervisor(s) relationship in both the production of high quality research and the development of the next generation of researchers, was clearly recognized and the roles and responsibilities of each were discussed.

Whilst key stages in supervision could readily be identified (Fig. 1), it was also agreed that there were many ways these stages can be approached.

The research work on different supervisory styles, conducted by Australian researchers Gatfield & Alpert

Figure 1
Key stages in the supervisory process

- Pairing of student and supervisor(s)
- Identification and approval of the research project proposal
- Induction and getting started
- Gaining a shared understanding of the expectations & processes of supervision
- Identifying and responding to training needs (both research focused and for personal development)
- Routine and ongoing monitoring and guidance
- Presenting, publishing research findings and producing the Thesis
- Preparation for and assessment
- Career coaching and guidance

(2002) and Gatfield (2005), acted as a trigger for discussions on the many ways supervisors chose to carry out their supervisory job. The research students also reflected on the way their own approaches and personalities drew particular supervisory responses from their supervisors at different stages in their work. It was interesting to see which of the words below (Fig. 2) the students associated with their own supervisors.

The changing relationship between student and supervisor became the focus of a significant piece of group work during the session. To set the context for discussion Kate introduced a simple model of the developing partnership (Fig. 3).

Students were asked to form three teams, Team 1, those who considered themselves to be at the beginning of their PhDs (1st years), Team 2, those in the middle (2nd and 3rd years) and Team 3, those approaching completion of their studies (3rd and 4th years).

Lively and animated, the students filled sheets of flipchart paper with their responses to the set task;

"With a group of colleagues discuss and flipchart the specific ways a supervisor can help you at this stage of your work...and include any ways you can think of that would make you an easier person to help."

The Outcomes

Below are the flipcharted comments that the groups presented back to a final plenary.

Team 1. First Years
From Supervisors

- Introduction — Informal/formal initial bonding between supervisor and student
- Expectations of supervisor and student roles discussed
- Discuss concept of the research work
- Null and alternative hypotheses
- Discuss practicalities of the work
- Time plan for the work
- Materials and resources needed and how to get them
- Methods and techniques to be developed and used (e.g. statistics)
- Recommended reading for literature review
- Availability of the supervisor and means of contacting, personal meetings etc
- Clarity on what happens if/when they are away

Availability of other support – technical support, mentors, advisors etc

Guidance on training

- what's available and when,
- what's expected, supported and advised

Figure 2
Which words describe your supervisor?

Supervisor Teacher Director Critic Guru Expert Guide Master Project manager Auditor Counsellor Colleague Trainer Senior partner Friend Editor Mentor Supporter Adviser Examiner Financer

List extended from one originally produced by Brown and Atkins (1988)

Self–Help

- Be proactive — maintain reading
- Act on initiative — lead on yourself from initial supervisor input
- Use 'To do' lists every day
- Manage your stress! — keep others informed
- Networking — seek opportunities to talk to people!
- Regular communication with supervisor
- Reports (Oral and Written)
- Progress and Concerns
- Devise a problem solving process
- Consider safety (COSHH)
- Team-working
- know how to cooperate
- encourage friendly relations (socialise)
- be friendly and approachable, fun and polite
- Know when to take a break (coffee break or holiday etc., to let off steam)

Figure 3
How does the supervisory relationship change over time — a simple model

- Student is...gaining research skills, methods & techniques
  Undertaking **Training**...Supervisor as **Trainer**
- Student is...developing professional skills & gaining in independence
  Undertaking a research **Apprenticeship**
  Supervisor as **Mentor**
- Student is...contributing to knowledge
  Undertaking **Scholarship**
  Supervisor as **Colleague**

Team 2.
Second and third year students.
Help wanted from Supervisors

- Structured meetings
- Clear constructive feedback
- Targets and deadlines
- Support in developing skills — presentations at meetings, publications etc
- Help to identify loose ends and address them
- Motivation
  - In general
  - To build confidence
  - To write up

Self-Help

- Be more proactive
- Be more understanding
- Set our own deadlines
- Take responsibility for our own work
- Be more positive — give solutions as well as problems

Team 3. Final year students
Help wanted from Supervisors

- Go over results and identify gaps
- Encourage to publish early to avoid stress
- Review the Thesis plan
- Build confidence for the *viva*
- Help prepare for the *viva*
  Mentor
- Help you to draw a line when to stop in the lab.
- Reading and giving feedback on draft chapters
- Reading and giving feedback on publications
- Advise on future jobs

Self-Help

- Maintain good communication with supervisors
- Be open to constructive criticism
- Be independent and expect minor supervision

The reporting back and subsequent discussion between groups of students at

different stages in their 'PhD journey' was both intelligent and supportive. With hindsight it is also striking how much the students' views aligned with the advice given by researchers in the field. For example, Estelle Phillips (co-author of 'How to Get a PhD" now in 3rd edition), had recently provided her 'Ten golden rules' for PhD students and advised student to:

- always leave a tutorial having agreed a date for the next one
- send supervisors a summary after each formal supervisory meeting
- make sure you do not have two supervisors with the same job/role
- yes, please be independent but you do need to conform, too
- agree arrangements for meetings with supervisors from the outset
- do not become romantically involved with your supervisor
- if anything is interfering with your work, let your supervisors know
- establish exactly what is being criticised and how to put it right
- ask direct, but positively constructed, questions
- tell your supervisor what you are discovering as you do.

(adapted from Phillips, 2005)

It would have been wonderful for the students' supervisors to hear their students mature and confident discussion — they would have been very proud.

The student session was viewed this year very much as a pilot exercise to see whether it would be worthwhile to make this an annual activity. The overwhelming feedback from the students who participating in it this year was that it was a great success and that it should be held every year. Sarah Hamill from Queen's University, Belfast commented: *"Personally I thought the making good use of your supervisor session was an excellent idea. The session was lead by Dr Kate Exley, a higher education specialist who immediately put everyone at ease. It was a great icebreaker, as it can sometimes be difficult for students to interact under normal conference circumstances. The content was well structured, encouraging each student delegate to define their relationship with their supervisor, and to identify any concerns. Kate discussed the differences between student and supervisor expectations, outlining potential problems and giving suggestions on how to overcome them. Discussion groups were organised, which allowed first and second year students to benefit from the advice of students in their final year. The discussion groups also meant students from similar disciplines but different universities could compare their experiences, something we would not usually have the opportunity to do.*

The take home message for me was that each student's relationship with his or her supervisor is different. Ultimately PhD students need to take responsibility for their own research project, but careful planning and regular communication should ensure a successful relationship with their supervisor."

SfAM would like to thank Kate for running this student session for us and the students who so energetically participated in it.

If you would like to find out more about the support and opportunities that we offer to students, please contact the Communications Officer, Dr Lucy Harper on +44(0)1234 326709 or email lucy@sfam.org.uk.

Dr Kate Exley* and Dr David McCleery**
*University of Leeds and **Safefood, Food Safety Promotion Board, Cork.

Suggested reading

for PhD students

Finn, J.A. (2005) *Getting a PhD: An action plan to help manage your research, your supervisor and your project*. London: RoutledgeFarmer Study Guide

Hunt, A. (2005) *Your research project: How to manage it.* London: RoutledgeFarmer Study Guide

Phillips, E. and Pugh, D.S. (2005) *How to Get a PhD: A Handbook for Students and their Supervisors.* Third edition. Buckingham: OUP

Murray, R. (2002) *How to write a thesis.* Buckingham: OUP

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for Supervisors

Ryan, Y. and Zuber-Skerritt, O. editors (1999). *Supervising Postgraduates from Non-English Speaking Backgrounds.* Buckingham: SRHE and OUP

Taylor, S. and Beasley, N. (2005) *A Handbook for Doctoral Supervisors.* London: Routledge

Tinkler, P. and Jackson, C. (2004) *The Doctoral Examination Process: A handbook for students, examiners and supervisors.* Maidenhead: The Society for Research into Higher Education and OUP

Footnote

Dr Kate Exley is a Consultant in HE and Senior Staff Development Officer for The University of Leeds. Kate has conducted research into the attitudes and practices of research supervisors in the UK and published on this and the guidance and training new supervisors receive.

She now runs a range of workshops for postgraduate students and their supervisors around the country (which seems a long way from doing her own PhD on transposable elements in Drosophila!). For further details email: kate@kate-exley.com or telephone: +44 1773 789209.
website: http://homepage.ntlworld.com/exleys/index.html

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CAREERS

Water Microbiology

Andy Gawler explores the work of a water microbiologist

AT THE TIME I LEFT COLLEGE and was considering a future career, the state of the environment wasn't seen as a particularly important issue. However things have changed dramatically and the environment is now a global issue. So how did I end up working for the Environment Agency? I suppose the path I have taken is typical of many people who are working within environmental organisations. Most would have started their careers in other areas.

Like many young people today I wasn't sure what I wanted to do when I left college. After a brief spell as a police cadet, I felt that a career in science was what I wanted to do. I had enjoyed science at college, especially Biology and decided to pursue this as a career.

I was fortunate to get a job as a trainee Medical Laboratory Scientific Officer (MLSO) with the local health authority pathology laboratory. This was a great opportunity to gain practical analytical skills across the spectrum of disciplines within pathology. When the time came to specialise I had already made up my mind that I wanted to be a microbiologist; I found the subject absolutely fascinating. The Public Health Laboratory Service and the local health authority jointly funded the Microbiology department. As my career progressed I worked for both organisations at different times but in the same laboratory. During this period I gained my fellowship of the Institute of Medical Laboratory Sciences and a position as a Senior Medical Laboratory Scientific Officer.

I guess I would have continued my career in the health service except for the changes that were occurring in the water industry.

In 1989 the water industry in England and Wales was privatised. As a consequence, a number of water companies were formed. The requirement for a regulator for the water industry and increasing public awareness of environmental issues led to the formation of the National Rivers Authority (NRA). The NRA was organised on a regional basis. In some regions the NRA inherited laboratories and their staff. However in the South West Region the laboratories stayed with the newly formed water company. The NRA decided to build a new laboratory at its Regional Head office in Exeter. The laboratory was designed to cater for the analytical workload for chemistry and microbiology which was associated with the various European Union directives which were coming to force and associated work.

The most important directive as far as microbiology was concerned was the Bathing Water Directive. This directive specifies that each designated bathing water in the UK should be tested 20 times during the bathing season. In England and Wales this is between 15 May and 30 September every year. There was also a requirement to test the bathing waters for the presence of enteroviruses and therefore the Exeter laboratory was designed to be a centre of expertise for environmental virology for the whole of the NRA.

In March 1991 I was appointed as Senior Scientist, Microbiology at the NRA laboratory in Exeter. This was quite a challenge as the laboratory was not finished, there was no equipment and staff had yet to be recruited. All this and a deadline to have the Laboratory ready to receive samples from the start of the bathing season, in May. This target was met, as was the target for provision of the national service for virology. This service was started on 1 April 1992.

My job as Senior Scientist was quite varied. As well as managing the microbiology laboratory at Exeter, I was also responsible for liasing with other microbiologists within the NRA to ensure that the microbiology service was delivered consistently across the Authority. It was as part of my role within the NRA that I first became involved in EU funded R&D projects.

The first such project was a three-year

project, which examined the analytical methods used in 12 European countries for analysing bathing water samples. This project gave me the opportunity to meet and work with other European environmental microbiologists and gain an insight into the challenges that they have to deal with.

In 1996 the NRA, Her Majesty's Inspectorate of Pollution (HMIP) and elements of local authorities associated with the regulation of waste were brought together to form the Environment Agency (EA). The NRA's National Laboratory Service (NLS) became the laboratory service for the EA, extending its role to include the analysis of waste samples.

By 2000, we had outgrown the Exeter laboratory and moved to a new site at Starcross, just across the Exe estuary. With the increased space at Starcross, the microbiology section was able to take 70% of the Agency's workload.

Today my job is quite diverse. The Starcross laboratory has 120 staff and a budget in excess of £4 million. As one of the section heads, I am part of the Laboratory Management team, so most Monday mornings are taken up with site management meetings.

As manager of the microbiology section I am responsible for the performance management of the services provided to the Agency and other customers. One of the most challenging issues is balancing the needs of a number of Environment Agency Regions in terms of wet weather surveys. The laboratory is resourced for a planned workload. However, occasionally colleagues from the Agency are required to carry out wet weather surveys to access the effect of run off on specific catchments. In times of general wet weather several Regions may want to carry out these surveys at the same time and I have to ensure that sufficient staff resource is available to cope with the increase in work. Sometimes this requires serious negotiation.

At the present time I am involved in three EU funded international projects. For the past three years I have been managing a Pilot Action within the ICREW Project. The lead partner is North West Region of the EA. This is a large project aimed at improving coastal and recreational waters. It has 7 Pilot Actions. I have been managing Pilot Action 3, which examined methods for microbial source tracking. At the present time we can use traditional microbiology, detect the faecal pollution of water, however, the tests we use at present can't tell us the source of the pollution. If we knew whether the pollution was from a human source or an agricultural one, it would be easier to investigate such events and remove the source of the pollution. Working with colleagues in France, Ireland and Portugal we have been developing molecular techniques for use in Microbial source tracking. Over the last two years especially this has been a major part of my job. Every six months there have been meetings between the partners. At these meetings apart from agreeing the way forward technically you also have to manage the budget and produce various reports track the progress of the project.

The second project is the Cycleau project. This is lead by South West Region of the EA and is researching river catchment management. There is a small research team working on this project at Starcross, which I am responsible for managing.

The third project is called Virobathe and this is lead by the University of Wales, at Aberystwyth. This project is developing and testing molecular methods for detecting *Norovirus* and Adenovirus in environmental waters. Because we need samples at a specific state of the tide and the sample point is close to where I live, I often take the samples on the way to work. I also represent the Agency on a number of committees and working groups.

Like most organisations today the National Laboratory Service is constantly trying to find better and more efficient ways of conducting its business. This always brings new challenges. My present challenges include the planning and implementation of an analytical service for microbiology to be provided from the Starcross laboratory for the whole of the Environment Agency. This has to be in place for the 2007 bathing season.

Once this has been achieved we will be looking ahead to the implementation of the new bathing water directive in 2015. This may seem a long way into the future but the new classification of designated bathing beaches will be based on four years analytical data. So new analytical methods will have to be selected, performance tested and available to the Agency by 2010.

Much of the work carried out in the Environment Agency's National Laboratory service is routine in nature. From a career perspective the work is similar to that of other laboratory services. However working within the EA does allow you to see how the results of that analysis are used to benefit the environment. The Environment Agency has a wide remit, ranging from protection of water quality to conservation and recreation to fisheries.

There can be opportunities for laboratory staff to move into other areas of the Agency and develop new career opportunities. Working for an organisation such as the Environment Agency is often challenging but also very rewarding.

References

- http://www.icrew.info
- http://www.cycleau.com
- http://www.virobathe.org

Andy Gawler
Senior Scientist, Microbiology, National Laboratory Service, Environment Agency

January Meeting 2007

a one day meeting on
Food and Health:

The Royal Society, Carlton House Terrace, London
Thursday 11 January 2007

A one day meeting with sessions on:

- Hospital Acquired Infections and
- Food Microbiology (in collaboration with the Chartered Institute for Environmental Health)

Topics to be covered will include:

Hospital Acquired Infections:
- the government perspective;
- infection control team's perspective;
- clinical microbiologist's perspective;
- *C.difficile*
- MRSA
- *Acinetobacter*

Simmering Questions in Microbiological Food Safety
- Is there a scientific basis for safe eating practice?
- How and why do enteropathogens make you ill?
- Norovirus and Hepatitus A — an important cause of foodborne illness?
- How do foodborne pathogens emerge?

CPD ACCREDITATION

Including:

The Denver Russell Memorial Lecture:
'Naturally Occurring Microorganisms and their Resistance to Physical and Chemical Agents' given *by Martin Favero, Advanced Sterilisation Products, Johnson & Johnson, USA.*

Please note that the meeting programme was correct at the time of going to press but may be subject to change.

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

Programme

10.00-10.30 **Arrival/Coffee/ Registration**

10.30-11.15 **The Denver Russell Memorial Lecture 'Naturally Occurring Microorganisms and their Resistance to Physical and Chemical Agents'**
Martin Favero, Advanced Sterilisation Products, Johnson & Johnson, USA.

11.15- 11.45 **What role can Government play in controlling hospital acquired infection?**
Brian Duerden

11.45-12.15 **Food poisoning – what are the real risks?**
Bob Adak, CDSC, Health Protection Agency.

12.15-13.15 **Lunch**

Afternoon: two parallel sessions of 5 talks

Session A. Hospital Acquired Infections

13.15-13.45 **'Infection Control Teams - friend or foe?'**
Martin Kiernan, Southport and Ormskirk NHS Trust

13.45-14.15 **Hospital acquired infections: a clinical microbiologist's Perspective.**
Kathleen Bamford (Hammersmith Hospital)

14.15-14.35 **Tea and Coffee**

14.35–15.05 ***Clostridium difficile:* current situation and prospects for the future.**
Jon Brazier, NPHS microbiology, Cardiff

15.05–15.35 ***Acinetobacter* outbreaks: how long before they are unmanageable?**
Kevin Towner , Nottingham University Hospitals NHS Trust

15.35–16.05 **MRSA: do we have the situation under control?**
Barry Cookson, Health Protection Agency

Session B. Simmering issues in food safety

13.15-13.45 **The Foodborne Disease Risk Matrix: have we got it right about the public health risk of foodborne disease?**
Joyce Brown, Operational Research, ASSRPD, Food Standards Agency

13.45-14.15 **Cross-contamination risks in the kitchen: problems with Salmonella and *Campylobacter***
Frieda Jorgensen, University of Bristol

14.15-14.35 **Tea and Coffee**

14.35-15.05 **Norovirus and Hepatitis A – important causes of foodborne illness?**
Mike Carter, School of Biomedical and Molecular Sciences, University of Surrey

15.05-15.35 **Microbiological criteria for foods**
Linden Jack, Microbiological Safety Division, FSA

15.35-16.05 **Emerging problems: new foods or new pathogens?**
Jim McLauchlin, Food Safety Microbiology Laboratory Centre for Infections, HPA

16.10 **Meeting closes**

Please note that the meeting programme was correct at the time of going to press but may be subject to change.

For the latest information, please visit us online at:
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BOOKING FORM and INVOICE

SFAM JANUARY MEETING THURSDAY 11 JANUARY 2007

Food and Health

Only ONE person per form please. If additional forms are required please photocopy this one. CLOSING DATE FOR REGISTRATIONS: Friday 22 December 2006. A LATE BOOKING FEE of £30.00 will be applied to all bookings made after this date.

If you are a Student (S), Honorary (H), Associate (A), or Retired Member (R), please enter the applicable letter (S, H, A or R) into the appropriate FEES BOX below:

FEES

Whole Conference Rate: inc. of registration fee, coffee breaks and lunch. **Please tick applicable box:**	**Full Members**	**Student, Honorary, Associate & Retired Members**	**Student Non - Members**	**Non - Members**	**CIEH/IBMS Members**
	£50.00 ☐	£30.00 ☐	£60.00 ☐	£100.00 ☐	£75.00 ☐

YOUR INTERESTS

Please indicate which of the two parallel sessions of 5 talks you wish to attend. Each session begins immediately after lunch at 13.15

Session A: Hospital Acquired Infections ☐ **Session B:** Simmering issues in food safety ☐

YOUR COSTS

Charges - please tick the applicable box(es)	Amount
☐ **Whole Conference Rate:**	£
☐ **LATE BOOKING FEE** Payable for all bookings made after Friday 22 December 2006	£30.00
TOTAL AMOUNT REMITTED:	£

YOUR DETAILS

Title:_____ **Family Name:**_____ **First Name:**_____

Organisation/Affiliation:_____

Address:_____

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_____ **Postcode:**_____

Tel No:_____ **Fax No:**_____ **Email:**_____

Please indicate any special dietary or other requirements (such as disabled access):_____

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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. Please note that AMERICAN EXPRESS and DINERS CARDS are NOT ACCEPTED.** However the following credit and debit cards are acceptable: VISA, Mastercard, Eurocard, Delta, Electron, JCB, Maestro and Solo.

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Tel: 01234 326661. Fax: 01234 326678. Email: meetings@sfam.org.uk

SUGGESTION: please photocopy this form to save mutilating your copy of the Microbiologist!

Broadening Microbiology Horizons

Manchester Metropolitan University
Wednesday 11 April 2007

CPD ACCREDITATION
Seven credits have been awarded for this meeting

Programme

09.30–10.30 **Arrival / Coffee / Registration / Trade Exhibition**

10.30–10.35 **Chairman's Welcome**

10.35–11.00 **"Lumping and Splitting" – latest developments in typing methods**
Andrew Fox, Health Protection Agency, Manchester

11.05–11.35 **Latest Developments in Detecting Yeasts and Fungi**
David Denning
South Manchester University Hospital NHS Trust

11.35–12.05 **Use of Bacteriophages as Treatments**
Geoff Hanlon University of Brighton

12.05–12.35 **An update on Microbiocides**
Jean Yves Maillard
Cardiff University

12.35–14.00 **Lunch / Trade Exhibition**

14.00–14.30 **Dental Microbiology**
Peter Gilbert University of Manchester

14.30–15.00 **Use of Silver in controlling wound infections**
Val Edwards-Jones
Manchester Metropolitan Manchester

15.00–15.30 **An Update on Rabies**
Tony Fooks
Veterinary Laboratories Agency

15.30–16.00 **Near Patient Testing**
Andrew Sails
Health Protection Agency, Newcastle

16.00 **Meeting Closes**

Please note that the meeting programme was correct at the time of going to press but may be subject to change.

DELEGATE FEES

If you are a non member and wish to attend this meeting we will give you **£10 off** your 2007 membership fee	Sfam Members	Sfam Student Member	Non - Member	IBMS Member
	£50.00	£25.00	£100.00	£60.00

To book your place at this meeting please visit us online at:
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society for applied sfam microbiology

MICROBIOLOGY IN THE REGIONS
Joint regional meeting

society for general Microbiology

6th UK Genetics and Molecular Mechanisms in Archaea

11 - 12 January 2007 ● University of York, UK

including a session sponsored by

the geneticssociety

Invited Speakers include: Zvi Kelman (Baltimore, USA) / Hans-Joachim Fritz (Goettingen, Germany) / Thorsten Allers (Nottingham) / Steve Bell (Cambridge) / Ed Bolt (Nottingham) / Bernard Connelly (Newcastle) / Hannu Myllykallio (Paris, France) / Malcolm White (St Andrews) / Dale Wigley (CRUK)
There will be a £200 Microbiology Communication Prize for the best presentation by a young scientist.

Email jpjc1@york.ac.uk for a registration form by 30 November 2006

CHRO 2007

2-5 September 2007, Beurs World Trade Center, Rotterdam, The Netherlands

14th International Workshop on *Campylobacter*, *Helicobacter* and Related Organisms

Preliminary programme

Plenary sessions

- *Helicobacter* pathogenesis
- *Campylobacter*: diversity and host specific markers
- Epidemiology of *Campylobacter* infections
- *Campylobacter* pathogenesis
- *Helicobacter:* the microbe's view
- *Campylobacter:* host-pathogen interactions
- Guillain-Barré Syndrome
- *Helicobacter* vaccine development

Symposia

- Epidemiology, typing and diagnostics of CHRO 1&2
- Genomics, metabolism, and physiology of CHRO
- Pathogenesis of CHRO
- Antimicrobial resistance of CHRO
- Animal models/vaccine development
- Risk Assessment, prevention and control
- Immune responses and disease

Workshops

Full-scale interventions to control campylobacteriosis
Immuno-pathobiology of post-infectious complications

The conference language will be English

Further information: http://www.chro2007.nl/

Summer Conference 2007

Microbiology of Fresh Produce

Park Plaza Hotel, Cardiff, UK
Monday 2 to Thursday 5 July 2007

CPD ACCREDITATION
has been applied for

Including:

The Lewis B Perry Memorial Lecture - 'Bacterial anti-cancer vaccines: a science frozen in time' given by *Dr Peter Green, NCIMB Ltd*

Call for Posters!

There will be an opportunity during the meeting to present posters in any relevant subject area. Abstracts of less than 500 words, to include aims and objectives, brief methodology, results, conclusions and implications of the work, should be submitted only as a Microsoft™ Word document attachment to an email addressed to julie@sfam.org.uk with the subject line 'Summer Conference 2007 submission'.

Including sessions on:

- Organisms and the plant
- Public health aspects of fresh produce
- Intervention strategies
- The industrial perspective

Please note that the meeting programme was correct at the time of going to press but may be subject to change.

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

Programme

Monday 2nd July

14.00 onwards - Arrive and Register

18.00-18.50 **Lewis B Perry Memorial Lecture (National Museum of Wales): 'Bacterial anti-cancer vaccines: a science frozen in time."**
Peter Green

19.00-20.00 **Drinks Reception – National Museum of Wales .**

20.00 Evening at leisure

21.30 Quiz Night

Tuesday 3rd July

09.00-09.35 **The problems with fresh produce: an overview**
Mike Doyle, University of Georgia, USA.

Session 1. Organisms and the plant

09.35-10.10 **Zoonotic pathogen interaction with the microflora of the growing crop phylloplane**
Bill Keevil, University of Southampton, UK

10.10-10.45. **Microflora of plant parts intended for consumption**
Jo Heaton, University of Lancaster, UK

10.45-11.15 **Coffee/ posters**

11.15-11.50 **Non-culture based approaches to examining the microflora of salad vegetables**
Chris Dodd, University of Nottingham, UK

11.50-12.25 **Measurement and modelling attachment of bacteria to plant surfaces**
Tim Brocklehurst, IFR Norwich, UK

12.25-13.00 ***Erwinia* soft rots**
Amy Charkowski, University of Wisconsin, USA.

13.00-14.00 Lunch

Session 2.

14.00-14.35 **Fungi quality and safety issues in fresh fruit and vegetables**
Maurice Moss, University of Surrey, UK

14.35-15.10 **Rapid methods to detect quarantine pathogens in imported produce**
John Elphinstone, Central Science Laboratory, York, UK

Public health aspects of fresh produce

15.10-15.45 **Prepared salads and public health**
Chris Little, Health Protection Agency, London, UK

15.45-16.15 **Tea/posters**

16.15-16.50 **Pathogens from organic wastes – incidence and survival**
Michael Hutchison, University of Bristol, UK

16.50-18.00 **Student Session**

17.30-19.30 **Trade Show**

Wednesday 4th July

Session 3.

09.10-09.35 **Microbial pathogens – strategies for survival**
Jay Hinton IFR Norwich, UK

09.35-10.10 ***Burkholderia cepacia* and other opportunistic pathogens**
Liesbeth Jacxens, University of Ghent, Belgium

10.10-10.45 **Risk Assessments for fresh fruit and vegetables**
Speaker to be confirmed.

10.45-11.15 Coffee/ posters

Intervention strategies

11.15-11.50 **Good agricultural practices**
Robert Gravaini, Cornell University, USA

11.50-12.25 **Novel physical methods for decontaminating**
Stephen James, FRPERC – Langford, UK

12.25-13.30 **Lunch**

Session 4.

13.30 -14.30 **Offered papers**

14.30 -15.00 **Tea/Posters**

15.00 -16.00 **Student presentations**

16.00 -16.30 W H Pierce Prize

16.30 -17.00 **AGM**

19.30-20.00 **Drinks reception, tour followed by Dinner at the Millenium Stadium, Cardiff**

Thursday 5th July

Session 5

09.00-09.35 **Chemical treatments**
Des O'Connor, Microsearch Laboratories, UK

09.35-10.10 **Modified atmosphere storage and packing**
Gail Betts CCFRA, UK

10.10-10.45 **Microbial transfer in fresh salad processing**
Debra Smith CCFRA, UK

10.45-11.15 **Coffee/ posters**

The industrial perspective

11.15-11.50 **EU microbiological criteria**
Kaarin Goodburn, Chilled Foods Association, UK

11.50-12.25 **Issues with organic produce**
Carlo Leifert, Tesco Centre, UK

12.25-13.00 **Suppliers' Perspective**
David Kennedy, Geest Ltd, UK

13.00-14.00 **Lunch & Close**

Please note that this programme was correct at the time of going to press but may be subject to change.

Microbial contamination of fruit and vegetables: evidence and issues

Keith Jones and **Joanna Heaton** review the facts

IS THERE A PROBLEM? Recent outbreaks show that there is. On September 14 2006 the US Food and Drug Administration issued the following press release:

FDA Warning on Serious Foodborne *E.coli* O157:H7 Outbreak. 'One Death and Multiple Hospitalizations in Several States. The U.S. Food and Drug Administration (FDA) is issuing an alert to consumers about an outbreak of *E. coli* O157:H7 in multiple states that may be associated with the consumption of produce. To date, preliminary epidemiological evidence suggests that bagged fresh spinach may be a possible cause of this outbreak.'

This and other recent outbreaks of food poisoning traced to eating fresh fruit and vegetables (table 1) suggest that there is a problem with this branch of the food chain.

The Center for Science in the Public Interest (CSPI) has shown that between 1990 and 2003 bacterial entero-pathogens associated with fresh fruit and vegetables caused 554 food poisoning outbreaks the US, more than poultry in the same period.

Yet microbiological surveys of fruit and vegetables at point of sale suggest that there is not a problem with fresh fruit and vegetables. For example, in the LACOTS/PHLS (Local Authorities Co-ordinating Body on Food Training Standards / Public Health Laboratory Service) Coordinated Food Liaison Group Studies (2001) on 'The Microbiological Examination of Ready-to-Eat Organic Vegetables from Retail Establishments', organic vegetables from supermarkets, health food shops, farmers' shops or markets, greengrocers, market stalls, and box schemes, were tested. Of the 3200 samples only 15 (0.5%) were of unsatisfactory quality. Unsatisfactory results were due to *E. coli* and *Listeria spp.* No *L. monocytogenes*, *Salmonella spp.*, *Campylobacter spp.* and *E. coli* 0157 were found. They suggest that overall agricultural hygiene, harvesting and production practices are good.

McMahon and Wilson tested commercially available organic vegetables for bacterial enteropathogens in Northern Ireland (*International Journal of Food Microbiology* **70**,155–162, 2001). They found no *Salmonella*, *Campylobacter*, *E. coli*, *E. coli* O 157 or *Listeria*, but *Aeromonas* was isolated from 34% organic vegetables and 41% of organic vegetables ready-to-eat after minimal processing (washing). They concluded that organic produce in Northern Ireland is of good microbial quality.

We did our own point of

sale survey in Lancaster and our results are in broad agreement. Monthly samples of salad vegetables were taken from a variety of retail outlets and tested for a range of bacteria. Neither *Campylobacter* nor *Salmonella* were isolated but *Aeromonas* and *Listeria* were frequently found on a range of products. Organic samples yielded higher numbers of faecal coliforms during the summer months. High numbers of *Listeria* (including *L. monocytogenes*) and *Aeromonas* were found on packaged, organic watercress.

The bottom line

It is striking that none of the surveys detected the outbreak-causing enteropathogens listed in table 1. It is clear that surveys, which are only snapshots of the enormous fruit and vegetable industry, do not tell the whole story, and that every now and again something goes seriously wrong!

Why should there be a problem with fresh fruit and vegetables?

(a) Increased exposure to enteropathogens

Government programs, such as the 'Five a Day' campaign in the UK and the 'Nine a Day' campaign in the US, together with the publics' awareness of the benefits of a healthy diet, have lead to increased consumption of fresh fruit and vegetables with the concomitant increase in exposure to the associated enteropathogens. However, not everyone enjoys eating five portions of fruit and vegetables every day and surveys suggest that we find them boring, expensive and too time-consuming to prepare. This has lead to a demand for more convenience products, such as bagged mixed salads, prepared vegetables and snack fruits, a demand which has been met by increased importation. Ninety percent of fruit and 29% of vegetables are currently imported into the UK. There is concern that imported fresh produce may be more contaminated than home grown produce.

(b) Switch to organically grown fruit and vegetables

There has also been increased consumption of organic food. The Soil Association states that the worldwide market for organic food reached £16.7 billion in 2005 with sales in the UK rising by 30% in the that year. As animal manures are used as fertilizer for organically grown fruit and vegetables, the potential for microbial contamination is higher than for conventionally grown crops. Rather surprisingly, there is little published evidence to support this.

(c) Lack of cooking and washing

Fruit and salad vegetables are different from most foods that we buy because they are not cooked and any disease-causing organisms present will be swallowed. The move towards pre-packed salads has made us careless about thoroughly cleaning fresh produce.

Which microorganisms are responsible for illness?

Any microorganism found in the faeces of humans, livestock and wild animals has the potential to contaminate fresh produce via manure or irrigation water. Indeed, most of the major enteric diseases - cholera, typhoid and dysentery, have been traced to fresh produce at some time or another. However, table 1 shows that a limited number of pathogens have been involved in outbreaks from fruit and vegetables in the last few years.

Salmonella is the most common cause of disease outbreaks linked to fresh fruit and vegetable products. Indeed, the CSPI has shown that in the USA, fruit and vegetables are responsible for more (and bigger) outbreaks of *Salmonella* than poultry. In the UK, around 40% of food poisoning outbreaks connected to the consumption of fresh produce are due to *Salmonella*. Several different species of *Salmonella* from a range of produce: sprouted seeds, cantaloupe melons, tomatoes, unpasteurised citrus juices, almonds, rocket and lettuce, have been responsible for outbreaks.

Most strains of pathogenic *Escherichia coli* have been associated with fresh fruit and vegetables, but reported outbreaks mainly concern *E. coli* O157:H7. The first UK outbreak was in women harvesting potatoes in a field fertilised with cattle manure. The biggest outbreak was in 1996 when 9,400 school children in Osaka, Japan, were infected with *E. coli* 0157 from white radish seed sprouts. Ten of the children died. The most recent outbreak in the USA (ongoing at the time of writing) has spread to 25 states and caused 175 cases of illness, 28 cases of severe kidney disease (haemolytic urinary syndrome), 93 hospitalisations and one death. The US FDA warns that more cases are expected (25/09/2006). It has been traced to spinach grown in California and it is thought that contaminated irrigation water is the likely route of infection. The guidelines developed by the FDA for the 'Lettuce Safety Initiative', in response to the number of lettuce-associated food poisoning outbreaks (20 since 1995 linked to spinach or lettuce in the US), have now been spread to spinach. This outbreak has also thrown the media spotlight onto the dangers of centralized distribution and the great distances our so-called fresh produce travels.

Norovirus associated with raspberries have caused several recent outbreaks in the EU. The virus is found in human faeces and it is thought that poor hygienic practices during harvest have lead to the contamination of the fruit. Poor hygiene is also thought to be responsible for other outbreaks caused by enteric viruses, for example, the 2004 hepatitis A outbreak linked to spring onions imported into Philadelphia from Mexico (table 1).

Several other pathogens are associated with fresh fruit and vegetable but have not caused recent outbreaks. For, example, *Listeria* is widespread on plants in the environment and is readily isolated from cucumber, potato, radish, leafy vegetables, sprouted seeds and tomato. Although there is a huge amount of literature dedicated to researching the attachment and survival of *L. monocytogenes* on salad vegetables, only two fresh produce-related outbreaks have been documented.

Aeromonas has been found on sprouted seeds, asparagus, broccoli, cauliflower, carrot, celery, cherry tomatoes, courgette, cucumber, lettuce, mushroom, pepper, turnip and watercress. However, although it is the most commonly isolated pathogen from vegetables, no outbreaks have been attributed to it.

Campylobacter jejuni is interesting in this regard. Epidemiology shows that eating salad vegetables is a major risk factor for *Campylobacter* infection, but hardly anyone has actually isolated it from fresh produce at point of sale.

Other pathogens associated with fresh produce include *Yersinia enterocolitica*, *Cryptosporidium*, *Giardia* and Cyclospora.

Fruit and vegetables are contaminated with large

numbers of harmless bacteria, but it should be noted that the outbreak strains of *Salmonella* and *E. coli* are almost never isolated during routine bacterial monitoring of fresh produce at point of sale. Some potential pathogens, such as *Listeria* and *Aeromonas*, are regularly found on fruit and vegetables.

What are the routes of contamination?

(a) Animal manures

Animal manures have been used as fertilizers since farming began. In the UK, 280 million tons of animal manure are applied annually to land: 80 million tons from housed animals and 120 from animals at pasture. As farm slurries contain $2.2x10^4$ to $3.2x10^6$, grazing cattle $2.3x10^5$ to $6.7x10^9$ and grazing sheep $2.8x10^9$ to $4.5x10^{12}$ *E. coli* per gram, this represents an awful lot of bacteria. Large amounts of sewage sludge are also disposed of onto farmland in the UK but not where fruit and vegetables are grown. Bacteria are transferred to growing crops by direct contact and by splash from heavy rain or irrigation with rain-guns. Regulations and guidelines monitored by DEFRA and the Environment Agency are designed to limit numbers and survival of enteropathogens in animal wastes put to land and to keep sludge and slurries separate from fruit and vegetables. It can be argued that growing crops are more likely to be contaminated by animal wastes by the wind blown aerosols formed during slurry spraying (especially that last burst high into the air as the operator clears the pipes) and from irrigation water contaminated by run-off from fields into streams and rivers, rather than wastes used as fertilisers.

Animal wastes used in organic farming are composted over lengthy time periods to minimize the survival of enteropathogens.

(b) Wild animals (including birds)

Wild animals, especially flocks of wild birds, can contaminate growing vegetables and fruit. The following enteropathogens have been detected in the faeces of wild birds:

Salmonella, *Campylobacter*, *Yersinia*, *Shigella*, *Vibrio cholerae*, *E. coli* 0157, *Listeria*, *Cryptosporidium*, *Giardia* and the faecal indicators - coliforms and enterococci. Wild birds contaminate pre-harvest crops while foraging in fields -especially those covered in sewage sludge or farm slurry, while roosting in green houses; and post-harvest crops while feeding and roosting in barns, storage buildings, warehouses and supermarkets. Birds are notoriously difficult to control and we are left to wonder whether crops grown under bird migration routes are susceptible to contamination by faecal bombing?

In Florida, *Salmonella* species normally associated with alligators and amphibians have been shown to contaminate outdoor grown tomatoes.

(c) Irrigation

The World Health Organisation (WHO) recognises that there is a link between contaminated irrigation water and contaminated fresh produce. In developing countries rivers used for irrigation, especially urban ones, can be highly polluted and contain upwards of 10^8 per 100 ml faecal coliforms. In developed countries surface waters contain fewer faecal coliforms at around 10^4 per 100ml. In the UK 51% of crops are irrigated with river water.

Three recent outbreaks have been caused by the use of contaminated irrigation water. The *E. coli* O157 outbreak in Sweden (2005) was caused by irrigation of lettuce with stream water contaminated by cattle faeces during a drought (table 1). The ongoing *E. coli* O157 linked to spinach in the USA is thought to be the result of contaminated irrigation water and the 2005 lettuce-associated *Salmonella* outbreak in the UK and Finland was caused by Spanish farmers using sewage effluent to irrigate the crop, also during a drought. The greater the distance that growers have to go to find new water sources the more likely it is to be contaminated, and in drought conditions, corners will be cut.

K. Obiri-Danso's group at the Kwame Nkrumah University of Science and Technlogy, Kumasi, Ghana, has shown that most fresh produce in this developing country is eaten locally but is not grown or stored in hygienic conditions. There are competing uses for irrigation water, such as washing, cooking and irrigation. Local produce is irrigated with contaminated water twice daily and post-harvest is washed in the same water and kept in water until sale. They note that contamination of fruit and vegetables increases with the amount of handling.

Water is not only used for irrigation, it is also used to cool crops in the field and to transport and wash crops post-harvest. This provides further opportunities for contamination if the water is not clean.

(d) Worker hygiene

The *Norovirus* and Hepatitis A outbreaks in table 1 are thought to be a result of poor worker hygiene. Prevention requires rigorous supervision and provision of field latrines and hand washing stations.

Table 1. Outbreaks of food poisoning from fresh produce 2004 - 2006

Causal organism	Crop	No. of cases	Country	Likely source
2006				
E. coli 0157	Spinach	175	US	Irrigation water?
4 outbreaks of Norovirus	Raspberries	43	Sweden	Chinese imports
2005				
E. coli 0157	Lettuce	120	Sweden	Stream water
Norovirus	Raspberries	500	Denmark	Polish imports
Norovirus	Raspberries	5	France	Unknown imports
S. Typhimurium DT104	Lettuce	96 & 56	UK & Finland	Spanish imports
S. Javiana & *S Braenderup*	Tomatoes	561	US & Canada	Post harvest washing
2004				
S. Thompson	Rocket	12	Norway	Italian imports
S. Newport	Iceberg lettuce	386	UK	Unknown source
Hepatitis A	Spring Onions	400	US	Poor harvesting hygiene

(e) Imports

Several of the outbreaks in table 1 are from imported produce, mainly from Europe. Outsourcing from the developing world does occur, but in the main, imported fresh produce from developing countries is grown under the control of supermarkets (or their agents) using rigorous enforcement of hygiene standards.

Issues of survival and infection

Research is ongoing on a series of issues concerning the ways in which entero-pathogens survive in manures and soils, colonise and survive on and in plants and survive cleaning processes and packaging.

(a) Protection increases survival

Two of the main physical factors controlling survival of enteropathogens in manures, soils and on plants are desiccation and UV radiation. Survival, therefore, is increased when enteropathogens are protected inside clumps of manure and soil, on the undersides of leaves, within plant surface structures, on root systems, by incorporation into phyllosphere biofilms, when inside protozoa and when internalised inside plants.

(b) Bacterial stress response

Spore-forming bacteria are well adapted to survival in the environment. This is the why they are so important as pathogens associated with herbs and spices. Gram negative enteropathogens such as *Salmonella* and *E.coli* 0157 are also well adapted to survival outside their natural animal hosts. Exposure to sub-lethal stresses triggers a cascade of overlapping genetically co-ordinated molecular responses that make the bacteria much tougher. Cross protection occurs because the overlapping stress responses enable bacteria exposed to one stress to become resistant to another. As bacteria that have passed through stationary phase are much hardier than those in log phase growth, there are implications for the measurement of survival. It should be measured on the hardier, stressed cells and not just log phase cells. There are also implications for the fresh produce industry. Enteropathogens on plant surfaces are already stressed, and stress is increased during processing and cleaning, potentially leading to very hardy bacteria geared to survival.

(c) Attachment

Bacteria attached to surfaces show different physiologies to those not attached, *E.coli*, for example, is much more resistant to chlorine when attached. Bacteria are actively involved in the attachment process. Recent studies show that the genes for motility and cell division are switched on by *L. monocytogenes* during attachment to cabbage and that the same genes required for virulence in animals are required for the colonisation of lettuce by *S. enterica*. This implies that the enteropathogens attached to salad plants are in a highly virulent state and goes some way to explaining the food-poisoning potential of fresh produce.

The degree of attachment has practical implications. Attached bacteria are difficult to wash off and it is generally accepted that approximately 10% of enteropathogens are not removed by washing. Washing can also lead to spread of bacteria. For example, if only one leaf of a head of lettuce is contaminated, the washing process transfers bacteria to all the other leaves.

(d) Biofilms

Biofilms occur naturally on field crops at levels of between 10^6 and 10^8 bacteria per cm^2. Inclusion in biofilms selects for physiologies that are more resistant to sanitation and removal by washing. For example, *S.* Newport adheres to alfalfa 10x more than normal plant-associated bacteria; *S.* Typhimurium aggregates with *Pseudomonas* on coriander and persists during dry periods; and *E. coli* O157 produces extracellular carbohydrates in response to environmental stress and can be observed as a biofilm on sprouted seeds after two days of growth.

(e) Internalisation

The presence of *E. coli* O157, *Salmonella* and *Listeria* inside plants has been demonstrated in tomato, apple, lettuce and spinach. The bacteria can enter via damaged surfaces, lenticels, during water-induced shrinkage (cooling) and washing. Bacteria inside fruit and vegetables cannot be removed and pose a special hazard. To date, it is not clear whether this is an important infection pathway, or simply an occasional occurrence.

(f) Sporadic cases

We know quite a lot about food poisoning outbreaks associated with fruit and vegetables, but little about sporadic cases. These are likely to vastly outweigh those from outbreaks.

Conclusions

The issues surrounding microbial contamination of fruit and vegetables are increasingly well recognised. The WHO (2003) reported that "there are increased outbreaks of disease epidemically associated with raw fruits and vegetables in industrialised countries due to changes in diet and increased food imports, and that in developing countries illnesses caused by fruit and vegetables are frequent and in some areas cause a large proportion of the countries' illness."

The number of recent outbreaks suggests that there is a problem with the consumption of fresh fruit and vegetables. This is not borne out by the results of microbiological surveys of fresh produce at point of sale. Nor is the commonly held view that organic produce is more contaminated than conventionally grown produce. There are several areas of concern, for example, the microbial quality of irrigation water; contamination by wild birds; microbial survival strategies; role of biofilms; internalisation of enteropathogens within plants; worker hygiene; imports; and the number of sporadic case, many of which will be addressed at the S*f*AM summer conference in Cardiff in 2007 (see page 26).

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Fermented fish anyone?

Martin Adams explores the microbiology of fermented fish products

THOUGH nutritionists enjoin us to eat more, many consumers recoil from fish unless it first assumes some form of bread-crumbed anonymity. This is unfortunate, as the unique perishability of fresh fish has meant that its preservation has long exercised human ingenuity and this has led to a huge diversity of interesting fish products. These employ the full range of traditional and modern food processing technologies, including fermentation.

One group of fermented fish products tends to be associated with colder latitudes. Fermented seal flipper, fish roe and narwhal blubber are popular among the Inuit people of the Arctic and they receive occasional wider notoriety when they are associated with cases of botulism caused by *Clostridium botulinum* type E. In Scandinavia, the fermented trout *rakefisk* of Norway and fermented herring, *surstromming*, in Sweden are widely regarded as delicacies, if something of an acquired taste. Fermented fish products are however most abundant and popular in East and Southeast Asia where the term 'fermented' encompasses products ranging from autolysed fish proteins to those in which the activity of lactic acid bacteria plays a significant role.

The common factor in all East and Southeast Asian fermented fish products is the inclusion of salt to reduce the product's water activity. The level of salt used can vary dramatically. Some fermented fish products such as the fish sauces and pastes are produced with very high levels of salt, often corresponding to saturation of the aqueous phase and a water activity (a_w) below 0.75 (Table 1, Figure 1). Whole, ungutted fish are generally mixed with dry salt and left in tanks to ferment at ambient temperature for periods of up to a year, during which time they are transformed into a liquid rich in amino acids and peptides (Figure 2 & 3). The fish sauces and pastes play a similar role to soy sauce and miso in the cuisine of many countries such as Thailand and as a result are probably the fermented fish product most familiar to a wider audience.

The low a_w and the anaerobic conditions prevailing during production, are sufficient to arrest the growth of all micro-organisms with the exception of the extreme halophiles. As a result the transformation that occurs during the production of fish sauces and pastes is thought to be largely non-

Table 1. Fish sauces and pastes of south-east Asia

Country	Sauce Amber/brown liquid, salty taste, cheese-like aroma	Paste Red/brown salty paste
Burma	*ngapi*	*nga-ngapi*
Indonesia	*ketjap-ikan*	*trassi-ikan; trassi-udang*
Cambodia	*nuoc-mam, nuoc-mam-gau-ca (livers only)*	*prahoc* *mam-ruoc* (shrimps)
Korea		*myulchljeot*
Laos	*nam-pla*	*padec*
Malaysia	*budu*	*belachan* (shrimps)
Philippines	*patis*	*bagoong*
Thailand	*nam-pla*	*kapi*
Vietnam	*nuoc-mam*	*mam-ca; man-tom* (shrimps)

microbiological and mediated by endogenous fish enzymes. It has been shown that acceptable products were still produced when microbial activity in the fish was eliminated by the use of antibiotics or irradiation but there is also some evidence that *Halobacterium* and *Halococcus* species such as *Halobacterium salinarium* multiply to high numbers (>10^8/ml) during the first three weeks of fermentation and that these may contribute both to the development of flavour and proteolysis.

Fish generally contain very little carbohydrate. So for a true microbial fermentation to occur, comparable to the lactic acid fermentations in milk, meat and vegetables, some exogenous source of carbohydrate must be added. This was used as the basis of a simple classification scheme which divided fermented fish products into fish/salt products such as the fish sauces and pastes and fish/salt/carbohydrate products (Table 2). The production of the fish/salt/carbohydrate products appears to be governed by two empirical rules which have a sound microbiological basis:

1. The use of higher salt levels results in a longer production phase but a better keeping quality product.

Initial salting of the raw material is crucial to inhibit the growth of the normal spoilage flora associated with the product and allow a dominant population of the lactic acid bacteria to develop and exert its antimicrobial effect. Much attention has been focused in recent years on novel antimicrobials produced by lactic acid bacteria such as the bacteriocins, and this has tended to detract from the fact that the principal antimicrobial factor produced by lactic acid bacteria is lactic acid. Its production is an inevitable consequence of the growth of lactic acid bacteria (LAB) on carbohydrate and explains why such a diversity of LAB produce a broadly similar effect – preservation - in so many products.

LAB differ quite widely in their relative salt tolerance, and it is a common observation in food fermentations that the salt level used markedly affects the lactic flora that develops. Clearly, increased salt levels will delay acid production and inhibit a number of lactic acid bacteria that might otherwise be active. This has been noted in model systems and in the production of the Thai product *pla-som* where the rate of pH decrease slows as the salt content increases from 6% to 9% and remains unchanged for 12 days when the salt level is 11%.

It is also apparent from an analysis of published data on the shelf life and composition of salt/carbohydrate products that salt content (reduced a_w) is the most important of the two major preservative hurdles operating (Table 3). Although the figures are very approximate, the mean salt level correlates far better with mean shelf life (r^2 0.85) than does the mean pH (r^2 0.12). This is rather similar to cheeses where the moisture content has a more pronounced impact on shelf life than acidity.

Increasing levels of salt in fish/salt/carbohydrate fermented fish also means that autolytic processes assume greater importance in terms of the product's sensory characteristics, so we have a balance between proteolysis and sourness which can be set at any required level by choice of an appropriate level of salt.

2. Inclusion of more carbohydrate produces a faster fermentation and a stronger acid taste.

Rice is the most common carbohydrate-containing ingredient in these products and it was widely assumed to be the principal source of fermentable sugar for the lactic acid bacteria present. This is almost certainly true in those products where the rice is added along with a

Figure 1. *Fish sauce and paste*

Table 2. Fish/salt/carbohydrate products of south-east Asia

Country	Product
Japan	*I-sushi, e.g., ayu-sushi, fana-sushi, tai-sushi*
Cambodia	*phaak, mam-chao, mam-seeing*
Korea	*sikhae*
Laos	*som-kay-pe-eun, som-pa, mam-pa-kor, pa-chao, pa-khem, som-pa-keng*
Malaysia	*peksam, cencalok*
Philippines	*burong-isda, e.g., burong-ayungi, burong-dalang, burong-bangus*
Thailand	*pla-ra, pla-som, pla-chao, som-fak*

Figure 2 & 3. *Production of the Thai fish sauce nam-pla*

Figure 4. *White burong-isda in traditiona banana leaf wrapping in the Philippines (Photo A.Reilly)*

Table 3. Salt and pH ranges of fermented fish/salt/carbohydrate products from Thailand

Product	pH	Salt (%w/w)	Shelf life
Pla-som	4.0 - 4.6	2.3 - 5.9	3 weeks
Som-fak	4.1 - 5.0	2.5 - 5.8	2 weeks
Pla-chom	5.0 - 6.1	3.8 - 4.8	2 weeks
Pla-chao	4.0 - 5.3	4.4 - 9.5	1 - 2 years
Pla-paeng-daeng	3.9 - 5.2	4.5 - 9.2	6 - 12 months
Pla-ra	4.7 - 6.2	7.8 - 17.9	1 - 3 years

Data from: Concise Handbook of Indigenous Fermented Foods in the Asca Countries 1986 Saono, S., Hull, R.R. and Dhamcharee, B. (eds)

Figure 5. *Red burong-isda (Photo A.Reilly)*

traditional saccharifying starter, such as *look-pang* or *koji*, which contains fungal amylolytic enzymes capable of degrading the starch to sugars readily fermentable by the lactic acid bacteria. The role such starters play in stimulating lactic fermentation can be seen in data relating to *burong-isda*, a product from the Philippines. *Burong-isda* is produced in two varieties, one containing the traditional red starter *ang-kak* which contains the mould *Monascus purpureus* and a white variety, which does not employ a starter (Figures 4 & 5). A survey showed that the red variety had a consistently lower pH (3.0-3.9) compared with the white variety (4.1-4.5), although both had similar levels of lactic acid bacteria.

In the absence of a saccharifying starter culture, the fermentation must rely on the endogenous enzymic activity from the substrates or their microflora for any starch degradation that occurs. Starch utilisation is not a common characteristic among the lactic acid bacteria, although amylolytic lactic acid bacteria have been reported, including some isolated from fermented fish. In one study only four out of forty four lactic acid bacterial strains isolated from Thai fermented fish products were capable of utilising the complex carbohydrates in rice, potatoes or maize starch. One of these strains, when tested, was unable to produce a pH decrease in a laboratory fish/salt/rice mixture suggesting that in the fermentation it served primarily to release sugars for other, more active, lactic acid bacteria to ferment.

Garlic is often used in significant quantities in fermented fish products such as the Thai product, *som-fak*. Until relatively recently its role was thought to be mainly that of a flavouring agent with perhaps some additional beneficial effects from the antimicrobial thiosulfinate, allicin, produced during the crushing or chopping of garlic. It has now been shown that the carbohydrate content of garlic, which can comprise high levels of fructans, plays a key role in the fermentation of some fish products. Garlic fermenting lactic acid bacteria have been isolated from both the ingredients and fermenting *som-fak* and have included both homo- and heterofermentative species. When garlic was omitted from a *som-fak* fermentation, the pH did not drop below 5.0 and the product rapidly spoiled, whereas the control achieved a pH below 4.5 within two days of fermentation.

Many fermented fish products are consumed without cooking, so in these cases product safety will depend on the quality of the raw materials used, the hygienic conditions of processing, the temperature and the level of salting and the degree of acidification achieved. The association of some Northern fermented fish products with botulism has already been mentioned and in Japan *I-sushi*, a fish/salt/carbohydrate product, has been the identified cause of botulism outbreaks since it was first reported in the early 1950s. Outbreaks have generally occurred in Japan's northern prefectures when home fermentations fail to produce the pH reduction (<4.6) necessary to control *C. botulinum* growth. Greater awareness of this, or perhaps a decline in home production, has led to fewer reported outbreaks in recent years.

Data are scarce on other forms of bacterial foodborne illness transmitted by fermented fish. Traditionally tropical fermented fish products are stored at ambient temperatures, which can be 30°C or more, so at lower salt levels the potential for growth of bacterial pathogens will be critically dependent on the pH. Generally if a pH below 4.5 is achieved rapidly then growth will be inhibited by the combined hurdles of salt and low pH. Survival of pathogens with a low infectious dose may however still be a problem. Many SE Asian fermented products use freshwater fish and the potential for them to transmit human parasitic infections has been recognised. Thorough cooking before consumption would virtually eliminate risk, although where this requires a change in food culture it may be difficult to introduce.

Admittedly it's hard to imagine some of the more exotic fermented fish products finding wider markets, but in view of the success of fish sauce and the constant imperative of the food industry to diversify, who knows?

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Stat Note 7

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

Chi-square contingency tables

PREVIOUS Statnotes describing the application of statistical methods to microbiological problems have been applied to measurement data (Hilton & Armstrong, 2005 a,b).

Measurement data are expressed in units; they are continuous variables and, in many cases, fulfil the requirements of the normal distribution (Hilton & Armstrong, 2005a). In some studies, however, the data are not measurements but comprise counts or frequencies of particular events. Such data are often analysed using the chi-square (χ^2) distribution. An example of the use of this statistic to test whether an observed distribution of frequencies came from a normal distribution ('goodness of fit test') was described in Statnote 1 (Hilton & Armstrong, 2005c). The objective of this Statnote is to extend these methods to the analysis of two different variables.

The Scenario

Methicillin-resistant *Staphlococcus aureus* (MRSA) is a significant cause of nosocomial and community morbidity and mortality that, over the past two decades, has become a worldwide problem exacerbated by the emergence of multidrug-resistant isolates. Such isolates demonstrate a reduced susceptibility to almost all clinically available antibiotics. It is generally accepted that sub-lethal exposure of bacteria to antibiotics can promote the rapid development of resistance and that this situation may be more likely to occur in a hospital setting than in the community. It might be hypothesised, therefore, that isolates of MRSA from a hospital (HA-MRSA) would demonstrate an enhanced resistance profile to antibiotics compared to MRSA isolated from the community (CA-MRSA).

To test this hypothesis, 197 isolates of MRSA consisting of 95 HA-MRSA and 102 CA-MRSA were isolated from soft tissue infections and screened for their sensitivity to a panel of 10 antibiotics using the British Society for Antimicrobial Chemotherapy (BSAC) disc diffusion method. Isolates were designated as resistant (R) or sensitive (S). If the hospital is providing an environment which promotes the development of antibiotic resistance then it might be expected that HA-MRSA would demonstrate a greater than average spectrum of resistance (i.e. ≥ 5 antibiotics of the 10 screened) than those isolated from the community. The potential significance of the association between antibiotic sensitivity and location of an isolate can be investigated using the chi-square (χ^2) test.

How is the test carried out?

First, the data are tabulated in the form of a 2 x 2 contingency table (Table 1). In Table 1, 44% of the HA-MRSA isolates were resistant to ≥ 5 antibiotics as against 4.9% of the CA-MRSA isolates. Is this difference sufficient to conclude that there is an association between the antibiotic sensitivity profile of the isolate and its location? Second, the expected frequencies are calculated for each cell of the 2 x 2 table and subtracted from the observed frequencies. Chi-square is the sum of the squares of these deviations divided by the appropriate expected frequency. The value of χ^2 is taken to the χ^2 table for 1 degree of freedom (DF) to obtain the probability that the value of the statistic would occur by chance if there were no differences between the isolates.

Interpretation of the results

The calculated value of χ^2 ($\chi^2 = 41.84$) is considerably greater than the value tabulated at the 5% level of probability. This is a value that would occur rarely by chance, in fact less than 1 in a 1000, and hence, we conclude that there is an association

Table 1. Is there an association between Hospital-Acquired and Community-Acquired MRSA antibiotic sensitivities (N >20)?

2 x 2 contingency table			
MRSA Isolate	**Resistant to ≥ 5 Antibiotics**	**Resistant to ≥ 5 Antibiotics**	**Total**
HA-MRSA	42	53	95
CA-MRSA	5	97	102
Total	47	150	197 = Grand tot.

1. The expected frequency (EF) in each cell is calculated as (Row Total x Column Total)/ Grand Total
2. Hence, the expected frequency of HA-MRSA isolates resistant to ≥ 5 antibiotics is (95 x 47)/197 = 22.66. This calculation is repeated for each of the four cells of the table.
3. Calculate $\chi^2 = \Sigma (O_F - E_F)^2/E_F$. In this cases $\chi^2 = 41.84$ (39.70 with Yate's correction) ($P < 0.001$) with 1DF.

between the antibiotic sensitivity profile of an isolate and its location. Caution is necessary when interpreting the results of χ^2 tests in observational studies (Snedecor & Cochran, 1980). There may be many factors that vary between a hospital and community setting that could influence the antibiotic resistance profile of a MRSA strain, some of which may be wholly or partly responsible for an observed significant difference.

To understand why a 2 x 2 table has only 1 DF, examine the deviations of the observed from the expected frequencies for each cell of the table. Examination of these deviations will show that they are all the same apart from their sign, i.e., in a 2 x 2 table there is only a single *independent* estimate of the deviation of the observed from the expected frequency. Another statistic that is sometimes given by statistical software is called 'phi-square' and is a measure of the degree of correlation between the two variables in a 2 x 2 table.

Yate's correction

Statistical software usually includes the option of calculating χ^2 with Yate's correction. This correction improves the estimate of χ^2 in a 2 x 2 table when expected frequencies are small (e.g., when expected frequencies < 10). The absolute value of the difference between the observed and expected frequencies is reduced by 0.5 before squaring. The effect of this is to make the estimate of χ^2 slightly more conservative when the table contains small frequencies. Yate's correction applied to the above example gives a value of $\chi^2 = 39.70$.

Table 2. Is there an association between Hospital-Acquired and Community-Acquired MRSA antibiotic sensitivities (N < 20)?

2 x 2 contingency table			
MRSA Isolate	**Resistant to ≥ 5 Antibiotics**	**Resistant to ≥ 5 Antibiotics**	**Total**
HA-MRSA	A	B	(A + B)
CA-MRSA	C	D	(C + D)
Total	(A + C)	(B + D)	Total (T) = N

1. If T < 12, calculate the probability (P) of this particular outcome among all possible outcomes with the same row and column totals:
i.e., P = A! x B! x C! x D!/ CA! x CB! x DA! x DB! x T!
2. If T is larger than 12, then a calculation based on logarithms can be used (see Fisher and Yates Table XXX, Dawkins 1975).

R x C contingency tables

It is possible to analyse two variables with a greater number of categories per variable and this is termed a rows (R) x columns (C) contingency table. For example, antibiotic resistance may have been tested for several different strains simultaneously. To make the test, the expected frequency is calculated for each cell of the table as in Table 1. The value of χ^2 is then calculated using the usual formula and the value of χ^2 compared with the χ^2 distribution, entering the table for (Number of rows – 1)(Number of columns – 1) DF (Snedecor & Cochran, 1980). If a significant χ^2 is obtained, the R x C table may need to be broken down into smaller tables to compare some of the isolates in more detail.

Fisher's 2 x 2 'Exact Test'

The χ^2 test described above is only an approximate test when applied to a 2 x 2 table and the approximation becomes poorer as sample size decreases. Hence, the test is inaccurate when the expected frequencies are low and it is usually considered inappropriate if the expected values fall below 5. One remedy is to apply Yate's correction as described above. An alternative to χ^2, called Fisher's 2 x 2 exact test, can be used, however, and is illustrated in Table 2. This test should be applied if the total sample size is less than 20 or if N lies between 20 and 40 and the smallest expected frequency is less than 5. When the total of the observations is small, say less than 12, the probability of a particular distribution of values in a 2 x 2 table being obtained, given the particular row and column totals, can be calculated directly from the data. If the total is larger than 12, then a more complex calculation can be made using logarithms (Dawkins, 1975).

Conclusions

When the data are counts or the frequencies of particular events and can be expressed as a contingency table, then they can be analysed using the χ^2 distribution. When applied to a 2 x 2 table, the test is approximate and care needs to be taken in analysing tables when the expected frequencies are small either by applying Yate's correction or by using Fisher's exact test. Larger contingency tables can also be analysed using this method. Note that it is a serious statistical error to use any of these tests on measurement data!

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MISAC Competition 2007

This year's topic was chosen because of the serious problems caused in hospitals by MRSA (methicillin resistant *Staphylococcus aureus*) and their extensive coverage by the media. Therefore, it was no surprise that the competition to write a newspaper feature on an hospital outbreak of MRSA proved to be a very popular one. It generated nearly 700 entries and involved more than 800 students from 105 schools and colleges drawn from England, Wales, Scotland and Northern Ireland.

Officers of SGM, the competition sponsor, joined the Chairman and other members of MISAC for the judging at SGM headquarters in Reading. This year the judging panel was particularly fortunate to benefit from the expertise of Alexandra Blair, Education Correspondent on *The Times* newspaper.

The judges looked particularly for attention to the guidance given to entrants on the writing of a news story including preparing the headline to catch the reader's attention, structuring the story to maintain interest while conveying essential information, and the appropriate use of pictures, diagrams and scientific terms. Other important features were evidence of scientific merit, the use of entrants' own words, and an appreciation of the importance bringing out the local interest. Many entries impressed the judges with their high quality, but a notable proportion were excluded from consideration because they did not adhere to the rules of the competition, particularly in using the format of an information factsheet instead of that of a newspaper article.

GCSE:Winner
Emma Pascall, Durham High School

GCSE 2nd place
Luke Hopper
St Anselms College, Birkenhead

Key Stage 3: Winner
Eleanor Tayler
The Abbey School, Reading

Key Stage 3: 2nd place
James McCarten
The London Oratory School

Key Stage 3: 3rd place
Jamie Wilson & **William Yeong**
The Grange School Hartford Ltd, Northwich

MISAC and the Society for General Microbiology express their sincere thanks to everyone who took part, including those whose efforts were not rewarded with a prize on this occasion and the teachers who organised the preparation and submission of the entries. We hope that this has been a beneficial experience, was enjoyable and has led to a greater interest in microbiology. Next year's competition, which is sponsored by SfAM, will concentrate on *Salmonella*. Details are now available on the MISAC website http://www.microbiologyonline.org.uk/misacomp.htm.

The winning entry from Emma Pascall

Luke Hopper's winning entry

James McCarten's winning entry

Am I eligible - can I apply?

Grants can be made available to ANY FULL member who is able to offer a suitable undergraduate student a work placement for a period of up to 10 weeks during summer. The grant is £160 per week for the student for a maximum of 10 weeks and up to £50 per week for lab costs for a maximum of 10 weeks. To apply, visit **www.sfam.org.uk/members/prizes.php**

GUIDELINES

1. Any full member of the Society who can offer an undergraduate student, or a recent graduate (within 6 months of graduation) a work placement is eligible to apply for this grant. The placement can last up to a maximum of 10 weeks, normally during the summer vacation.

2. The Grant will normally provide support at the rate of £160 per week for the student and up to £50 per week for lab costs. The monies will usually be paid to the Department in which the student/graduate works unless a specific request is made for an alternative method of payment.

3. Applications should be made by the supervisor using the PDF form provided on the website or the paper form obtainable from the Society Office.

4. Successful applicants and their students/graduate must write a report on the placement within 4 weeks of completing their placement which will be published in *Microbiologist*. Photographs of the applicant and/or the work done during the placement are desirable. These should be supplied as (a) digital images at a size of not less than 4 inches square at a resolution of not less than 300 pixels per inch, or (b) original photographic prints which will be scanned and promptly returned.

5. Normally a member may not apply for a further grant until a period of two years has elapsed.

6.There is no closing date for this Grant and applications can be made any time during the year. Applicants must apply at least 6 weeks before the proposed start date.

www.sfam.org.uk/members/prizes.php

Inhibitory effects of citrus essential oils

Katie Fisher reports on her project

I I WAS NEARING THE completion of my three-year BSc. Biology (Hons) degree at the University of Northampton and during my final year project I investigated the inhibitory effects of citrus essential oils (EOs) on two bacteria.

The results proved to be very interesting, especially as little research had been carried out in this area previously. I had a desire to study this topic in more depth so when the opportunity arose to carry on with the research for a further 10 week period, as funded by the Students into Work grant, I was delighted to undertake the project.

Citrus EOs have potential bactericidal properties not only against yeast, moulds and spore bearing bacteria but food-poisoning bacteria as well (Deans & Ritchie, 1987). Limited research has been carried out on this group of oils. Recent investigations have shown that limonene is one of the major components of citrus oils and ranges from 88% to 95% of the oil. Levels in bergamot are lower, ranging from 32-45%. Citral has also been highlighted as a compound in citrus fruits which is active against decay caused by *Penicillium digitatum* (Caccioni *et al.*, 1998). A factor that must be considered when looking at essential oils is that they are made of volatile components; therefore their use as an antimicrobial has a limited life span, as they evaporate rapidly.

The aims of the research were:

- to assess the effect of lemon, orange (sweet) and bergamot essential oils against a variety of Gram-postive (*Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli* 0157, *Campylobacter jejuni* and *Arcobacter butzeri*) food borne pathogens, using a disc diffusion method,
- to determine the minimum inhibition concentrations (MIC) of the oils against each of the bacteria using an agar dilution method and
- to assess the effectiveness of the main components of the oils including limonene and citral.

The results gained from the *in vitro* experiments were then used to assess the effectiveness of the EOs when applied to food. During the project the research widened to evaluate the effect of the vapours of the EOs *in vitro* and on food. This project has highlighted the ways in which research can develop and expand during the life of an investigation, as the study of EO vapours was an avenue that I had not considered until I began to carry out experiments and analyse the results.

The inhibitory effects of citrus oils were greatest against Gram-positive bacteria with bergamot, citral and linalool being the most effective. The vapours of the oils showed an inhibitory effect on food after 7 to 12 hour exposures. Further investigation would have to be carried out to establish how essential oils affect the organoleptic properties of the foodstuff.

This project has allowed me to develop my laboratory and investigatory skills

further and allowed me to try new techniques, which lead to me to devise my own methods when testing vapours. It has also illustrated the importance of exploring new possibilities and keeping an open mind when investigating. The other main area of development for me during this project is that of my journal writing skills, of which I had very little experience previously. This work has given me insight into what a career in research would be like. It has given me a hunger for investigative work and has influenced my future career as I am now studying for a PhD — an option that I had not contemplated before. I would like to thank *Sf*AM for funding this project and allowing me to develop my skills and

broaden my horizons. I would also like to thank Professor Carol Phillips for her support throughout this project.

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Detection of biocide resistance genes in animal associated staphylococci

Claire Wright reports on her project

THE STDENTS INTO WORK programme came at an opportune time for me as I am in between my second and final year of a Biomedical Sciences (Hons) degree at University of Bradford.

I completed a ten week project during the summer. The placement was invaluable to me as I have had the opportunity develop valuable laboratory and research skills in advance of my final year, as well as gain confidence working in a research environment.

The Division of Biomedical Sciences teaches a broad range of biology based subject, but I am most interested in Microbiology and particularly antimicrobial resistance. The project for which I received funding was with Dr Jeremy Ross and was to look at antimicrobial resistance in clinical veterinary isolates, of *Staphylococcus aureus* and its close relative *Staphylococcus intermedius*. *S. aureus*, is a well documented human pathogen, which is commonly found residing as a commensal organism in the nasal passages. Infections can result in conditions such as impetigo, abscesses, and toxic shock syndrome, among many other conditions. *S. intermedius* is a coagulase positive, close relative of *S. aureus*, frequently isolated from canines and other animals as part of their normal flora. The organism is an opportunistic pathogen of animals causing conditions such as pyoderma, abcesses and conjunctivitis. In some cases *S. intermedius* has been shown to be pathogenic to humans causing infections of canine inflicted wounds and opportunistic infections of immuno-compromised patients. The organism is regarded as a zoonotic pathogen.

There is a lot of concern over the overuse of antibiotics in human medicine, and biocides in domestic consumer products, but the same compounds are used in veterinary medicine and pet animals are increasingly exposed to antibacterial products present in numerous pet care products from shampoos to dog blankets. The idea of transfer of bacterial strains between pet animals and humans has been raised and it has been suggested that pet animals may harbour a reservoir of antibacterial resistant bacteria that may transmit resistance genes to human pathogens. The reverse is also true and human associated bacterial strains and resistance genes may transfer to animals. Already the human associated pathogen, MRSA has been transferred to the veterinary world and is causing infections in animals.

My project involved investigating antibiotic and biocide resistance in clinical *S. intermedius* and *S. aureus* strains isolated from pet animals by a local veterinary diagnostic laboratory (IDEXX, Wetherby). These strains included a number of MRSA isolates. In particular we were interested in strains which showed resistance to biocides such as cetrimide, chlorhexidene, and benzylkonium chloride as these are disinfectants used in both veterinary and human facilities. We also looked for resistance to triclosan, although we did not discover any resistant strains.

The techniques that I used on this placement were numerous and a source of many new skills for me. After using breakpoint concentrations of biocides to screen the isolates I determined the Minimum Inhibitory Concentrations (MICs) of each compound to those strains exceeding the breakpoint by the agar dilution method, using NCCLS guidelines.

Resistance to several disinfectants in staphylococci, including cetrimide and benzylkonium choride, is encoded by qac genes, (***q***uaternary ***a***mmonium ***c***ompounds), which also give resistance to the dye ethidium bromide. These genes are efflux pumps which sit in the bacterial membrane and remove the compounds from the cells. The genes can be found on plasmids or in the chromosome. I used oligonucleotide primers specific for *qac* A/B, C (also known as smr), G, H and J to look for the presence of these genes in the veterinary staphylococci identified as resistant to biocides. To do this I used the polymerase chain reaction. In order to look for the *qac* genes I had to extract chromosomal and plasmid DNA, which could then be used in a PCR reaction to amplify and identify the gene should it be present in the isolate, the amplified products were visualised using gel electrophoresis.

I was able to find both *qac*A/B and *qac*G genes present in the staphylococcal isolates, in one case present in an MRSA isolate and identified several small plasmids which appear to carry *qac* genes from *S. intermedius*. I was also involved in investigating the mechanism of resistance to the antibiotic erythromycin in these animal staphylococci using PCR, so I was extremely busy.

I would like to thank not only SfAM for funding this placement, but also Dr Jeremy Ross for allowing me this opportunity in the first place and not throwing me out of the lab during my numerous catastrophes. I would also like to thank Mick Rich at IDEXX Veterinary Laboratories, Wetherby, for supplying the clinical isolates. The placement has given me the opportunity to develop confidence in a research environment, which is completely different from undergraduate practicals. This has encouraged me to apply for PhD projects once I complete my degree course.

Claire Wright
University of Bradford

Differential efficacy of biocides on MRSA and MSSA

Michelle Finnegan reports

AFTER GRADUATING FROM university with a degree in Biochemistry, I chose to do a PhD in Pharmaceutical microbiology at Cardiff University and although I had taken many elective modules in microbiological science, I was still under-confident in my ability to undertake such a large project.

To remedy this, my supervisor applied for the SfAM grant so I could obtain experience in pure microbiology during the summer before registering as a postgraduate student. As the PhD project I undertook involved the investigation of bacterial reactions to Biocides, a study of methicillin-resistant *S. aureus* (MRSA) resistance to common antiseptics was a suitable foundation.

The aim of my project was to investigate the susceptibility of MRSA to four different biocides, at various concentrations, over time, in order to establish the inactivation kinetics of a MRSA and Methicillin-susceptible *S. aureus* (MSSA). Two strains of MRSA were used, MRSA I57 collected from the chronic venous leg ulcers of Heath hospital patients, and the MRSA E72 Heath hospital recommended control strain. In addition to this two MSSA strains were tested, MSSA D76, collected from the chronic venous leg ulcers of Heath hospital patients, and MSSA (NCTC) 6571 control strain recommended by the British Society of Antimicrobial Chemotherapy.

The non-control strains of MRSA and MSSA (I57 & D76 respectively) used in this experiment were collected from the chronic venous leg ulcers (CVLUs) of Heath hospital patients. CVLU's occur as a consequence of incompetent valves, which cause venous hypertension in the lower legs where it is thought that leucocytes get trapped in the capillaries and block them. The increased pressure causes fibrinogen to leak through the capillaries and be deposited in the extracellular matrix, causing hypoxia of the surrounding tissues.

The chronic nature of many venous leg ulcers makes them more prone to infection which can occur with the disruption of skin integrity, patient immunocompromisation, or antibiotic resistance. This allows entry to opportunistic pathogens such as *Staphylococcus aureus*, various strains of which are estimated to be present in 88% of non-infected leg ulcers. Although it is uncertain whether a correlation exists between specific species of bacteria and non-healing wounds, the load of bacteria in a wound is fundamental to its healing outcome. Non-healing wounds are associated with a load of more than 10^5 bacteria per gram of tissue.

Disinfectants are an integral part of the control policies designed to eradicate and prevent the spread of MRSA. Concern has arisen regarding the potential of co-resistance to antibiotics and antiseptics, as this could lead to a situation whereby antibiotic resistant strains are selected for by the use of disinfectants.

Bacterial response to biocides is determined essentially by the nature of the chemical agent and the type of organism involved. Factors such as temperature of contact, environmental pH and the presence of organic matter can also exert a considerable effect on the activity of an antibacterial agent as well as the concentration and mode of action of the biocide itself. Gram-positive MRSA becomes less susceptible to the toxicity of biocides by a process called 'active efflux'. These *S. aureus* efflux genes are plasmid-encoded, for example *qac*A/B gene encodes for an efflux determinant found in several strains of *S. aureus* and gives resistance to intercalating agents, and quaternary ammonium compounds.

The common disinfectants chosen to test the inactivation kinetics of MRSAs' susceptibility to different biocides were potassium permanganate, chlorhexidine, silver nitrate and povidine iodine. The inactivation kinetics were determined using suspension tests, which were performed to assess the bactericidal efficacy of each biocide. The bactericidal activities of the disinfectants were expressed as logarithmic reductions in viable organisms and were calculated by: RF(reduction factor) = Log CFU (control) - Log CFU (treated). These log reductions were plotted as a function of time using Statview and statistical comparisons were performed using simple ANOVA followed by Fisher's *post-hoc* test.

Although time was too limited to allow the five repeats of each suspension test which would allow any statistical significance in log reduction of cfu/ml to be viable, the results obtained in this set of three repeats gave valuable insight into the efficacy of these common biocides.

The results obtained confirmed that both silver nitrate and povidine iodine are effective enough to meet the requirements of the 'european suspension Test EN 1276' at their in-use concentration, and display enough efficacy to be of use in situations where patients' sensitivity will only allow lower doses to be applied. The effect of Chlorhexidine 0.2% w/v was gradual but powerful with a 5-log reduction achieved after a contact time of 30 minutes for MRSA and MSSA strains, as such it is an effective agent for use against resistant *S aureus* strains.

The in-use concentration of Potassium permanganate 0.01% w/v was found to be ineffective against both MRSA and MSSA strains, but with markedly lower bactericidal ability on MRSA. However a concentration of 0.05% w/v of potassium permanganate was found to be sufficient to cause a 5-log reduction in the cfu/ml of all four MRSA and MSSA strains, making it quite an effective biocide.

The six weeks I spent working on this project were both challenging and rewarding, I gained further experience in aseptic techniques, working with containment level 2+ microbes, general culture techniques and experimental planning.

I would like to thank my supervisor Dr. Jean-Yves Maillard at Cardiff University and SfAM for affording me this opportunity to contribute to such an important area of research.

Michelle Finnigan
Cardiff University

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4. Completed applications must include an abstract of any intended contribution to be made at the meeting and must be received by the Society Office not less than six weeks before the date of the event.

5. Student member applications must be supported by their supervisor and include the contact telephone number(s) and email address(es) of the supervisor or head of department who is supporting their application.

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9. The award of this grant is at the sole discretion of the Hon President of the Society.

10. The applicant must write a short article of between 400 - 600 words within 4 weeks of the meeting, the content of which will be agreed with the Editor of sfam *Microbiologist* and will be published in the magazine. Photographs of the applicant and/or the subject of the article are desirable. These should be supplied as (a) digital files in TIFF or JPEG format at a size of not less than 4 inches square at a resolution of not less than 300 pixels per inch, or (b) original photographic prints which will be scanned and promptly returned to the applicant.

Adaptive evolution in *Erwinia carotovora*

ERWINIA CAROTOVORA subsp. *carotovora* (Ecc) is a Gram-ve member of the Enterobacteriaceae and a phytopathogen. *Ecc* causes soft rot in potato and is an economic problem for farmers (Tournas, V. H., 2005).

One of the most important virulence factors of Ecc are the many secreted **p**lant **c**ell **w**all **d**egrading **e**xoenzymes (PCWDEs) (Herron, S.R. *et al.*, 2000) this bacteria produces. These damage the stability of the plant cell wall which subsequently leads to cell lysis. In order to protect the nutrients released during plant cell lysis from competetors, *Ecc* strain ATCC 39048, produces a β-lactam antibiotic, 1-carbapen-2-em-3-carboxylic acid (Car) (Coulthurst, S. J. *et al.*, 2005), that is thought to kill other bacteria. Car production in Ecc is encoded for by the *carABCDEFGH* gene cluster. The first five genes, *carABCDE*, are involved in Car biosynthesis. Car auto-resistance is encoded for by *carFG*. The last gene, *carH*, has no known function. Carbapenem antibiotics are important in treating clinical infections because they are resistant to most classes of β-lactamase that are responsible for β-lactam antibiotic resistance. Chemically synthesised carbapenems such as imipenem are used in hospitals, but it is hoped that by learning about Car production in *Ecc*, Car could be more effectively mass produced with possible benefits in antibiotic production.

Previously, regulators of Car production have been identified, one of which is the carIR quorum sensing system, which activates Car production at high cell densities. However, our group has never identified any repressors of Car production.

In an effort to isolate mutants that were up-regulated in production of Car, I employed a positive selection strategy. A β-lactamase translational fusion was cloned into *carH* that encodes for a periplasmic protein. The CarHϕBlaM protein chimera enabled *Ecc* to grow in the presence of low concentrations of ampicillin (Ap). By growing the strain in the presence of high concentrations of Ap it was possible to isolate mutants with increased Ap resistance. Surprisingly, these mutants possessed IS*10* insertion sequences (Kleckner, N. *et al.*, 1996) immediately upstream of the *carH::blaM* reporter construct. It appears that expression of CarHϕBlaM was increased because of the powerful P_{out} promoter from IS*10* enabling transcription of *carH::blaM* to be increased.

*IS*10 was introduced to *Ecc* strain ATCC 39048 when we first started working on Car production. The transposon Tn*10* (which contains IS*10*) was introduced to generate a restrictionless derivative of ATCC 39048, to aid in genetic manipulation of this strain. In order to enable Tetracycline to be used as a marker for subsequent experiments, the restrictionless derivative of ATCC 39048 was exposed to fusaric acid to select for a Tetracyline sensitive spontaneous mutant which was designated ATTn10. *Ecc* strain ATTn10 has been used in our lab for many years as a model organism in which to study quorum sensing, Car production and pathogenesis, without any knowledge that remnants of Tn*10* remained in the genome. This study demonstrated the potential pitfalls in using transposons and should hopefully serve as a word of caution to others.

An award from the President's Fund, which allowed the above work to be presented at the thrice rebuilt XII International Congress on Molecular Plant-Microbe Interactions, is gratefully acknowledged.

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Steven Bowden

The adjuvant properties of *Bordetella pertussis* adenylate cyclase toxin

BORDETELLA PERTUSSIS IS A Gram-negative bacterium that causes whooping cough in humans and this may be especially severe in young infants.

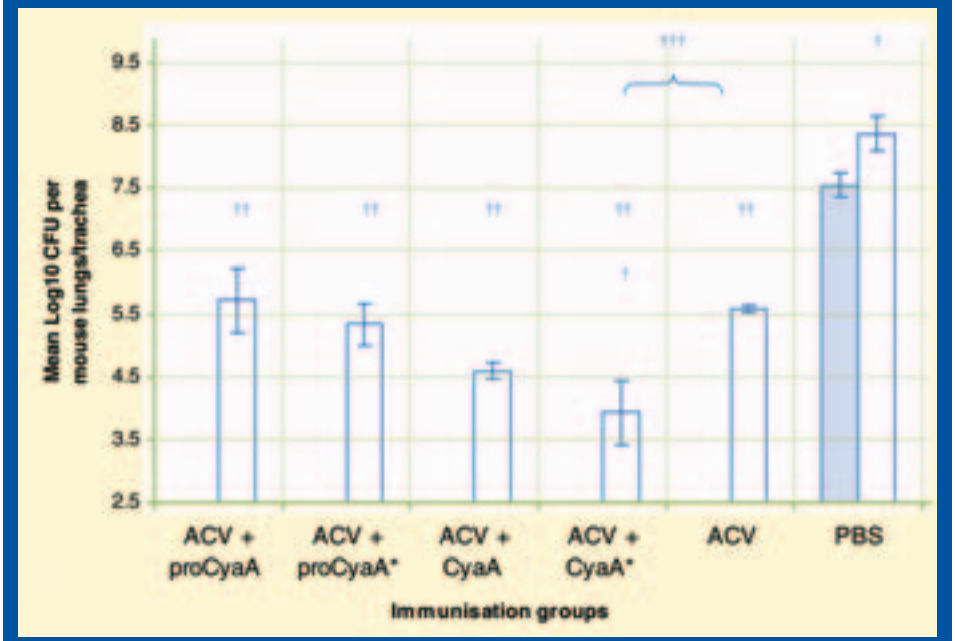
Several virulence-associated factors have been implicated in the disease process including toxins such as lipopolysaccharide (LPS), pertussis toxin (PT) and the adenylate cyclase toxin (CyaA) and adhesins such as filamentous haemagglutinin (FHA), pertactin (PRN) and fimbriae. However, disease can be prevented by immunisation with whole-cell pertussis vaccines (WCVs) and with the newer acellular pertussis vaccines (ACVs) containing up to five purified *B. pertussis* antigens, namely detoxified pertussis toxin (dPT), FHA, PRN, and fimbriae (types 2 and 3). ACVs are less reactogenic than WCVs, and this is presumed to be due to reduced toxin activity and fewer inflammatory molecules such as LPS. However, some ACVs may be less efficacious than WCVs. In mice and humans, it has been suggested that WCVs may preferentially prime Type-1 $CD4^+$ T (Th1) cell responses that favour cell-mediated immunity, compared with ACVs that promote Type-2 $CD4^+$ T (Th2) cells and favour humoral immunity (Redhead *et al.*, 1993; Barnard *et al.*, 1996).

CyaA, a 177kDa protein endowed with adenylate cyclase (AC) and cell-invasive abilities, is synthesised as a protoxin (proCyaA) that is post-translationally acylated by a separate protein, CyaC. CyaA has two functional domains: the C-terminal domain (of about 1300 amino acids) which has membrane-targeting and pore-forming activity and the 400 amino acid N-terminal domain which has AC enzymic activity (Ladant and Ullman, 1999). Interaction with, and invasion of, mammalian target cells which express the CR3 (CD11b/CD18) receptor (Guermonprez *et al.*, 2001), is facilitated by acylation of CyaA and, upon entry into the cell, the N-terminal AC enzymic moiety is activated by host calmodulin to produce supraphysiological levels of cyclic AMP (Confer and Eaton, 1982). In immune effector cells, this impairs their phagocytic and bactericidal capabilities and induces apoptosis, features that are assumed to assist survival of the bacterium in the initial stages of respiratory tract colonisation (Gueirard *et al.*, 1998). Anti-CyaA antibodies have been shown to enhance phagocytosis of *B. pertussis* through neutralisation of CyaA which normally inhibits phagocytosis by neutrophil polymorphonuclear leukocytes (Weingart *et al.*, 2000). An immune response to this toxin may therefore be important in preventing colonisation of the host by *B. pertussis*.

Previous work showed that CyaA could act as a protective antigen (Betsou *et al.*, 1993; Hormozi *et al.*, 1999) and as an adjuvant towards co-administered antigens, such as ovalbumin, by enhancing the serum IgG responses to the bystander antigens in mice (Hormozi *et al.*, 1999). My work was designed to assess the relative contributions of the enzymic and pore-forming/invasive activities of the toxin for adjuvant activity towards an ACV for both humoral and cell-mediated responses and against *B. pertussis* infection. Four different forms of highly-purified recombinant CyaA, very low in LPS, were prepared. These were: fully functional CyaA; CyaA lacking adenylate cyclase enzymic activity (CyaA*); and the non-acylated forms of these toxins, proCyaA and proCyaA*. Our studies, done in collaboration with Dr. Dorothy Xing, NIBSC (National Institute of Biological Standards and Control, Herts) showed that, when mice were immunised twice with a sub-protective dose of ACV together with CyaA*, there was significantly enhanced protection afforded against intranasal challenge with virulent *B. pertussis* compared with mice immunised with ACV alone (Fig. 1).

Pooled sera from each of the immunised groups immunised with a CyaA preparation neutralised the haemolytic, cytotoxic and, to a lesser extent, the enzymic activity, of CyaA. Co-administration of all CyaA forms with ACV caused a general increase in IgG2a antibody levels against the ACV components, compared with levels in mice immunised with ACV alone. Again, this effect was most pronounced with CyaA*. Spleen cells and peritoneal macrophages from mice at two weeks post-immunisation were tested for cytokine or nitric oxide production,

Fig. 1. *Protection of mice against intranasal challenge with B. pertussis by immunisation with ACV alone or in combination with different CyaA forms.*



Groups of 5 mice were immunised on days 0 and 28 with ACV alone or ACV + CyaA forms at 25 μg then challenged intranasally with B. pertussis 18.323 on day 42. Mice from a phosphate buffered saline (PBS) control group were sampled at 2 h post-challenge for enumeration of bacteria in lungs and tracheas (grey bar). All remaining mice were sampled at 7 days post-challenge (white bars). Results represent the means of five mice per group with the SEM (bars). Symbol: †, $P < 0.05$ (groups vs ACV: ANOVA) or ††, $P < 0.05$ (groups vs PBS: ANOVA) or †††, $P < 0.05$ (as linked by brackets).

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respectively, after *in vitro* stimulation with a mixture of *B. pertussis* antigens or with heat-killed *B. pertussis* cells. With *B. pertussis* antigens, spleen cells from mice immunised with ACV + CyaA* secreted higher amounts of IL-5, IL-6, IFNγ and GM-CSF than cells from other immunised mice including those from mice immunised with ACV alone or ACV + CyaA. Macrophages from mice immunised with ACV + CyaA* produced higher levels of nitric oxide than macrophages from other immunised mice. These findings suggested that the enhancement of protection provided by CyaA* was due to an augmentation of both Th1 and Th2 immune responses to *B. pertussis* compared to the responses in mice immunised with ACV alone. The adjuvant properties of the CyaA* derivative suggest that it may have potential as an acellular vaccine component.

I am grateful to S*f*AM for the award of a President's Fund Grant that allowed me to present some of my work at the European bacterial toxins workshop (ETOX 12) held in Canterbury, UK, 2005.

Gordon Cheung
University of Glasgow

Arcobacter species – the third in the family?

The RNA Superfamily VI of the Proteobacteria consists of three genera: *Helicobacter*, *Campylobacter* and *Arcobacter*. *Arcobacter* spp. is the least well-known of the three and has only been recognised since the early 1990s.

The original classification as 'aerotolerant campylobacters' came about because they are similar in morphology to campylobacters and also grow under microaerobic conditions. However, they also grow optimally in oxygen at 30°C and, in comparison to the pathogenic thermophilic campylobacters, they are not able to grow at 42°C.

A. butzleri, *A. skirrowii*, *A. cryaerophilus* (Groups 1A and 1B) and *A. cibarius* have been associated with animals and humans while *A. nitrofigilis* and a newly suggested species, *A. halophilus* appear to be free-living (Snelling *et al.*, 2006). *Arcobacter* spp have been isolated from a range of sources including water, healthy dairy cattle, pigs and sheep, with poultry being the most significant reservoir, although the organism does not appear to colonise the gut.

A. butzleri and *A. cryaerophilus* are most commonly associated with human disease (Phillips, 2001) with only isolated cases of *A. skirrowii*-associated diarrhoea having been reported. The first described case of an *Arcobacter*-related human infection was in 1988 and throughout the 1990s there were reports of human infections caused by both *A. butzleri* and *A. cryaerophilus*. Arcobacters were isolated from diarrhoeal cases, sometimes associated with underlying chronic conditions. *A. butzleri* and *A. cryaerophilus* 1B were also isolated from cases of bacteraemia. Recently there have been reports that *A. butzleri* may be an underestimated pathogen and in a 8-year study carried out in Belgium *A. butzleri* was the fourth most common *Campylobacter*-like organism isolated, making up 3.5% of the total (Vandenburg *et al.*, 2004).Therefore, although the epidemiological evidence linking *Arcobacter* infection with human illness is not large, there is increasing evidence of the association of *Arcobacter* spp. with human illness, lending weight to the argument of their importance as human pathogens.

There are two probable reasons for the lack of information on the epidemiology and extent of *Arcobacter* infections in humans. Firstly, the most common symptoms of infection are acute watery diarrhoea accompanied by abdominal pain, nausea and vomiting, which are similar to those of *Campylobacter* infection, and therefore the two might be confused symptomatically. Secondly, because arcobacters are differentially sensitive to antimicrobial agents present in selective media used for *Campylobacter* isolation, routine techniques used for *Campylobacter* spp. isolation from clinical samples will not necessarily isolate arcobacters.

The external environment can present itself as potentially hostile, threatening the viability of the organism through a variety of bactericidal mechanisms. Therefore the capacity of *Arcobacter* spp to induce disease is dependent on their ability to survive and multiply within the host and within the environment.

Preservatives added to food, changes in temperature or pH, the presence of other organisms in the environment, whether in food or within the human gut are all potential stressors. Theory surrounds the possibility that a sub-lethal stress induces an adaptive tolerance response (ATR) and may provide protection towards subsequent exposure to lethal stress. The ability to survive an up-shift in temperature might constitute an important survival mechanism when the organism moves from environmental conditions, for example, from food to the human gut. Similarly, the ability to survive

acidic conditions might indicate an ability to move through the stomach, enter the intestine and hence produce gastrointestinal symptoms.

An ATR mechanism has been identified in a number of pathogens including *Campylobacter jejuni* which produces such a response to aeration, acid adaptation and thermal stress (Murphy *et al.*, 2003). We have shown that *A. butzleri* is able to produce an ATR in response to heat in that when *A. butzleri* is heat shocked to 70°C no viable cells can be recovered, although when pre-treated at 50°C for 10 minutes before heat shock a proportion of cells do survive, with stationary cells being more resistant than exponential cells and spent medium having a protective effect, similar to that seen in *C. jejuni*. This differential response between exponential and stationary cells is also seen when *A. butzleri* cells are exposed to acid. After 30 minutes at pH5 followed by acid challenge at pH3 some stationary cells survive whilst neither pre-treated exponential cells nor unadapted cells survive similar conditions.

The presence of other bacterial species and/or their culture medium or peptides produced by these species, known as bacteriocins, can inhibit the growth of *A. butzleri*. Nisin, a commercially available bacteriocin produced by *Lactobacillus lactis* subspp *lactis*, is mainly active against Gram positive bacteria, although Gram negative bacteria may be susceptible if the outer layer is disrupted with chelating agents such as tri-sodium phosphate or EDTA. Studies carried both in culture medium and on a food matrix (chicken skin) have shown that the combination of nisin with EDTA/TSP or sodium lactate inhibits the growth of *A. butzleri* and together with low temperatures would provide an effective method of reducing contamination (Long & Phillips, 2003; Phillips & Duggan, 2001).

There is increasing interest in the interaction of bacterial species with each other as a means of preventing disease, both in the scientific arena and more generally, as witnessed by the plethora of 'good bacteria' drinks etc on supermarket shelves. Like *H. pylori* (Krausse *et al.*, 2005) and *C. jejuni* (Chaveerach *et al.*, 2004) *A. butzleri* also is inhibited by the presence of other bacteria such as *Lactobacillus spp.* and *Bifidiobacteria* although this inhibition varies according to the *A. buzleri* isolate. In one study we have carried out on three strains, the chicken isolate proved to be the least susceptible to the presence of a number of effector bacteria. The greatest inhibition zones were seen between the type strain NCTC 1248 and *Bifidiobacterium adolescens* or *L. plantarum*.

I would like to thank the Society for Applied Microbiology for providing funds to enable me to attend the 13th *Campylobacter, Helicobacter* and Related Organisms conference (CHRO 2005) in the Gold Coast Australia in September 2005 where we were able to present some of these findings.

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The use of peptide nucleic acid probes to detect legionellae in mains water distribution supplies

LEGIONELLAE ARE widespread environmental bacteria commonly associated with aquatic environments.

Several studies have indicated their presence in drinking water distribution systems but most concern is directed at the control of their distribution within building systems. Outbreaks of Legionnaire's Disease are often linked to poorly maintained air conditioning systems or cooling towers. Populations existing in the mains distribution system may act as a reservoir and lead to future contamination of buildings. With the exception of indicator species (e.g. *E. coli*) there is little information on the survival and distribution of potential pathogens within potable water systems. Biofilms in mains supply drinking water systems may act as a refuge for bacteria, protecting them against chemical disinfection or mechanical disruption. It is possible that pathogenic species can make use of these protective characteristics and hence, survive within the biofilm structure. In the current study we were primarily concerned with detecting pathogens within such biofilms. Legionellae are known to be a biofilm species and as they are microaerophiles which prefer a low oxygen atmosphere, the biofilm with its reduced redox potential could provide a suitable environment for their persistence.

One of the major problems relating to the study of environmental biofilms is a lack of efficient and effective detection methods. It is well-documented that the standard culture method of detection of legionellae has many limitations. It can take up to 14 days to get a result, it does not necessarily allow the growth of stressed cells and because the cells must be removed from the biofilm, this method provides no information regarding the location and distribution of cells. We wanted to develop a method which would be specific, rapid and provide details on the location of individual cells.

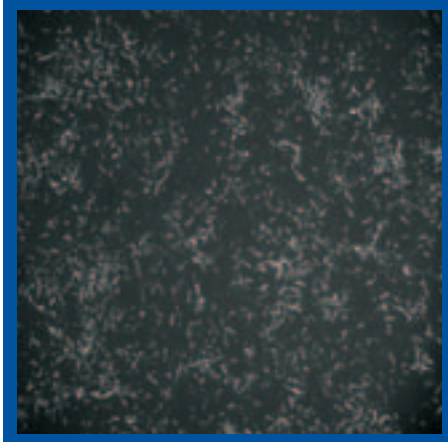
The approach taken relies on a modified fluorescence *in situ* hybridisation (FISH) assay and a

specialised microscopy technique. FISH assays have been widely used but are not preferred when analysing environmental samples due to problems with non-specific labelling and interference from autofluorescence of the target or sample material. To date, the majority of FISH assays have relied on the use of DNA probes to target specific regions of the 16S rRNA molecule of a certain bacterium. We have been developing a range of target-specific peptide nucleic acid (PNA) probes. PNA probes share many of the same characteristics as DNA probes (e.g. obeying Watson-Crick hydrogen bonding rules, allowing for sequence specific binding to DNA and RNA) but are synthetic molecules and have a number of unique properties (Stender *et al.*, 2002). The phosphodiester backbone is replaced by a 2-aminoethyl-glycine linkage which gives the molecule a neutral charge compared to the highly charged DNA molecule. Their neutral charge allows them to access inaccessible areas of the 16S rRNA molecule where more variable sequence regions are located. PNA probes exhibit a high thermal stability and are resistant to ionic changes. These features are advantageous when analysing direct environmental samples. In such samples, the environmental material itself may lead to ionic and pH changes which can affect hybridisation efficiencies.

As part of the EC Framework Programme 5-funded SAFER project (EVK1-CT-2002-00108), we have designed two PNA probes for the detection of legionellae in environmental samples; the first targets all *Legionella* species while the second is specific to *L. pneumophila*. These probes were based on existing DNA probes (LEG 226, Manz *et al.*, 1995 and LEGPNE1, Grimm *et al.*, 1998) but were shortened to 15-mers - the optimum probe length for PNA probes. The probes were synthesised and labelled with a fluorophore. They were then tested on a range of *Legionella* and non-Legionella species. These laboratory tests confirmed the specificity of the probes and indicated that there was no non-specific labelling. These experiments also demonstrated the thermal stability of these PNA probes and hybridisation occurred over a 15 degree temperature range. Direct comparisons were made with the original DNA probes and in all cases, the PNA probes performed more efficiently (fig. 1).

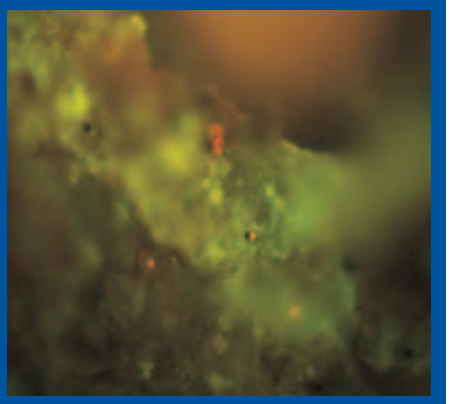
These preliminary results were encouraging but our aim was to develop a method which could be used directly on environmental samples, and specifically drinking water pipes, to show the distribution of bacteria within the associated biofilm. The first problem to overcome was how to examine a piece of pipe. Using a specialised microscope system, the episcopic differential interference contrast/epifluorescence (EDIC/EF) microscope (Keevil, 2003), it is possible to examine opaque and solid materials. The microscope uses long working distance objectives and a specialised lighting and filter system. By cutting pipe sections, it is possible to examine them directly under the microscope. We have examined biofilms on polyvinylchloride (PVC) and cast iron pipe samples. The new PNA probes have been applied directly to pipe sections to assess whether they can penetrate the biofilm and specifically label individual bacteria within the structure. Cast iron pipes can also have extensive corrosion deposits which could affect the efficiency of the hybridisation procedure and background fluorescence could affect the visualisation of individual labelled cells. Some cast iron pipe samples have been found to have deposits up to 2.5 cm in depth. By combining the PNA hybridisation protocol and the specialised microscopic system of the EDIC/EF microscope, we have been able to locate individual *L. pneumophila* on all the pipe samples analysed (fig. 2). This method will now be used to quantify the population found on potable water pipe surfaces. This method could provide invaluable information on how biofilms may act as a refuge and reservoir for potential pathogens.

Fig. 1



Legionella pneumophila cells labelled following hybridisation with novel PNA probe. Magnification x 600 (from Wilks et al. 2006)

Fig. 2



A composite EDIC/fluorescence image of the surface of a drinking water pipe. Cells of L. pneumophila have been labelled with a PNA probe with a red fluorophore attached. Magnification x 1000.

I am grateful to S*f*AM for awarding me the President's Fund. It enabled me to attend Legionella 2005 in Chicago were I presented this work.

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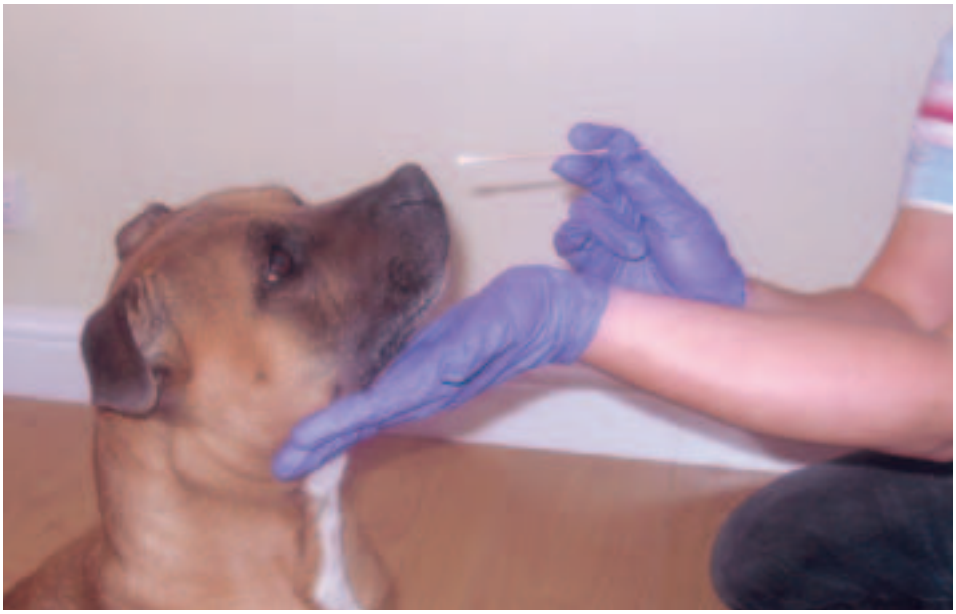
Reservoir Dogs?



ARE DOMESTIC PETS AND larger animals a potential reservoir for infectious disease within our community and family home? Are they harbouring an army of unwanted microscopic enemies ready to invade when least expected? Or are they just the next in a long line of Methicillin Resistant *Staphylococcus aureus* victims?

Methicillin Resistant *Staphylococcus aureus* (MRSA) has become a serious worldwide hospital pathogen and over the last decade has been reported throughout the community in healthy patients with no prior hospital contact. Methicillin Resistant *Staphylococcus aureus* is transmissible via human-to-human contact but reports of carriage in companion animals have opened up a new potential reservoir for MRSA zoonotic transmission within our family homes and veterinary practices. As a nation of pet lovers we often forget that animals are ideal hosts for asymptomatic carriage of a variety of bugs and a range of animals have been reported to carry MRSA worldwide, including dogs (van Duijkeren *et al.*, 2004), cats (Weese *et al.*, 2006), cattle (Devriese and Hommez 1975) and horses (Seguin *et al.*, 1999). Human and animal transmission can work in either direction but the most likely source of origin is man with increasing MRSA prevalence in our population over-spilling into other animal groups.

The Health Protection Agency reassures us that carriage of MRSA in companion animals is of limited risk to an individual unless they are in a diseased state, only then may animal carriage act as a potential reservoir for human infection. Patients with MRSA infections may suffer possible relapse after treatment due to asymptomatic carriage of MRSA within a pet or family member. A recent case in the Netherlands mirrors this scenario (van Duijkeran *et al.*, 2004) where colonisation of a nurse after antibiotic treatment was caused by an isolate genetically identical to that carried by a pet dog. Each time the nurse was cleared of MRSA, re-colonisation of the same strain occurred some weeks later. Elimination of the strain long term was only achieved when the dog was also treated and cleared of the organism. It was assumed that the nurse transferred MRSA to the dog through contact, then acted as a canine reservoir for re-colonisation.

In a similar case (Manian, 2003) a diabetic patient and his wife had re-occurring staphylococcal infection. A genetically identical strain of MRSA was found in a pet dog's nose and re-infection was once again only prevented when MRSA was eradicated from both pet and owners. These cases are not only limited to the family home, the removal of a colonised cat in one London Hospital ward combined with infection control measures resolved an MRSA outbreak in a geriatric ward (Scott *et al.*, 1988), a reason why pets are no longer free to roam our hospital corridors!

Methicillin Resistant *Staphylococcus aureus* infection in animals is not common but risk increases when associated with veterinary exposure, open wounds, and immuno-suppression. Transmission is by no means uni-directional and human MRSA colonisation can also pose a threat to animal health, with reports of identical strains residing in both staff and visiting animals at veterinary practices (Baptiste *et al.*, 2005). Studies have shown a high rate of MRSA colonisation in veterinary staff in the UK, with a common epidemic hospital strain, EMRSA-15, isolated in staff, dogs and the surrounding environment (Loeffler *et al.*, 2005). Transmission of MRSA between horses and humans has been reported in the US (Seguin *et al.*, 1999) where horses from different farms developed genetically similar MRSA infections after treatment at the same veterinary hospital. Three staff members were also colonised with the same strain implicating hospital staff to be the primary source of infection.

Worryingly strains encoding Panton Valentine Leukocidin (PVL), a toxin associated with severe community MRSA infection have been isolated from companion animals such as dogs, cats, rabbits and parrots (Rankin *et al.*, 2005). Strains producing PVL will pose a great threat to the host carrier if infection was to precede colonisation and also to those in close contact with the colonised animal. Panton Valentine Leukocidin is highly cytotoxic causing leukocyte destruction and necrosis of soft tissue and eradication of such strains is difficult due to their potent invasive qualities. Continued surveillance of PVL-positive strains within the animal population must be maintained to minimise associated risk of transmission.

As MRSA rates increase within our population it is inevitable that the incidence of animal carriage will also rise. Most cases reported are from clinical outbreaks, little has been reported on colonisation rates as a whole but for the meantime studies indicate that the prevalence of MRSA in the animal population is still quite low (Lefebvre *et al.*, 2006). The Department for Environmental Food and Rural Affairs are currently monitoring the situation with the help of University Veterinary Hospitals. Veterinary practices must employ infection control measures to

eradicate MRSA colonisation in staff and animals to prevent post-treatment infections. The ability of MRSA to transmit zoonotically between animals and humans is of clinical importance to both public and animal health and must be taken into consideration if we are to truly control MRSA community transmission in the future.

Jessica Rollason
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Carbohydrate Active Enzymes of *Streptococcus pyogenes*

GROUP A STREPTOCOCCI (GAS) are infective human pathogens capable of causing a wide range of clinical manifestation such as scarlet fever, pharyngitis, rheumatic fever, streptococcal toxic shock syndrome (STSS) and severe invasive infections such as necrotizing fasciitis.

Streptococci produce a range of virulence factors associated with their infectiveness including pyrogenic exotoxins involved in the systemic toxicity of the bacteria, a hyaluronate capsule which allows for evasion of the host defences and surface M proteins which are a major component in the adhesion of the bacteria during infection (Medina, 2004). The surface M proteins also form the basis for the classification of GAS. The M protein classifications are identified by a numbering system. M1, M3 and M18 GAS are of particular importance due to their relative abundance in human infections.

- M1 are commonly seen in invasive infections and have been seen to be responsible for GAS epidemics.
- M3 are the prevalent cause of STSS and necrotizing fasciitis.
- M18 has been seen to be found in persistent sequelae such as rheumatic fever.

Streptococcus pyogenes is a Group A Streptococcus. The strain SF370 is a M1 *Streptococcus* and as such is capable of causing invasive infections and has been seen to be one of the primary causes of GAS epidemics (Banks *et al.*, 2002). In streptococcal species there is a high degree of genetic variation which contributes to the diversification and evolution of the species. Phage or phage-like elements cause the majority of this variation. The bacteria are polylysogenic, containing material from multiple phages, GAS M1 serotype contain four such elements which accounts for 7% of the total genome and encodes 172 coding sequences (Banks *et al.*, 2002). These coding sequences often encode for enzymes that have been acquired by the streptococcus through evolution and have an implication in their ability to infect. It is this polylysogeny that causes such a wide degree of variation among streptococci from the same M group.

Bacteriophages use tail fibre proteins to bind to host receptors during an infection. An example of such viral fibre structures is the triple beta helix. The triple beta helix is a left handed turn with a triangular cross section. As the name suggests each turn is composed of three strands which are connected by short linkers with hydrogen bonds across the strands. The strands cause angles of approximately 60^0 between them. The structure has a central longitudinal hydrophobic core. The highly twisted structure means that 57% of the accessible surface of the monomer is buried when in the trimer. This structure has been shown to form the puncturing needle of the phage. Such fibre proteins have been shown to be enzymatically active and this is the case of HylP1 from *S. pyogenes* looked at in this study. Like hyaluronan degrading enzymes from streptococcal species, such enzymes from bacteriophages are believed to be involved in adhesion and invasion. Hyaluronidases from bacteriophage are believed to be involved in the degradation of the hyaluronan capsule allowing for infection of streptococcus (Baker *et al.*, 2002).

Carbohydrates have great structural and functional diversity in nature. From this ubiquity it is apparent that there must be a plethora of enzymes involved in the biosynthesis, catalysis and utilization of carbohydrates. This vast diversity of such enzymes has led to the development of a classification database called CAZy (Carbohydrate Active enZymes), which can be found at http://afmb.cnrs-mrs.fr/~pedro/CAZy/db.html. This database classes carbohydrate active enzymes based on their activity or putative activity, into five groups: glycosidase and transglycosidases, glycosyltransferases, polysaccharide lyases, carbohydrate esterases and carbohydrate binding modules. The classes are further split into families which contain proteins with a degree of sequence similarities.

Polysaccharide lyases catalyse the cleavage of the beta1-4 glycosidic bond via a beta elimination mechanism causing the formation of an unsaturated carbon-carbon double bond at the non-reducing end of the saccharide. Glycoside hydrolases also cleave the glycosidic bond between disaccharides but by the use of activated water. There are two mechanisms of action which result in either the retention or the inversion of the anomeric structure.

This study looks at such carbohydrate active enzymes from *S. pyogenes*. Three phage encoded genes, *HylP1*, *HylP2* and *HylP3*, from *S. pyogenes* have been cloned and expressed using cloning vectors and recombinant technology. HylP1 has been characterised as a polysaccharide lyase against hyaluronan from umbilical cord and the structure solved using x ray crystallography showing the characteristic structure of the tail fibre of the bacteriophage containing a unique triple beta helix at its core. *HylP2* and *HylP3* have been cloned and expressed but are yet to be characterised.

A further enzyme from *S. pyogenes* is encoded by the gene *Ugl*. The encoded enzyme is thought to be from a unique class of enzymes, the unsaturated glucuronyl hydrolases. These fall into the class of glycoside hydrolases but are unique in that they will only target unsaturated residues from polysaccharide lyase degradation removing this residue and allowing for further degradation (Hashimoto *et al.*, 1999). Ugl has been cloned and expressed and has shown some activity against unsaturated hyaluronan produced using *HylP1*.

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Plasmid Biology

Barbara E. Funnell and Gregory J. Phillips (Eds)
ASM Press, Washington DC. 2004
ISBN 1-55581-265-1 pp 638
Price $120
Reviewed by: Judith Evans

Plasmids are extrachromosomal pieces of DNA capable of autonomous replication within their host cell. They generally carry at least one gene that is beneficial to their host, such as antibiotic resistance genes in bacterial cells. The study of plasmids, their transfer and replication has allowed a greater understanding of activity within cells at the molecular level. This book provides an in-depth study of plasmids aimed at under and post-graduate students, their instructors on courses such as microbial genetics, ecology, bacterial pathogenesis and biotechnology. It is also an extremely

useful resource for researchers in those fields.

The first of 29 chapters starts the book off with a review of the study of plasmids and provides a comprehensive walk through the history of plasmid research in easy to read sections covering replication, mobility and maintenance of plasmids. This overview emphasises the achievements of the early pioneers and highlights discoveries that laid the foundations for further progress.

The rest of the book is broken into six sections covering plasmid replication systems, plasmid maintenance and inheritance, specific plasmid systems, virulence and antibiotic resistance plasmids, plasmid ecology and evolution and finally, plasmids as genetic tools. Each chapter can therefore be read as a separate unit or the well planned index makes it straightforward to go directly to a specific area of interest.

Copy number is a major factor in the persistence or loss of plasmids, the first section examines three means of replication control available to plasmids; models of regulation of ori containing plasmids, control by antisense RNAs and control in rolling circle replication.

The second section is concerned with plasmid maintenance and inheritance. It details methods used by plasmids to ensure segregation at cell division is successful, such as partition systems or genes symbiosis. There is a fascinating chapter on the disadvantages of circular DNA in replication and its sensitivity to rearrangements caused by homologous recombination and subsequent plasmid loss. Further chapters address DNA configuration and metabolism, conjugation between bacteria (Gram positive and Gram negative are dealt with separately) and the role plasmids play in mobilisation of usually non-transferable elements within cells.

I found the next section the most interesting of the book, with chapters describing how plasmids can expand their host range for a better chance of survival, linear plasmids, a description of benign parasitism exhibited by some plasmids and an overview of viral plasmids that integrate with host mammalian cells. The rest of the chapters illustrate the differences plasmid carriage can make to a host cell, with a range of examples ranging from degradative plasmids enabling the host to utilise carbon from organic sources, carriage of virulence genes, antibiotic resistance genes and iron uptake gene carriage. The next section focuses on evolution and population genetics, and the presence of large plasmids and megaplasmids in some bacterial genomes. The final section is given over to the use and future of plasmids as genetic tools.

The figures and tables throughout the book are clear and the coloured section, where the reader is directed where black and white would be inadequate, is a useful one. Another feature that worked well was the deliberate emphasis on previously published reviews and papers, meaning the reader is not left to work out which references to pursue. The majority of chapters end with some kind of concluding remarks that emphasise where current knowledge is patchy and where the authors think future work is headed. This allows addendum in proof where relevant; making it clear that whilst the book is an excellent reference, the subject is fluid and more research is published continually.

Freshwater Microbiology

Biodiversity and Dynamic Interaction of Microorganisms in the Aquatic Environment. David C. Sigee
John Wiley & Sons, Ltd.
December 2004. £35
ISBN 0 471 48529 2
Reviewed by Chris Hodgson

This book brings together the fascinating microbiological communities found in the freshwater environment. It focuses on the interactions and dynamics of viruses, bacteria, fungi, actinomycetes, protozoa and algae. It is essentially a one stop shop that reflects the growing interdiciplnarity that is today's science.

The book is made up of ten chapters. The first two chapters are general introductions. Chapter one; Microbial diversity and freshwater ecosystems, introduces us to the global water supply and moves swiftly to a general description of the biodiversity of microorganisms covering their size and trophic status. At the end of this chapter we are introduced to the first of many case studies. Case study 1 looks at the microbial food web associated with an algal bloom. The use of case studies is a brilliant way to both enforce what has gone before in the chapter and brings some interest to what could be a dull academic text book.

Interestingly this text book purports to be invaluable to both students and researchers in the fields of freshwater biology, algology, microbiology, environmental biology and conversation ecology. It would be valid to conclude that all these 'ologies' are catered for within this tome however, it would not be the first choice for a student who is new to these academic disciplines. On the other hand it should be a stable text for those undertaking research in any of these academic spheres.

Molecular analysis and standard culture techniques are explored throughout the text for the various microbiological communities, ensuring the books relevance to the current knowledge in the various fields. This is demonstrated quite eloquently in pages 291 – 294 where classic taxonomic analysis is compared and contrasted with molecular analysis, the later exemplified by Case Study 6.1 which looks at the work of Findlay *et al.*, (2003) who studied substrate-mediated shifts in the biological properties of stream sediment bacteria in relation to both function (extracellular enzyme activity) and molecular composition (RAPD analysis).

In the opening paragraph of this review I intimated that without the case studies this could be a dull text book and it does have that soporific effect. There is a tendency towards repetition in terms of process description and the text is perhaps dominated by all things algae. There is to my mind a fundamental problem with the photographs found throughout the text; why are they not in colour? For example Figure 4.20 depicts the macrophyte domination of the seasonal wetlands at the edge of Fogg dam floodplain, North West Australia, the description that the follows the figure legend paints a wonderfully colourful scene — the actual picture is disappointingly 'foggy'!

In terms of value for money I think that this text book has to be exceptional, having a plethora of both relevant tables and figures and that winning formula of case studies in abundance. Its contribution across the combined academic fields is at this time second to none, in that it is unique.

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