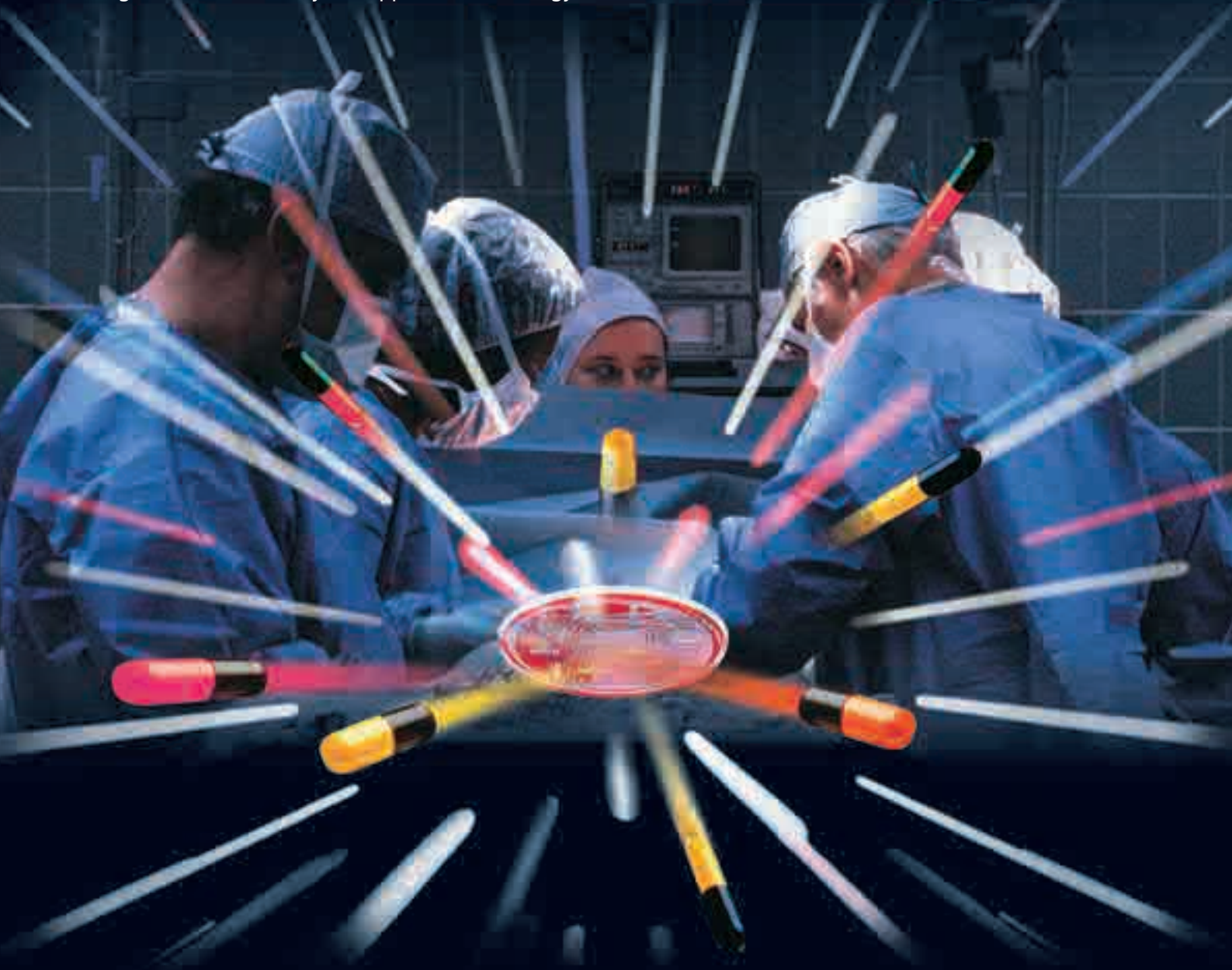
Microbiologist

The magazine of the Society for Applied Microbiology ■ December 2005 ■ Vol 6 No 4



MRSA INFECTION

The fight goes on!

ALSO IN THIS ISSUE:

- 2006 Summer Conference Programme
- *Clostridium difficile:* return of the old enemy
- We interview *Bifidobacterium lactis*
- The history of the UK's oldest microbiological Society

excellence in microbiology

don whitley scientific
excellence in microbiology

We are the friendly face of microbiology!

Don Whitley Scientific is a leading supplier of innovative equipment and contract & consultancy services for microbiologists worldwide. If you work in clinical, pharmaceutical, research or industrial microbiology, please phone one of the friendly faces in our technical sales team on:

+44 (0)1274 595728

www.dwscientific.co.uk

Microbiologist

The magazine of the Society for Applied Microbiology ■ **December 2005** ■ Vol 6 No 4 ISSN 1479-2699

Microbiologist
Vol 6 No.4
December 2005
ISSN 1479-2699

Publisher: Society for Applied Microbiology.

Editor: Lucy Harper
lucy@sfam.org.uk

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

Advertising:
Julie Wright
Telephone: 01234 326661
julie@sfam.org.uk

Art and Design & layout:
Pollard Creativity

Production and printing:
Pollard Creativity.
All technical questions should be addressed to:
sfam@pollardcreativity.co.uk
Tel: 01933 665617

Society for Applied Microbiology, The Blore Tower, The Harpur Centre, Bedford MK40 1TQ, UK

Tel: +44 (0)1234 326661
Fax: +44 (0)1234 326678
email: info@sfam.org.uk
www.sfam.org.uk

REGULARS

MEETINGS

BOOK YOUR PLACE ONLINE NOW!

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: lucy@sfam.org.uk

Microbiologist
Vol 6 No.4
December 2005

Contact the Editor:
lucy@sfam.org.uk

Microbiologist copy Dates: contributors please note that the final copy dates in 2005/2006 will be:

Vol 7 No.1 March 2006
Friday 16 December 2005

Vol 7 No.2 June 2006
Friday 10 March 2006

Vol 7 No.3 Sept 2006
Friday 7 July 2006

Vol 7 No.4 Dec 2006
Friday 15 Sept 2006

How to submit material
Please submit all articles, reports, meetings notifications, letters etc., as plain text (*.txt) or rich text files (*.rtf). Please submit all images as original photographic prints or transparencies rather than scanned images and these will be processed by us and returned to you promptly. If your images are only in digital format please make sure they are supplied at a resolution of 300dpi (dots or pixels per inch at a size of not less than 100mm (4 inches) square.

Advertisers: if you wish to advertise in *Microbiologist* you should contact the Society Office in the first instance. Advertising rates and Guidelines on how to submit advertisements are given on the website and are also obtainable by emailing the editor at: **lucy@sfam.org.uk**

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

THIS ISSUE OF THE *Microbiologist* touches on one of my 'pet' subjects—the reporting of science in the media. Everyday, we see attention-grabbing headlines in the media which are used to 'sell' stories and encourage you to read further (see 'Media-watch', page 6). As scientists, we don't generally receive our scientific information from the newspapers. However, the general public do and I believe it is our responsibility as scientists to do our bit to ensure that science is reported accurately and in an informative, less sensationalist way. This is where the growing and increasingly important field of science communication comes into play.

On the topic of communication of science, our regular Med-Vet-Net column gives an overview of the internship that's currently underway. The network has appointed two interns from two of the Partner Institutes of Med-Vet-Net to study all aspects of science communication during a three month visit to the UK. See page 10 for details.

Continuing on this topic, this issue reports on two pathogens which have both been described by the media as hospital 'superbugs'—methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile*. Some may say that these bacteria have become a problem due to inadequate disinfection and the overuse of antibiotics. It is debatable whether or not all the media attention afforded to MRSA is a good thing. From one point of view, highlighting any deficiencies in hospital hygiene and the over-prescription of antibiotics in hospitals may result in these issues being tackled further at policy level. However, at the same time we have to ensure that the general public aren't 'scared' by media hype. An article in this issue of the *Microbiologist* discusses the importance of rapid methods of detection of MRSA (page 26), and we also have an overview on *C. difficile* (page 33). One method of therapy for CDAD (*C. difficile* associated disease) is the use of probiotics. After seeing the very promising CV of the probiobiotic *Lactobacillus acidophilis* in the June issue of the magazine (*Microbiologist*, June 2005 Vol 6 No 2), this issue sees another CV and job interview of his cousin *Bifidobacterium lactis* (page 16).

Our president takes us through the history of the society as we near our 75th anniversary year. I had no idea of some of the events that took place in 1931, the year the Society for Applied Bacteriology began. Read her column to find out more (page 8). On this note, we are extending the deadline for the SfAM anniversary competition. To celebrate 75 years of the Society for Applied Microbiology, we are running a writing competition to find the most interesting, entertaining and informative article describing an historical event in Microbiology. We are looking for 500-700 words of your opinions, thoughts or memories of a famous microbiological breakthrough, historical event or a personally significant tale. The only other rules of the competition are that the article is microbiological in nature and that it is based upon an event of the last 75 years. So, for all you writers out there like Milton Wainwright (*Microbiologist*, September 2005, Vol 6, No 3 pp26 - 29) who have witnessed or are merely fascinated by a particular Microbiological breakthrough or event in History, then see page 7 and send in your entries to me.

I hope you enjoy your SfAM Christmas card and small gift , which leaves me to wish you all a very merry Christmas and a great 2006!

COMMITTEE MEMBERS 2005 - 2008

HON PRESIDENT: **Dr Margaret Patterson**, Agriculture and Food Science Centre, Newforge Lane, Belfast BT9 5PX
margaret.patterson@dardni.gov.uk

HON GENERAL SECRETARY: **Dr Anthony Hilton**, School of Health and Life Sciences, Aston University, Birmingham B4 7ET
a.c.hilton@aston.ac.uk

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

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

HON EDITOR: Journal of Applied Microbiology
Professor Arthur Gilmour, Agriculture and Food Science Centre, DARD and Queen's University, Newforge Lane, Belfast BT9 5PX
arthur.gilmour@dardni.gov.uk

HON EDITOR: Letters in Applied Microbiology
Dr Jean-Yves Maillard, Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff CF10 3XF
maillardj@cardiff.ac.uk

HON EDITOR: Microbiologist
Dr Lucy Harper, Society for Applied Microbiology, The Blore Tower, The Harpur Centre, Bedford MK40 1TQ
lucy@sfam.org.uk

ORDINARY COMMITTEE MEMBERS until July 2006

Dr David McCleery, Chief Specialist Microbiologist, Safe Food, Food Safety Promotion Board, 7 Eastgate Avenue, Little Island, Cork, Ireland
dmccleery@safefoodonline.com

Dr Shona Nelson, Faculty of Applied Sciences, University of West of England, Coldharbour Lane, Bristol BS16 1QY
Shona.Nelson@uwe.ac.uk

Professor Diane Newell, Veterinary Laboratories Agency, New Haw, Addlestone, Surrey, KT15 3NB
dnewell.cvl.wood@gtnet.gov.uk

ORDINARY COMMITTEE MEMBERS until July 2007

Dr John Coote, Infection and Immunity Division, Glasgow University, Joseph Black Building. Glasgow G12 8QQ
j.coote@bio.gla.ac.uk

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

Dr Karen Stanley, Biosciences, Faculty of Health and Wellbeing, Sheffield Hallam University, Sheffield S1 1WB
k.stanley@shu.ac.uk

Dr Susannah Walsh, H3.09b Hawthorne Building, School of Pharmacy, Faculty of Heath and Life Sciences, De Montfort University, The Gateway, Leicester LE1 9BH
swalsh@dmu.ac.uk

ORDINARY COMMITTEE MEMBERS until July 2008

Dr Tony Worthington, Department of Phartmaceutical and Biological Sciences, Aston University, Birmingham B4 7ET
T.Worthington@aston.ac.uk

Dr Andrew Sails, Health Protection Agency, Institute of Pathology, Newcastle General Hospital, Westgate Road, Newcastle-upon-Tyne NE4 6BE
andrew.sails@hpa.org.uk

SOCIETY OFFICE STAFF

CHIEF EXECUTIVE OFFICER: **Philip Wheat** **pfwheat@sfam.org.uk**

MEMBERSHIP CO-ORDINATOR: **Julie Wright** **julie@sfam.org.uk**

EVENTS ORGANISER: **Rachel Downey** **rachel@sfam.org.uk**

MEDIA*watch*

SfAM has been incredibly busy over the last few months responding to requests by the media for comments from experts on the many 'microbiological' news stories we see in the media. One such story was generated from a survey performed by *'Top Gear'* magazine which claimed that 'Boy Racers' (males under 25) and people with dogs have the greatest number of bacteria in their cars. Our very own Honorary General Secretary, Dr Anthony Hilton was interviewed *twice*, once by Radio 5 live and later the same day by Radio 2. The Editor of *Microbiologist*, Dr Lucy Harper was also involved in a Radio 1 broadcast on the same subject. To give our members an idea of the nature of the story, below is the transcript from Anthony's interview for Radio 2:

Anthony Hilton Dirty Cars Interview. Radio 2, 1 September 2005; 5.20pm

Interviewer: The car is a sort of mobile home to about 500 colonies of germs according to a survey in this months Top Gear magazine. The dirtiest cars are driven by dog owners with the bacterial count of boy racers coming next. The cars of mature motorists are least likely to provoke plague. So just how dirty are our cars? Dr Anthony Hilton, senior lecturer at Aston University joins me now, welcome.

Anthony: Hello, thank you.

Interviewer: What kind of germs can you find in the average car?

Anthony: I think the survey found a number of organisms that you would expect to be there. The ones that are often found on the hands and skin of humans, therefore when they're touching the steering wheel and gear stick those bacteria are left there. A number of organisms were found in the footwells which are often associated with dust or with soil, so again you might expect those to be left from peoples feet as they get in and out of the car. But I think the most worrying is when you have organisms of a faecal origin. Those can easily be spread into the car by animals, dogs for example having taken them for a walk—I think those are the most concerning.

Interviewer: So this could be real cause for concern?

Anthony: I don't think it's particular cause for concern. The problem is that if, for example you had a dog in your kitchen then you might be likely to clean your kitchen thoroughly before you prepare food and eat in it, but in a car, people don't really consider cleaning the inside of a car so if these organisms are there, they might persist and then they might cause a problem.

Interviewer: Child seats are very dirty as well—does that fit with what you'd expect?

Anthony: I would think so, if you imagine the average child seat's probably full of bits of biscuits and other sources of nutrients for organisms to grow, so I think that's possibly where they come from.

Interviewer: The survey talks about *Staphylococcus*, and *Bacillus*—what kind of things do they do and what are they?

Anthony: Of the first one you mentioned, *Staphylococcus*, the main one you might have heard of is *Staphylococcus aureus*, or *Staphylococcus Epidermidis* and both of these are organisms that are commonly found on the hands. They live there all the time and in many people they don't cause any problems at all. They may cause spots and blemishes in adolescents but on the whole they're not really much of a problem. *Bacillus*, and the main one is *Bacillus cereus*, that's an organism that's found mainly in soil and dust and it can be associated with food poisoning but it's unlikely to be problem in cars really. I think the ones to worry about are those that people might have heard of such as *Salmonella*, *Campylobacter* and *E.coli.* All three of these are associated

with faeces and therefore can be a problem.

Interviewer: *Staphylococcus* it says here is mainly associated with nose picking—is that right?

Anthony: It's true that it's found in large numbers in the nose, also under the armpits and in the groin, but it's also found on the arms and hands. It tends to accumulate in moist areas of the body so that's probably why it's found there.

Interviewer: Boys racers—or young men—are pretty dirty. There were five times more germs than in the seat of a girl's car and twenty times more than the seats of the mature motorist. Does that surprise you?

Anthony: It doesn't. A car, to many young men, becomes a bit of a mobile room really, they eat, sleep in it and probably get up to all manner of things in it, so it doesn't surprise me that the number of organisms are higher than for someone who uses their car to get to work and lives mostly in a house (laughter).

Interviewer: That's very true, thank you very much Dr Anthony Hilton, the General Secretary of the Society for Applied Microbiology. Your views already coming in on this.

Stuart says: "I am a boy racer who works on a farm —it sounds like I'm doomed"!

References

Here are the references to more news stories of a microbiological nature:

■1. "Going down to the woods? Wear socks"—an interesting article warning of the danger of ticks and Lymes disease in the british countryside this summer. *The Times*, 8 August 2005

■ 2. "Saddam germ bomb spores date from outbreak in 1930s"—a cow contaminated with the same strain of anthrax as a CIA report as Saddam's choice for bioweapons research. *The Times*, 9 August 2005.

■ 3. "Oxford team raises hopes of HIV vaccine in 10 years" Short article based on a presentation from the Health Protection Agency's annual conference. *The Guardian*, 13 September 2005.

■ 4. "Could Mental illness be infectious?" Is there a link between viral infection and mental illness? *The Times*, 30 September 2005. http://www.timesonline.co.uk/article/0,,8124-1803397,00.html

■ 5. "We can't afford to be caught napping again" Professor John Oxford gives his opinion on avian flu. *The Times,* 20 October 2005: http://www.timesonline.co.uk/article/0,,1072-1833794,00.html.

■ 6. "Superbugs kill 400 in one year" An example of an attention-grabbing headline! http://www.timesonline.co.uk/article/0,,2090-1849557,00.html.

■ 7. "MRSA league tables meaningless" http://news.bbc.co.uk/1/hi/health/4383044.stm

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

75th Anniversary Competition

Next year will mark the 75th Anniversary of the Society for Applied Microbiology. To celebrate we are running a writing competition to find the most interesting, entertaining and informative article describing an historical event in Microbiology. We are looking for 500-700 words of your opinions, thoughts or memories of a famous microbiological breakthrough, historical event or a personally significant story. The only other rules are that your article should be microbiological in nature and based upon an event that occurred within the last 75 years.

A panel of judges will then sift through the entries and choose the winner, who will receive a bottle of the finest champagne and have their article published in *Microbiologist*. Three runners up will also see their work feature in the *Microbiologist* during our anniversary year. To enter, simply fill in the registration form below and send it to us together with your article before the closing date of **Friday 20 January 2006.** Good Luck!

The closing date for entries is **Friday 20 January 2006**.

Forename:_____ Surname:_____

Address:_____

_____

Title of your article: _____

_____

Simply photocopy this page and send it to: '75th Anniversary Competition', Society for Applied Microbiology, The Blore Tower, The Harpur Centre, Bedford MK40 1TQ, UK

Dr Margaret Patterson takes a look at the History of the Society in the first of her columns as our new President

WE WILL SOON BE ENTERING 2006 and the 75th Anniversary year of the Society. You will have already seen our new SfAM 75 logo, which we will use throughout 2006. Things were very different 75 years ago.

Our records show that there was a meeting of the Committee of Dairy Bacteriologists held on 9 September 1930 in which it was recommended that 'an autonomous association entitled the *Society of Agricultural Bacteriologists* (SAB) should be formed.' The next committee meeting took place the following year in Reading on Wednesday 16 September 1931, followed by the general meeting on 17 September 1931 where Dr R Stenhouse-Williams was elected as the first President of SAB and the annual subscription rate was set at ten shillings (equivalent to around £23 today).

1931 was notable for other events as well—it was the year that the original Tube Map of London was designed by London Transport employee Harry Beck and it was also the year the game of *Scrabble* was invented (it was originally named Criss Cross but was renamed *Scrabble* in 1948). In 1931 an early version of the $33^{1/3}$ rpm long playing record was demonstrated in New York. The first recording was of Beethoven's 5th Symphony. Unfortunately the venture failed due to the high cost of record players and was not revived until 1948. 1931 was also the year the first ice vending machine was introduced into the USA, in Los Angeles—ice cost 15 cents for 25lbs (equivalent to around £6.30 today). *Alka Seltzer* went on sale for the first time in early December (in time for the party season) and Rolla Harger, a biochemist, invented the Drunkometer—the forerunner of the breath test—for measuring alcohol content in the blood. 1931 was also the year that the first electron microscope was constructed by Ernst Ruska and Max Knoll. Ruska continued his work on electron microscopes and in 1986 was awarded a Nobel Prize in Physics for his efforts. Notable deaths in 1931 included Kitasato Shibasaburo, the Japanese bacteriologist who, with Alexandre Yersin, co-discovered *Pasteurella pestis* (now *Yersinia pestis*) as the infectious agent of bubonic plague. Aristides Aramonte y Simoni also died that year. He was a bacteriologist and was associated with the discovery of the role of mosquitoes in the transmission of yellow fever.

Many things have changed, mostly for the better, over the last 75 years. Science has advanced significantly since then and our knowledge and understanding of microbiology is no exception. If you can think of a notable event in Microbiology that took place since 1931, why not tell us about it? We are looking for 500-700 words on your opinions, thoughts or memories of a famous microbiological breakthrough or a personal story related to microbiology. Details of how to enter and possibly win a bottle of anniversary champagne can be found on page 7.

Society members in 1932 at the Summer Conference

Back now to the present. SfAM is very proud to be the oldest microbiology society in the UK and we are planning a series of special events throughout the year to mark our anniversary. The year will start with our Winter meeting on Thursday January 5th, in London. The themes of global infectious disease epidemiology and vaccines are very topical as avian flu and TB, to name but two, are constantly in the news. We will also be holding the first Denver Russell Memorial Lecture, to be given by Professor Sattar, University of Canada, at this meeting. There is still time (just about) to register for this meeting. Full details and a booking form can be found on page 20, or you can book online via our web site or by contacting the Society office directly

The summer conference will be held in Edinburgh on 3-6 July 2006. We believe that the theme *Living together; polymicrobial communities*—has something to interest everyone and we hope as many members as possible will make the effort to attend this special event. Full programme details can be found on page 24. Professor Max Sussman, a past President, will deliver the Lewis B Perry Memorial Lecture on the first evening. This will give us a preview of a publication that Max, along with Grahame Gould, Fred Skinner and David Post, are writing on the history of the Society. I am sure if anyone has special memories of the Society over the years, Max and his fellow archivists would be delighted to hear from you (you can contact him via the Office). This will be published towards the end of 2006.

Finally, I want to mention the SfAM Anniversary Fellowships (see page 13 for details). These have been especially established to mark our 75th year. We were aware that laboratory based staff can often find it difficult to obtain funding for external training. SfAM will provide generous funding for such staff to visit another laboratory for specific training for up to four weeks. We hope that many of our members will take the opportunity to apply for these prestigious awards.

On behalf of the Officers, I would like to wish you all a very healthy, happy and successful 2006 and I hope to see as many of you as possible during our special year of events.

Dr Margaret Patterson
President of the Society

Philip Wheat talks about his first six months as the Societys new Chief Executive Officer

Hello again from your Chief Executive Officer. This column will become a regular feature of the *Microbiologist*. The intention is to give you, as members of the Society, an idea of the activities I have been involved with on your behalf.

My first six months in post have been extremely challenging but rewarding. As well as having to understand and assess the operational activities of the office I have attended numerous exhibitions and meetings, spreading the word that our Society represents the voice of applied microbiology. The first exhibition I was involved with was the annual meeting of the American Society for Microbiology (ASM) in June, where the venue was a hot and sticky Atlanta. This exhibition was a great success with over one hundred enquiries for details of membership, all of which were followed up once I returned to the UK. In addition, several corporate members were enrolled into the Society.

The second international meeting I attended was in July where again the Society exhibited at the International Food Technology (IFT) annual meeting which was attended by over eighteen thousand delegates. The meeting was held in the New Orleans Convention Centre only six weeks before Hurricane Katrina struck. Once again, there was significant interest with over seventy enquiries concerning membership. Details were also sent to six companies who enquired about corporate membership. Straight after IFT the Honorary President and Secretary went straight onto the third international exhibition for the Society, the International Union of Microbiology Societies meeting in San Francisco.

June and July were particularly challenging months because as well as attending ASM and IFT I was also present at the first annual three day meeting of Med-Vet-Net in Winchester. This was quickly followed by our own four day annual meeting in Brighton. I thoroughly enjoyed meeting some of you at Brighton and was especially pleased to meet all the officers of the Society for the first time.

SfAM also exhibited at the Chartered Institute of Environmental Health meeting in Cardiff in September. This meeting enabled the promotion of the Society to environmental officers with an interest in microbiology. A further meeting I attended in September was the three day Institute of Biomedical Science (IBMS) Congress held at the International Convention Centre in Birmingham. The Society sponsored one of the Scientific Sessions on *Escherichia coli* and this enabled a large number of Biomedical Scientists working primarily in Clinical Microbiology laboratories to be exposed to SfAM. The meeting generated interest in both individual and corporate membership.

In addition to these formal scientific meetings, I have represented the Society at the Annual Delegates meeting of the Federation of European Microbiology Societies (FEMS) which was held over two days in Bologna in September. In October I also attended the Governing Board meeting for Med-Vet-Net which was held in Paris.

I have also held meetings and had discussions with other learned Societies. These include meeting representatives from the Society for General Microbiology (SGM) and Institute of Food Science Technology (IFST). These exploratory meetings were called to assess how the organisations could collaborate on issues of mutual interest which affect their members. In addition, I have met with representatives of the Science Media Centre, an organisation which acts as the conduit of communication between scientists and all areas of the general media.

My day-to-day activities have included meetings with the many professionals who provide services to the Society. These meetings have included discussions with bankers, accountants, stockbrokers and the solicitor.

I welcome comments and suggestions from members to improve the services offered by the Society. I can be contacted at the Society office by telephone or email at: pfwheat@sfam.org.uk.

I wish you all a happy Christmas and a prosperous 2006.

Philip Wheat
Chief Executive Officer

MED•VET•NET

Teresa Belcher reports on training researchers in science communication

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 of 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 5 years.

Part of the remit of the **Med-Vet-Net** Communications Unit is to train up to nine researchers from the partner Institutes in science communication. A Science Communication Internship has been developed, and the first interns began the three-month programme on 19 September 2005. The two current candidates are Dr Emmanuelle Bensaude, who currently works at the Veterinary Laboratories Agency (VLA), Weybridge, UK and Dr Sonia Tellez from the Universidad Complutense of Madrid (UCM), Spain (see biographies).

The Internship consists of a 12-week period of full-time training / tutorials in various aspects of science communication including:

- Communicating with government and industry
- Communicating with the media
- Presentation skills
- Internet and website design
- Writing skills and publications
- Communicating with the public and children
- Organising events and exhibitions

The first six weeks of the course has encouraged debate about why science needs to be better communicated. Discussions have examined the inter-relationship between policy, public opinion and the media and how this affects how science is perceived and reported in the news.

In the first week, the interns attended the EURAGRI conference in York where the future direction of the European research in agriculture was being discussed. They actively spoke to attendees about **Med-Vet-Net** and the poster being presented, as well as conducting interviews with Professor Antoinette Betschart, the Associate Administrator of the American Agriculture Research Service (ARS) and Professor Howard Dalton, the Chief Scientific Advisor at DEFRA, UK.

The second week, a delegation consisting of the interns, Teresa Belcher and Lucy Harper from the Communications Unit, Claire Cassar, **Med-Vet-Net** Deputy Project Manager, and SfAM's external affairs advisor, Professor Nigel Poole, set off for Brussels. Here we visited the industry association EuropaBio, the European Commission to discuss EC communications, large biotechnology company Pioneer/Dupont and the MEP Phillip Whitehead. All these visits were very thought provoking and created great discussion about the considerable number of influences there are affecting science policy decisions.

The interns have also undertaken a number of courses, covering the communications between media-public-industry, writing press releases, public speaking, and networking.

They have visited the Science Media Centre in London, an organisation which aims to act as the link between journalists and scientists, and will assist in the running of an upcoming media event.

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**

They have also had some intensive training in the development and management of websites.

The remaining time of the internship is equally busy and will equip the interns with a full range of skills to enable them to become effective science communicators. In the last six weeks, they will be attending the 'Communicating European Research' (CER) conference in Brussels, will visit the Dutch **Med-Vet-Net** partners, complete a desktop publishing course, and visit some further biotechnology companies. Another aspect of science communication that will be examined is communicating science to children. This will encompass a visit to the science museum Techniquest in Cardiff where regular practical sessions for schools are run. The interns will also learn more about exhibits, exhibition design, and online learning resources.

Following completion of the 12-week training, the interns will return to their previous jobs. For three months, they will work at least 50% applying the skills learnt by communicating the work of **Med-Vet-Net** as well as their institute, and also assist the **Med-Vet-Net** Communications Unit with the dissemination of information throughout Europe. Following that, we hope they will continue to be a point of 'communications' contact for the Communications Unit.

So far, the internship has proved to be very successful. It is anticipated that a further four interns will be recruited to be trained for three months in Autumn of 2006. These positions will be advertised soon. As part of the communications strategy for **Med-Vet-Net**, we hope to train at least one representative from each of the partner countries. These trained scientists will return to their institutes with the communication skills necessary to assist in the further dissemination of **Med-Vet-Net's** research.

Biographies

Emmanuelle Bensaude

Emmanuelle studied Chemistry and Biochemistry at the University of Paris VI, before doing an MSc in Molecular Biology and Plant Biotechnology at Toulouse III. She has been working at the Veterinary Laboratories Agency since 1998, where she studied for a PhD on the molecular interactions between classical swine fever virus and host cells. Emmanuelle's areas of scientific expertise are molecular diagnosis of pestiviral diseases, and viral strategies to evade their host innate immune responses. She is currently one of the lucky **Med-Vet-Net** interns studying Science Communication. Outside work, Emmanuelle enjoys hiking, cooking for friends and playing with her two-year old son.

Sonia Tellez

Sonia is a veterinarian, with a B.Sc. and Ph.D. from Universidad Complutense of Madrid, Spain. The title of her Ph.D. was "Detection frequency and characterization of *Salmonella* spp. isolated from reptiles and amphibians" and was completed in 2003. Since she finished her B.Sc. she has been working as a researcher in clinical microbiology, studying zoonotic bacteria such as *Salmonella*, *Campylobacter* and *Clostridium* at the Animal Health Department of Veterinary Faculty at Complutense University. Between 2001 and 2002 she has worked at the Investigation Centre of Animal Health (CISA) researching bioterrorism-related anthrax.

Sonia's main areas of expertise are foodborne pathogens, clinical microbiology of livestock and exotic animals and disease epidemiology. She will soon begin work as the manager of the Communication Department at the soon-to-be-formed Complutense Institute of Animal Health (ICSA).

Teresa Belcher
Med-Vet-Net Communications Director

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

Dr V Korouk

India

Dr G. P. Rao

Northern Ireland

Mr K McGreeghan-Crosby

United Kingdom

Corporate: Barloworld Scientific; Quadratech Diagnostics Ltd

Mr D Bramston; Dr A Brown; Dr J Crowe; Prof. A. J. Fox; Mrs J Hook; Dr P Humphreys; Mr M J Hurst

USA

Mr A Ciralsky; Dr K Decker; Mr G Kirker; Dr R Montalvo; Mrs C Smith

Sponsor a new Member of the Society and win a £50 Book Token!

Could you be the next winner of the 'SfAM Sponsor of the Year' Award?

If you feel you could be our next winner for 2005, and would like some promotional material to help you recruit new members please contact Julie Wright, Membership Co-ordinator on 01234 326661 or email julie@sfam.org.uk.

New Committee Members

We welcome two new members of Committee, Dr Andy Sails and Dr Tony Worthington

Andy Sails

Andy is a clinical scientist based at the Health Protection Agency Laboratory in Newcastle upon Tyne where he is Head of the Research and Development unit. He began his microbiology career in 1991 as a trainee biomedical scientist at Preston Public Health Laboratory. Further postgraduate training at Preston resulted in a Masters degree in Biomedical Science from Manchester Metropolitan University. In 1996 he was seconded into the PHLS *Campylobacter* Collaborating unit at Preston to work on a project investigating the use of PCR to overcome the difficulties of detecting campylobacters in food and related products. The project formed the basis for his PhD studies which he completed in 2000.

He then left the PHLS and UK to take up a Postdoctoral Fellowship in the Food borne and Diarrhoeal Diseases Branch at Centers for Disease Control and Prevention (CDC) in Atlanta, USA where he worked on DNA sequencing-based methods for fingerprinting food borne pathogens. He left CDC in 2003 to take up his current post here at Newcastle, where he lead the development and evaluation of new technology and methods for microbiological diagnosis, identification and typing within the laboratory. He also assists in their transfer into routine service use. His main research interests include the detection and epidemiological fingerprinting of bacterial pathogens and the application of PCR to clinical microbiology to aid the diagnosis and management of infectious disease.

He has been a member of the Society for Applied Microbiology for approximately ten years. He is looking forward to serving on the SfAM committee to support the society in its work and to help it to achieve the goals set out in the society mission statement. In particular he would like to promote society membership to colleagues in both clinical and food, water and environmental microbiology laboratories. In addition he would like to work with the Society to promote the public understanding of microbiology, both to raise its profile to the general public and to encourage students to seek careers within microbiology.

Tony Worthington

Tony began his career in Microbiology in 1985 as a trainee Biomedical Scientist in the Pathology Laboratories at Birmingham Heartlands Hospital. Between 1985 and 1991, he undertook a

Higher National Certificate in Medical Laboratory Sciences (specialising in Medical Microbiology) at the University of Central England, Birmingham, and achieved State Registration as a Biomedical Scientist. In 1991, he became a Fellow of the Institute of Biomedical Sciences (FIBMS) following successful completion of the IBMS fellowship examination in Microbiology.

Between 1991 and 1999, Tony worked as a Senior Biomedical Scientist in the Department of Clinical Microbiology and Infectious Disease at the University Hospital Birmingham NHS Trust; one of the UK's leading teaching hospitals which specialises in organ transplantation. In addition to his full-time post as a Senior BMS, in 1996, he undertook a part-time PhD at Aston University to investigate the serological response to staphylococcal short-chain lipoteichoic acid in patients with infected central venous catheters and graduated in 2000. Whilst undertaking his PhD, in 1999, Tony was appointed Clinical Research Scientist (grade B) within the UHB NHS Trust and managed several clinical research projects which focused primarily upon the rapid diagnosis of infections associated with indwelling intravascular devices and prosthetic joints, skin disinfection and also epidemiological studies on antibiotic resistant microorganisms associated with hospital acquired infections including MRSA, vancomycin resistant enterococci and *Clostridium difficile*.

In 2004, Tony was appointed as Lecturer at Aston University where he is continuing to undertake research into skin disinfection and *Clostridium difficile*. Tony has presented his research at national and international scientific conferences and currently has in excess of 30 publications in peer reviewed journals. In addition to research at Aston University, Tony is the undergraduate admissions tutor for Biology and his teaching comprises clinical microbiology and infectious disease, treatment of infection and gastrointestinal parasitology.

On a personal level, Tony lives Solihull in the West Midlands. He is married and has a two-and-a-half-year-old daughter. Tony is also quite a keen sportsman and whilst he enjoys partaking in the occasional football match with his colleagues and students at Aston University, he much prefers to watch the game from the sidelines as an avid Birmingham City FC supporter and season ticket holder.

Archive Documents

The society archivists would like to thank to Jan Crabtree who kindly donated a number of the Societys proceedings and journals on behalf of her father Geoff Walker. Geoff and Jan were keen to see the journals go to a good home. Geoff was a member of the Society from 1941 – 1987 when he retired. He continues to speak highly of his membership with us.

Good News for Ex-President!

Not content with no longer having a regular column in the *Microbiologist*, our previous president, Peter Silley appears here with two pieces of excellent news!

We are delighted to announce that Peter Silley has been appointed as Visiting Professor of Applied Microbiology within the School of Life Science at the University of Bradford. Peter has been teaching for a number of years and now looks forward to playing a more prominent role within the University. The appointment comes at an opportune time as Peter is also leaving Don Whitley Scientific in order to concentrate more fully on MB Consult, his highly successful microbiological consultancy business. MB Consult is very much involved in the veterinary pharmaceutical sector and Peter will bring this experience to the University, giving students an awareness of some of the issues facing microbiologists in industry. Paul Walton, DWS managing director, said: *"Our sincere congratulations to Peter on this new appointment. He has been an enormous influence within Don Whitley Scientific for over fifteen years, contributing very significantly to our growth, development and profitability – directors and staff wish him and his family well for the future."*

The second piece of good news is that, on 21 August this year, at the General Assembly of the European Federation of Biotechnology (EFB), now *Professor* Silley, was elected onto the Executive Board. Peter was elected as a member from Industry and he will represent the Society through his role as Custodian Trustee. Congratulations, Peter, from all at SfAM.

SfAM WEBSITE www.sfam.org.uk

Have you visited the SfAM website lately? As well as keeping you up-to-date with SfAM news and activities, it offers full SfAM members many other services. If you are a Full Member or Full Student Member, log on, using your SfAM username and password, to:

- advertise microbiology job opportunities (free!)
- post your CV (free!)
- advertise your microbiological skills or consultancy (a small annual fee is required)

Have you forgotten your username and password? Go to the website and click on 'Services' - 'Member Log on' and then follow the instructions on that page to have your username and a new password emailed to you.

Don't miss out on our wide range of grants, including our newest grant, the SfAM Fellowship. Details and application forms can be found at:

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

Coming soon – online booking for the 2006 January Meeting. You'll find the complete programme online and on page 21 in this issue of *Microbiologist*.

New corporate members join SfAM

society for applied sfam microbiology

We are extremely pleased to announce that the last six months has seen the recruitment of six new corporate members to the Society. We welcome **Acolyte, Astra Zeneca, Barloworld scientific, Mast, Pro-lab** and **Quadratech** to the Society.

Could YOU benefit?

Did you know that the Society has many generous grants and prizes available to members? To find out if you are eligible and could benefit visit the website at: **www.sfam.org.uk**

Advertise!

With a highly targeted circulation of 2000 copies, *Microbiologist* is a cost-effective way for members and non-members to reach qualified microbiologists in industry, academia and public services, both in the UK, and worldwide.

For more information about the benefits and costs of advertising your products or services in *Microbiologist* please contact the Society Office or visit the website.

BUGS CV

Lucy Goodchild looks at the CV and asks *bifidobacterium bifidum* a few pressing questions to see if he's up to the job!

Job Title:

Keeping the immune system healthy

Name:

Bifidobacterium bifidum (recently identified as *B. lactis*)

D.O.B

1900 (Discovered by Tissier)

Relatives in the trade:

Lactobacillus acidophilus is a cousin of mine. My two closest relatives are *B. longum* and *B. infantis.*

Appearance:

Gram positive, branched, rod-shaped. This is down to my genome, which is very similar to that of *B. longum,* which is circular and over 2 million base pairs long. It has a high G-C content, around 60%.

Address:

Mucous membrane lining of the large intestine and in the vaginal tract of humans. I live with about 400 other species. I can sometimes survive the acidic conditions of the stomach. I also live in food like yoghurt, cottage cheese, buttermilk, soy sauce, miso, tempeh and even in fermented vegetables like sauerkraut.

Skills:

I can digest lactose and produce lactic and acetic acid. I also digest fructooligosaccharides and many other molecules that humans cannot digest.

Employment:

I am currently used in many 'probiotic' foods and drinks, including products like *Yakult.* I am also used in probiotic pills. There has been research into employing me in probiotic ice cream and in beads for oral use (See referees).

That was an impressive CV, so we decided to bring him in for interview. Let's see how he performs

Interview

Interviewer: So let me just check something first. What is your name? I'm a little confused.

B.bifidum: Well, I don't blame you, I have lots of names! I am best known as one of the friendly bacteria, but many others share that nickname. Some people also call me a probiotic. My birth name is *Bifidobacterium bifidum*, but it has recently been changed to *Bifidobacterium lactis*.

Interviewer: Why is that?

B.bifidum: Well, taxonomy and classification are always changing. I am from the family *Bifidobacteriaceae* and my genus is *Bifidobacterium.*

Interviewer: I see. So, do you come from a large family?

B.bifidum: Oh yes. I have many relatives. *Lactobacillus acidophilus* is one you may know. I am also closely related to *B. longum* and *B. infantis*.

Interviewer: Do you live with them?

B.bifidum: Yes, I mostly reside in the mucous membranes of the large intestine along with about 400 other species. I also stay in the vaginal tract and in some foods.

Interviewer: Goodness, it must be crowded!

B.bifidum: It can be, but that's good.

Interviewer: Why is that?

B.bifidum: Well, if there are a lot of us friendly bacteria, then pathogens have nowhere to live. And they are the kind of bacteria you definitely do not want around! *Salmonella, Shigella, E. coli* and C. *perfringens* all try to get space in the intestine, but if there are lots of us they stay away.

Interviewer: So presumably that is the reason you are being used in health supplements?

B.bifidum: Absolutely. There are lots of other reasons too. I am particularly good at digesting lactose, like many of my relatives, and I can produce beneficial acids like lactic and acetic acid, that lower the pH of the intestine and even prevent constipation and diarrhoea. I can also digest fructooligosaccharides, which is something humans cannot do.

Interviewer: What are they?

B.bifidum: They are short chains of sugar molecules, found in bananas, garlic, honey, leeks, spinach and lots of other foods. Eating them helps us friendly bacteria to grow in the intestine.

Interviewer: Interesting. So what other useful skills do you have?

B.bifidum: I absorb ferrous ions, certain forms of iron. Pathogens need these to live so I stop them from growing that way. I can also help the body absorb different minerals like calcium, magnesium and zinc.

Interviewer: So you have lots of plus points for health. But how do you propose people increase your number?

B.bifidum: The most popular way at the moment is through probiotic drinks like *Yakult.* I can sometimes survive the acidic conditions in the stomach so I do occasionally end up in at home in the intestine. I can also be put into pills and I have even been stored in ice cream!

Interviewer: So we can see that you are good for digestion, but what about preventing colds?

B.bifidum: Well, a study has recently been conducted by Michael de Vrese and his team at the Federal Centre of Nutrition and Food in Kiel, Germany, showing that taking probiotic supplements can reduce the effects of infections.

Interviewer: How did they come to that conclusion?

B.bifidum: They compared the effects of normal vitamin supplements to those of a probiotic, *Multibionta*, on colds in 479 healthy 18 to 76 year olds. Colds were shorter and less severe in the people taking probiotics.

Interviewer: That sounds very interesting indeed. I think you should be employed, on a trial basis initially. It would appear that you are highly suited to the job of keeping the human digestive system healthy, but we do not have much evidence for your effect on the immune system just yet.

Referees

- http://www.dsmz.de/bactnom.nam0541.htm
- http://jds.fass.org/cgi/content/abstract/75/6/1415
- http://open-access-biology.com/probiotics/reuter/reuter.html
- Kushal, Ruchi, Studies on Microentrapped Co-culture of *Lactobacillus Acidophilus* and *Bifidobacterium Bifidum* for Inhibition of Enteropathogens, National Dairy Research Institute (www.shaping-the-future.de/pdf_www/125_paper.pdf)
- Bacteria may ease common cold, *The Times*. www.timesonline.co.uk/printFriendly/0,,1-2-1777221-210,00.html

Adenylate Kinase Assay may provide Key to more Rapid Analysis of Bacterial 'Fitness'

Acolyte Biomedica develops proprietary diagnostic systems for clinical microbiology.

FOR FURTHER INFORMATION see page 15, or visit: www.acolytebiomedica.com/

TECHNOLOGY originally developed by the UK Defence Science and Technology Laboratories (Dstl) at Porton Down for battlefield based bio-warfare agent detection, has formed the basis of a new rapid bacterial detection assay. Researchers at The Wellcome Trust Sanger Institute have used it to examine the growth kinetics of *Salmonella serovar* typhi containing different mutations associated with resistance to the fluoroquinolone group of antibacterial agents.

The assay, *RapiTECT GN* from *Acolyte Biomedica*, uses an Adenylate Kinase (AK) mediated bioluminescence detection system as a means of quantifying the number of bacteria in a liquid sample. AK is a constitutive enzyme that catalyses the conversion of ADP to ATP and is found in all bacteria (and other living cells). The assay method involves the addition of a bacterial lysis reagent to release the AK into the surrounding matrix. ATP is then generated by the AK enzyme following the introduction of an excess of ADP. Firefly luciferase/luciferin is added to the sample, light is emitted in the presence of the ATP and the photon emission is measured using a luminometer. Although certain aspects of the assay may be recognisable to those who already perform traditional ATP bioluminescence assays, the sensitivity of this method is improved due to the equilibrium reaction being artificially driven to overproduce ATP by the addition of an excess of ADP (Fig. 1). Moreover, unlike traditional ATP assays AK levels are constant and do not fluctuate with metabolic state, providing a more reproducible, quantifiable signal which closely correlates with cell numbers. These features enable small changes in the microbial population to be detected at a very early stage of the growth cycle.

The target enzymes for the fluoroquinolone antibacterial agents are topoisomerases. Several mutations in topoisomerase enzymes have been linked to the development of low level resistance both *in vivo*, and high level resistance *in vitro*. It is possible that high level resistance is counter selected in vivo after the antibiotics are withdrawn. Point mutations were introduced into the GyrA, and ParC subunits of *S. typhi* to generate isogenic mutants with high and low level resistance. The AK detection assay was then used to compare the growth rate of 24 strains containing different mutations, further experiments are also planned to measure the time-kill

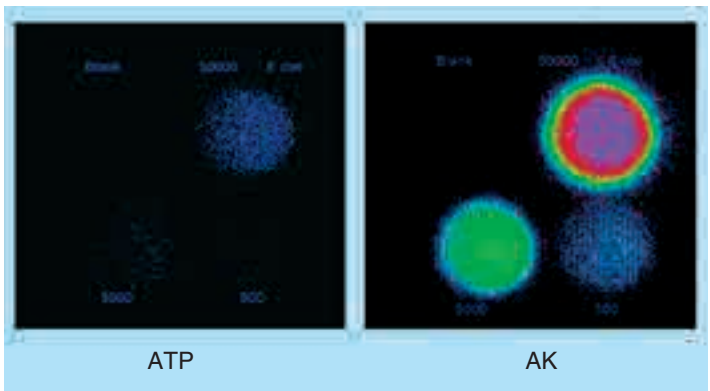


Fig. 1. *- Comparison of traditional ATP and AK bioluminescence*

properties of fluoroquinolones against each mutant. As the effects of the combination of mutation and the exposure to fluoroquinolone on cell size and clumping was unknown, the use of optical density was not considered appropriate. Furthermore the counting of bacteria down to 10^2 CFU/ml from an inoculum of 10^6 CFU/ml is necessary to establish bactericidal activity. This cannot be achieved by optical density measurement. It was on this basis the AK detection assay was assessed for suitability for this purpose.

An attenuated strain of *S. typhi* was used as the background for the introduction of mutations into target enzymes. The strain is dependent on aromatic amino acids (aro) and will grow only if these are added to the media. Using a Thermo LuminoSkan single tube luminometer the growth curve in Isosensitest broth (Oxoid, UK) plus aro supplement, was followed by RLU for the susceptible parental strain. This was carried out using the standard AK assay protocol from *Acolyte Biomedica*. In order to assess the reliability of the method this was compared to a plate count of colony forming units (CFU) calculated using a 20μL surface drop method on LB agar plus aro supplement. The results showed good correlation (R^2 = 0.993) between CFU and RLU over a growth curve from 10^5 to $2x10^6$ CFU/mL (Fig. 2).

Two low level and two high level topoisomerase mutants were then tested in triplicate using the AK assay alone. The slopes of the resulting growth curves were calculated using *Prism4 for Windows* (Graphpad software Inc.). The results indicate that the susceptible parental strain behaved more reliably than the mutants, so it was concluded that the variation was not methodological.

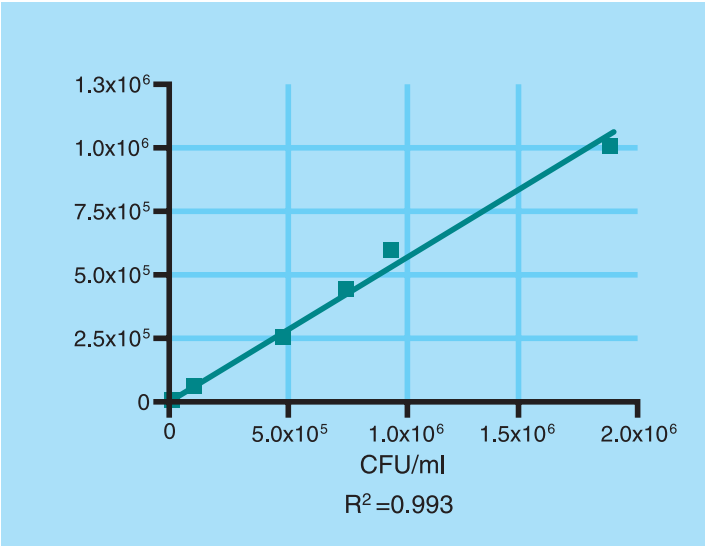


Fig. 2. *– Correlation between CFU and AK assay RLU for attenuated strain of S. typhi*

Although variation was seen there was a detectable, but not statistically significant, difference in the growth rate of the strains. More repeats using very carefully standardised conditions are necessary before definite conclusions can be drawn.

The measurement of growth rate has been applied to the analysis of bacterial cultures since bacteriology began. Growth rate has been used as a surrogate marker for virulence and as a phenotype representing the fitness of bacterial isolates. Techniques for the measurement of growth rate; colony counting and measurement of optical density, however are fraught with problems. For example: Contamination of slow growing organisms such as *Mycobacterium tuberculosis*, clumping, or filament formation on exposure to different test conditions effects the estimation of bacterial numbers by both CFU or measurement of optical density, in unknown ways. Automated instrument assays for measuring growth rates by measurement of the activity of a constitutively expressed enzyme, such as AK, has several advantages. These include a high level of sensitivity (10 organisms) which would allow the accurate quantification of live bacteria down to levels found towards the end of standard time-kill assays. Starting with an inoculum of 10^6 bacteria a four fold reduction in live count (normally considered to represent killing) would require the estimation of bacteria at 10^2 per ml. This is impossible by optical density measurements and very time consuming by culture as it requires numerous dilutions and replicates for reliable data. Full automation of growth curves, including killing curves, would be of immense benefit in several fields of bacteriology. Whilst this is not available with the current assay format (as it is necessary to sacrifice the cultures in order to count bacteria) multisampling of the experimental culture vessel using multi-channel pipettes for sampling and an automated luminometer allows the use of 96 well microtitre plate format. The ability of the AK assay to accurately measure small changes in microbial biomass mitigates the need for numerous sample testing points over a time course.

For the estimation of bacterial numbers in cultures over a wide range detection of AK has exciting possibilities. Further testing of this assay for the detection of survival of bacteria after exposure to antibiotics is currently underway. Moreover as AK is present in all living cells this technology can be adapted to provide an equally rapid, accurate means of detecting and enumerating any prokaryotic and eukaryotic cell types. This presents numerous possibilities within bioengineering, pharmaceuticals and cell culturing, replacing many of the lengthy, laborious techniques currently being used.

Dr John Wain
The Wellcome Trust Sanger Institute

a one day meeting on

Epidemiology and vaccines

The Royal Society, Carlton House Terrace, London
Thursday 5th January 2006

CPD ACCREDITATION

A total of 7 IBMS credits has been awarded to this conference

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 attachement to an email addressed to info@sfam.org.uk with the subject line 'January 2006 meeting submission'.

- Including: **The Denver Russell Memorial Lecture**

Parallel Sessions on:

- Vaccines
- "From postcodes to pandemics – global infectious disease epidemiology on your doorstep"

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

January Meeting 2006

Programme

10.00-10.30 Arrival/ Coffee/ Registration

10.30-11.15 The Denver Russell Memorial Lecture: The use of Microbiocides, in Infection Control: a critical look at safety, testing and applications.
Prof. S. Sattar, University of Ottawa, Canada.

11.15-11.45 Combating the Inevitable - Pandemic Influenza
Dr. J Robertson, NIBSC, UK.

11.45-12.15 How epidemiology informs vaccine design.
Prof. Martin Maiden, University of Oxford, UK.

12.15-13.15 Lunch

Afternoon: two parallel sessions of five talks (thirty minutes each)

Session A: Postcodes to pandemics: new perspectives in global disease epidemiology.

13.15-13.45 Appending neighbourhood codes to name: lessons microbiology can learn from health promotion practitioners.
Prof. Richard Webber, University College London, UK.

13.45-14.15 Epidemiology of TB in Asian and UK communities.
Prof. Peter Hawkey, HPA Birmingham, UK.

14.15-14.35 Tea

14.35–15.05 Epidemiology of gastrointestinal pathogens in the Grampian Regions
Dr. Norval Strachan, University of Aberdeen, Scotland.

15.05-15.35 Spatio-temporal statistics for infectious disease data.
Prof. Peter Diggle, Lancaster University, UK.

15.35-16.05 Impact of global climate change on infectious disease epidemiology.
Prof. Paul Hunter, University of East Anglia, UK.

16.10 Meeting closes

Session B: Current Vaccine Issues

13.15-13.45 Conjugate vaccines in the infant immunization programme.
Dr. Andrew Pollard, Dept of Paediatrics, University of Oxford, UK.

13.45-14.15 MMR
Dr. Mary Ramsay, CDSC, HPA Colindale, London, UK.

14.15-14.35 Tea

14.35-15.05 Bioterrorism Vaccines.
Prof. Richard Titball DSTL, Porton Down, UK.

15.05-15.35 Human Papilloma Virus vaccines
Speaker TBA.

15.35-16.05 Latest developments in TB vaccines.
Dr. Doug Lowrie, NIMR, Mill Hill, UK.

16.10 Meeting closes

Please note that the above paper titles and speakers were correct at the time of going to press but may be subject to change.

CPD ACCREDITATION

A total of 7 IBMS credits has been awarded to this conference

The Denver Russell Memorial Lecture is sponsored by

CTFA

Ciba

RECKITT BENCKISER

Book online!

Members and Non-members who wish to book their place at this meeting can now book online via the website using our secure online booking form, or by printing out and completing our PDF booking form. To book and pay online simply point your browser at: www.sfam.org.uk/janmeet.html

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

BOOKING FORM and INVOICE

SFAM JANUARY MEETING 2006 THURSDAY 5th JANUARY 2006

a one day meeting on Epidemiology and vaccines

Only ONE person per form please. If additional forms are required please photocopy this one. CLOSING DATE FOR REGISTRATIONS: Thursday 1st December 2005. A LATE BOOKING FEE of £20.00 will be applied to all bookings made after this date.

FEES

Whole Conference Rate: inclusive of registration fee, coffee breaks and lunches.	Full Members	Student, Honorary, Associate & Retired Members	Student Non - Members	Non - Members
	£50.00	£30.00	£60.00	£95.00

YOUR INTERESTS

Below is a list of some of the meeting topics. Please indicate which topic you would like to attend by ticking the appropriate box(es). Places will be reserved on a first-come first-served basis, but we will try to accommodate your preference

- ☐ **Session A:** Appending neighbourhood codes to name: lessons microbiology can learn from health promotion practitioners.
- ☐ **Session A:** Epidemiology of TB in Asian and UK communities.
- ☐ **Session A:** Epidemiology of gastrointestinal pathogens in the Grampian Regions.
- ☐ **Session A:** Spatio-temporal statistics for infectious disease data.
- ☐ **Session A:** Impact of global climate change on infectious disease epidemiology.
- ☐ **Session B:** Conjugate vaccines in the infant immunization programme.
- ☐ **Session B:** MMR
- ☐ **Session B:** Bioterrorism Vaccines.
- ☐ **Session B:** The Challenges in Developing a Vaccine for Immunocompromised Patients: 'The StaphVAX Story.'
- ☐ **Session B:** Latest developments in TB vaccines.

YOUR COSTS

Charges - please tick the applicable box(es)	Amount
☐ **Whole Conference Rate:**	£
☐ **LATE BOOKING FEE** Payable for all bookings made after Thursday 1st December 2005	£20.00
TOTAL AMOUNT REMITTED:	£

YOUR DETAILS

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

Address:_____

_____ **Postcode:**_____

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

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

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.

☐ **Cheque enclosed** ☐ **Please charge my *Mastercard/Visa card /Debit card*** *(please delete inapplicable items)*

TOTAL Amount enclosed/ to be debited: (*Remember to include your LATE BOOKING FEE if booking after 1st December 2005) **£** _____

Card number: ☐☐☐☐ ☐☐☐☐ ☐☐☐☐ ☐☐☐☐ Expiry Date: ☐☐☐☐

Issue No. ☐☐☐ Start Date: ☐☐☐☐ *(Debit cards only)*

Signature: _____ *Date: _____ Cardholder's address to which credit card statement is sent:

_____

_____

Please return the completed form by fax (post if you are enclosing a cheque) to:

The Society for Applied Microbiology, The Blore Tower, The Harpur Centre, Bedford MK40 1TQ, UK.

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

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

Scientific programme

The overall aim of the conference is to promote understanding of the complex microbial ecosystems that are present in the digestive tract of man and animals, and their interactions with the host.

It is therefore of interest to those working to improve human and animal health and nutrition through dietary manipulation, and to any scientist concerned with the microbial ecology of the digestive tract and the interplay between micro-organisms and their hosts.

Abstracts

Abstract submission and symposium proceedings. All abstracts are to be submitted electronically via the website.

Deadlines

Abstract submission: 31 January 2006
Final registration: 31 March 2006.

Registration

Those interested in attending the conference are requested to pre-register on the RRI-INRA2006 website.

Information

Mrs V. Smith
RRI–INRA Secretariat, Rowett Research Institute, Greenburn Road, Aberdeen AB21 9SB, UK.

Aberdeen 21 - 23 June 2006

Conference website: www.rowett.ac.uk/RRI-INRA2006

ICFMH Working Party on Culture Media (WPCM)

The WPCM is organising a Workshop on 29 August at 'Food Micro 2006' in Bologna

The principal task of the workshop will be the revision of all the monographs on culture media published in the Pharmacopoeia of Culture Media (see *Handbook of Culture Media for Food Microbiology*, Elsevier Science, Amsterdam). To assist this work Assessors are required, particularly for the following monographs:
All purpose Tween agar, Bile oxalate sorbose broth, Brigg's agar, Cellobiose polymyxin B colisitin agar, Differential clostridial agar, Enterococcosel agar/broth, Iron sulphite agar, LS differential agar, M17 agar, Preston broth, Rapid perfringens medium and Sulphite cycloserine agar.
Any worker who is able to share QA results for any of the media with the Working Party is asked to contact the Convenor, Gordon Curtis, at gdwcurtis@onetel.net, from whom further details may be obtained.

A limited number of places will be available at the Workshop. Priority will be given to those who have offered to assess Monographs. Full details of Food Micro 2006 can be found at: www.foodmicro2006.org/home.asp

Bologna 29 August 2006

Conference website: www.foodmicro2006.org/home.asp

CPD ACCREDITATION
applied for

75th Anniversary Conference 1931 - 2006

Living together: polymicrobial communities

Apex International Hotel, Edinburgh, UK
Monday 3 to Thursday 6 July 2006

- Including: **Lewis B. Perry Memorial Lecture**

There will be sessions on:

- Physiology and functionality of polymicrobial communities
- Influencing polymicrobial communities
- The gut microflora
- Bioremediation

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 attachement to an email addressed to info@sfam.org.uk with the subject line 'Summer Conference 2006 submission'.

Please note that the conference 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 3rd July

Arrive and Register.

18.00-19.00 **Lewis B Perry Memorial Lecture: Out of a dusty archive – from SAB to SfAM, the first 75 years.**
Professor Max Sussman.

19.00-20.00: **Drinks Reception.**

From 20.00: **Evening at leisure.**

Tuesday 4 July

Session 1. Physiology and functionality of polymicrobial communities.

09.00-09.35 **Interspecies signalling communication.**
Dr Miguel Camara, University of Nottingham, UK.

09.35-10.10 **Co-ordination and Competition in specialised microbial communities.**
Dr Andrew Whiteley, University of Oxford, UK.

10.10-10.45 **Adaptation and evolution in a two-species structured community.**
Prof Soren Molin, Technical University of Denmark.

10.45-11.15 **Coffee/ posters.**

11.15-11.50 **The role of niche differentiation in the community assembly and coexistence of uncultured bacteria from the genus *Achromatium.***
Dr Neil Gray, University of Newcastle, UK.

11.50-12.25 **Genomics, ecophysiology and interactions of yet uncultured nitrifying bacteria.**
Dr Holger Daims, Vienna, Austria.

12.25-13.00 **Living together while being eaten: *Bdellovibrio* predation in polymicrobial communities.**
Dr Liz Sockett, University of Nottingham, UK.

13.00-14.00 **Lunch.**

Session 2. Influencing polymicrobial communities.

14.00-14.35 **Combating polymicrobial communities: learning from Nature.**
Prof. Peter Steinberg (Director, Centre for Marine Biofouling & Bio-Innovation).

14.35-15.10 **Probiotic modulation of the oral flora.**
Prof. Jeffrey Hillman, University of Florida, USA.

15.10-15.45 **Using synbiotics to address major gut problems.**
Prof. Stig Bengmark, University College London, UK.

15.45-16.15 **Tea/posters**

16.15-16.50 **Impact of antibacterial usage on polymicrobial communities.**
Prof. P Gilbert, University of Manchester, UK.

16.50-17.25 **Impact of antimicrobial residues on gut communities: are the new regulations effective?**
Prof. P. Silley, MB Consult, UK.

17.30-19.00 **Trade Show**

Wednesday 5th July

Session 3. The gut microflora

09.00-09.35 **Bacterial metabolism and interactions in the gut.**
Prof. Harry Flint, Rowett Research Institute, Aberdeen, UK.

09.35-10.10 **Probiotics and gut biofilms.**
Dr Sandra MacFarlane, University of Dundee, UK.

10.10-10.45 **The gut flora in early life.**
Dr Christine Edwards, University of Glasgow, UK.

10.45-11.15 **Coffee/ posters.**

11.15-11.50 **Intestinal bacteria and ageing.**
Dr Emma Woodmansey, Smith and Nephew Research Centre, York, UK.

11.50-12.25 **Microbial interactions with the gut immune system.**
Dr Elizabeth Furrie, University of Dundee, UK.

12.25 -13.30 Lunch.

Session 4.

● Offered papers ● Student presentations I WH Pierce Prize ● Annual General Meeting.

19.30-20.00 **Drinks reception followed by Conference and 75th Anniversary Dinner.**
The Hub, The Royal Mile.

Thursday 6th July

Session 5. Bioremediation

09.00-09.35 **Bacterial and fungal transformations of metals, minerals and metalloids.**
Dr Geoff Gadd, University of Dundee, UK.

09.35-10.10 **Contaminant degradation in terrestrial environments: multiple roles of fungi and protests.**
Dr Hauke Harms UFZ, Germany.

10.10-10.45 **Phenolic degrading communities: functional phylogeny, assembly and stability.**
Dr Andrew Whitely CEH, Oxford, UK.

10.45-11.15 **Coffee/ posters.**

11.15-11.50 **Polymicrobial community strategies for mediating the bioremediation of complex organic mixtures.**
Dr Mike Larkin, Queen's University, Belfast, UK.

11.50-12.25 **Themes and variation: emerging patterns in microbial remediation of spilled oil.**
Dr Ian Head, University of Newcastle, UK.

12.25-13.00 **Natural attenuation (or lack of it) in two highly contaminated UK aquifers.**
Dr Roger Pickup, CEH, Lancaster, UK.

13.00-14.00 Lunch & Close

Steve Davies reports on the continuing war against Methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals and the community

THE WAR AGAINST methicillin-resistant *Staphylococcus aureus* (MRSA) in the UK continues both in the hospital and community environment. Recently published figures highlight the fact that Hospital Associated Infections, of which MRSA is the most notorious, cause approximately 5000 deaths annually and cost the NHS around £1 billion per year.

The recent European Antibiotic Resistance Surveillance System (EARSS, 2004) figures demonstrate that presently the UK has one of the worse MRSA records in Europe, despite the increased emphasis the Department of Health have afforded it. Additionally, increased problems with community MRSA are being seen, particularly those expressing the Panton Valentine Leukocidin (PVL) gene, which is currently causing devastating morbidity in individuals worldwide (CDR, 2005).

One characteristic that many microbiology laboratories still seem to overlook is the need for rapid results. Microbiologists' desire to be scientifically correct, by ensuring that techniques that are being used have the required sensitivity and specificity, tend to overshadow the length of time such accuracy takes. If one considers the question: Which poses the greater infection control risk, a patient or staff member with heavy growth of MRSA in their nose or one with a very light colonisation that may or may not be there if the sample is repeated a day later? The answer is obvious. However, whilst many laboratories are now using direct inoculation onto solid media as their main detection method, others persist in using broth enrichment and subsequent subculture onto solid media as their sole method, introducing a 24 hour delay on all samples, including those from patients/staff that are heavily colonised/infected. In my opinion, this desire to slightly increase sensitivity at the expense of responsiveness is ludicrous and is one of the reasons why little headway appears to have been made in reducing the number of MRSA infections.

Throughout science, molecular techniques that provide sensitivity, specificity and speed are becoming more widely available. MRSA detection is no different. Now available are molecular techniques that can provide a Negative Predicted Value of 100% for negative results after 24 hours (Kearns *et al.*, 1999), allowing better bed management of patients. Recently, however, a molecular technique that detects positive results in as little as two hours from receiving the swab, has become commercially available (GeneOhm Sciences) and for nasal swabs, it has the required sensitivity and specificity of 92.5% and 96.4% respectively. If these values are repeated in routine use and over a greater range of sample types, this could prove to be the advanced 'weapon' microbiology departments have been waiting for and would certainly enable us to create safer hospital bed usage.

At present, however, the expertise and expense of providing such a service routinely on all screening swabs is prohibitive to most routine laboratories. Initially, one imagines that high risk wards, such as general intensive care units, or surgical wards, such as orthopaedic surgery, may be given preference with the introduction of a more comprehensive and rapid pre-admission screening programme. It has been shown on many occasions, particularly in the Netherlands, that confirming that a patient's negative MRSA status, before admitting them onto the main ward reduces MRSA infection dramatically. A turnaround time of two hours for a MRSA result certainly makes this approach more feasible.

Another breakthrough in MRSA detection was the introduction of the MRSA-Screen Latex Agglutination Test that detects Penicillin Binding Protein 2a (PBP2a). MRSA contain the mecA gene, which is responsible for PBP2a production, which continues the biosynthesis of the organism's cell wall in the presence of cloxacillin/flucloxacillin (Livermore, 2001), enabling it to be indifferent to the presence of the antibiotic and hence resistant to it. This methodology allows the determination of whether a strain of *S. aureus* isolated during routine culture media (e.g. blood agar) is methicillin-resistant, or not, providing there are sufficient colonies of the organism in question. The procedure requires no specialist equipment and takes 20 minutes to perform and as such, should be an essential addition to the armoury of every microbiology department (Cavassini *et al.*, 1999). In other words, we can determine whether a proven *S. aureus* is a methicillin-sensitive (MSSA) or MRSA in 20 minutes rather than waiting the traditional 24 hours for the results of the overnight

SA INFECTION

fight goes on!

susceptibility testing to become available. Our laboratory routinely uses this methodology on samples from patients on moderate to high risk wards, who previously have not had MRSA and who have sufficient growth of *S. aureus* present to allow the test to be performed accurately. Fortunately, an in-house computer programme produces a test list based on these criteria, which results in between five to ten tests per day, of which there are usually daily positives. We have used this procedure for more than two years, which has again resulted in the detection of MRSA 24 hours earlier than previously. It is, however, important to be aware that coagulase-negative staphylococci (CNS) can also contain the mecA gene and hence produce PBP2a. This means that an unnoticed mixture of MSSA and MR-CNS could produce a false positive result.

The mainstay of all MRSA screening at present is the solid agar plate. There are several solid media that have been designed to isolate MRSA. Historically, many of these use mannitol fermentation to differentiate *S. aureus* and CNS and incorporate methicillin/oxacillin into the media as a selective agent. Unfortunately, like MRSA, many CNS ferment mannitol and are also methicillin resistant. This results in a loss of both sensitivity and specificity, with a sensitivity of only 60% found in one of our studies (Zadik & Davies, 2001). Previously we have recommended the use of Baird-Parker plus ciprofloxacin (BPC), as the best media where the epidemic strains, EMRSA 15 and 16 predominate (Davies & Zadik, 1997). This is due to the increased sensitivity, specificity and speed that it provided, when compared to mannitol-based media. Baird-Parker Medium is traditionally used to differentiate *S. aureus* and CNS in the food industry. It contains potassium tellurite, which inhibits most other organisms and egg-yolk. Unlike CNS, *S. aureus* have the ability to produce both lipase and lecithinase that breaks down the egg-yolk and produces a single halo-effect, and subsequently a double-halo effect, around the black colonies, after 24 and 48 hours respectively. In addition, EMRSA 15 and 16, as well as being methicillin-resistant, are also ciprofloxacin–resistant, whereas the vast majority of MSSA are ciprofloxacin-sensitive, and hence ciprofloxacin-resistance can be used as a marker for methicillin-resistance. The use of nasal swabs inoculated onto this media was also found to be the most cost effective screening procedure, when all papers were statistically reviewed.

Recently, however, the introductions of media containing chromogenic substrates that differentiate *S. aureus* from CNS have moved this discussion forward. The first to be introduced for *S. aureus* was CHROMagar™Staph aureus, which differentiates *S. aureus*, CNS and *Enterococcus* species based on chromogenic activity. They produce pink, white and blue colonies respectively. This has been previously recommended as an alternative to routine culture media for human clinical samples (Gaillott *et al.*, 2000). We have previously evaluated its suitability for MRSA screening by incorporating firstly methicillin and then ciprofloxacin, into the media. Unfortunately the methicillin-containing medium was too inhibitory (presumably due to not having an increased salt concentration in the media) and the ciprofloxacin-containing medium performed worse than Baird-Parker plus ciprofloxacin. The ease of recognition of MRSA isolates, however, was noted (Davies *et al.*, 2000).

Recent developments in the antibiotic disc sensitivity field have apparently changed this. For many years, confirming MRSA using disc susceptibility has been problematic and has required either incubating the sensitivity plate at 30°C instead of 37°C, increasing the salt concentration in the medium or a combination of both (Brown, 2001). Several papers, however, have been published that show that antibiotic discs, containing a Cephamycin or Moxalactam, appear to accurately confirm methicillin-resistance, without the need for either a reduction in incubation temperature or increase in salt concentration. (Skov *et al.*, 2003). Cefoxitin, a Cephamycin, has since been recommended at a concentration of 4 mg/l for agar incorporation techniques of antimicrobial susceptibility testing.

The next obvious advance is to combine the two apparent breakthroughs. Several commercial companies have realised this potential and have produced media containing both *S. aureus* differentiating chromogenic substrates and cefoxitin and many such media are becoming available for MRSA screening. So far the results appear very optimistic although there is a relative lack of published reports. We have recently performed an in-depth evaluation of two such media and compared them to four other solid media, including Baird-Parker plus ciprofloxacin. The results demonstrate statistically superior results for both chromogenic substrates containing media (awaiting publication), compared to the other four media. The vast majority of isolations were detected after 24 hours incubation, with excellent sensitivity and specificity. A multi-centred comparison of the numerous MRSA chromogenic media would be of value.

Moving onto the debate as to whether broth enrichment is necessary when screening for MRSA. Previously, we and

many others have shown that the use of overnight broth culture followed by subculture onto routine media has been proven to increase MRSA detection (Davies & Zadik, 1997), but the slight increase in sensitivity achieved has to be offset by the 24 hour delay that is often created in both confirming both a positive and negative result, as discussed earlier. An attempt to improve this situation is by the introduction of the antibiotic-containing mannitol salt indicator broth.

In theory, this broth approach attempts to combine the increased sensitivity of using broth enrichment with the mannitol-fermentation property of certain solid media. If successful, this indicator broth approach should allow a negative report to be issued after 24hours, if no mannitol fermentation has taken place. It also would allow more than one swab from the same patient to be inoculated into one broth, saving both on consumables and time. *S. aureus* (and some CNS) ferment mannitol, and in doing so produce acidic conditions, which in turn turns the phenol red indicator yellow. Therefore, any broths that remain red can be reported as MRSA not present after 24 hours and any that turn yellow or orange after 24 hours incubation are sub-cultured for confirmation, delaying a positive report but giving a good negative report turnaround time. Although this approach does seem quite appealing, we did evaluate one version of this approach in the two chromogenic substrate study described above and the reality appears to be a mixture of too many false-positives and a lack of sensitivity for the lighter MRSA colonisation/infections. I am confident, however, that commercial companies will try to improve this approach further.

Recorded history shows that humans have always suffered infections caused by *S. aureus* and before the advent of antibiotics it was a common cause of fatal hospital-acquired infection. The majority of *S. aureus* isolates soon developed resistance to penicillin and interestingly methicillin resistance was first detected in 1961, only two years after its introduction, but it was not until the 1990s that MRSA got a stranglehold in the UK.

Whether to screen for MRSA is a hotly disputed subject and this is highlighted in MRSA guidelines, which recommend that infection control teams adopt a more flexible approach based on local circumstances, and suggests risk assessments for each ward type. These guidelines also identify three components of good infection control practice, which are effective hand hygiene, a clean environment and appropriate protective clothing. All of which are essential, but I would like to add a fourth essential component and that is rapid microbiology laboratory results.

As discussed, laboratory techniques are now available that allow the detection of the majority of MRSA strains within 24 hours. It is essential that laboratories take this opportunity to update their methodologies accordingly. The recently reported vancomycin-resistant *Staphylococcus aureus* (VRSA) (CDC, 2002) has heightened the fear that a fully-resistant *S. aureus* is a probability, rather than a possibility. It is in everyone's interest that microbiology laboratories realise the need for speed in the war against MRSA.

Steve Davies
Sheffield

References

- Brown D F J (2001) Detection of methicillin/oxacillin resistance in staphylococci. *J Antimicrob Chem*, **48**, Suppl. S1, 65-70
- Cavassini M, Wenger A, Jaton K, Blanc DS & Bille J (1999) Evaluation of MRSA-Screen, a Simple Anti-PBP 2a Slide Latex Agglutination Kit, for Rapid Detection of Methicillin Resistance in *Staphylococcus aureus*. *J Clin Microbiol*, **37**, 5, 1591-1594
- Centres for Disease Control and Prevention (2002) *Staphylococcus aureus* resistant to vancomycin – United States. *J Am Med Assoc*, **288:** 824-5.
- Communicable Disease Report Weekly (2005) Community MRSA in England and Wales: definition through strain characterisation, 15, No 11
- Davies, S and Zadik, PM (1997) Comparison of methods for the isolation of methicillin resistant *Staphylococcus aureus* *J Clin Pathol,* **50**, 1-3
- Davies, S, Zadik, PM, Mason, C M and Whittaker, S J (2000) Methicillin-resistant *Staphylococcus aureus*: evaluation of five selective media, *J Med Microbiol,* **57**, 269-272
- Domin M A (1998) Highly virulent pathogens: a post-antibiotic era? *Br Journal Theatre Nurs*, **8** (2), 14-18.
- Duckworth, G. J., Cookson, B., Humphreys, H. and Heathcock, R (1998) Revised guidelines for the control of methicillin-resistant *Staphylococcus aureus* infection in hospitals. *J Hosp Infect.* **39** (4): 253-290
- European antibiotic resistance surveillance system (EARSS) (2004) MRSA cases continue to increase in Finland. *Eurosurveillance Weekly*, **8:** 38 (Sept 16)
- Gaillott, O, Wetsch M, Fortineau N & Berche P (2000) Evaluation of CHROMagar Staph aureus™, a new chromogenic medium for isolation and presumptive identification of *Staphylococcus aureus* from human clinical specimens. *J Clin Microbiol,* **38**, 1587-91.
- Kearns AM, Seiders PR, Wheeler J, Freeman R & Steward M (1999) Rapid detection on methicillin-resistant Staphylococci by multiplex PCR. *J Hosp Infect*, **43**, 2, 33-37
- Kunori T, Cookson B, Roberts JA, Stone S & Kibbler C (2002) Cost-effectiveness of different MRSA screening methods. *J Hosp Infect*, **51**, 189-200
- Livermore, D (2001) Antibiotic resistance in staphylococci. *Int J Antimicrobial Agents*, **16**, Supp.1, 3-10.
- Mercier, C. (1996) Stopping the superbug. *Prac Nurse*. **12**(8), 508-511
- National Audit Office (2004) Improving patient care by reducing the risk of hospital acquired infection: A progress report. *Report by the Controller and Auditor General.* **HC876** Session; 2003-2004: 14 July
- Skov R, Smyth R, Clausen M, Larsen AR, Frimodt-Moller N, Olsson-Liljequist B & Kahlmeter G (2003) Evaluation of a cefoxitin disc on Iso-Sensitest agar for detection of methicillin-resistant *Staphylococcus aureus*. *J Antimicrobial Chem*, **52**, 204-207
- Woodford N, Livermore D (2003) And a 'new' classical method: cefoxitin screen for MRSA. *Antibiotic Resistance Monitoring and Reference Laboratory Newsletter* **2003**; Summer: 4
- Zadik PM, Davies S, Whittaker S et al (2001) Evaluation of a new selective medium for methicillin-resistant *Staphylococcus aureus*. *J Med Microbiol*; **50:** 476-479

Testing the difference between two groups

In the third of a series of articles about statistics for biologists, **Anthony Hilton** and **Richard Armstrong** test the difference between two groups

Stat Note 3

IN STATNOTE 1 (*Microbiologist*, June 2005), we described a test to determine whether a sample of measurements came from a normal distribution.

If a variable is normally distributed, it is referred to as a *'parametric'* variable. In Statnote 2 (*Microbiologist*, September 2005) we showed that if a variable is parametric, then a sample of observations can be described by the sample mean 'X*' ('central tendency') and standard deviation (SD) 's' ('spread'). We also described two statistical procedures based on the normal distribution. Firstly, whether an individual measurement is typical or atypical of a larger population of measurements and secondly, we showed that the mean of a small sample of measurements also comes from a normal distribution, viz. that of the population of sample means. The degree of spread of this distribution can be described by the standard error (SE) of the mean. This information was used to calculate a confidence interval (CI) for a sample mean, which is a measure of the error associated with a sample mean as an estimate of the 'true' population mean. In this Statnote, these statistical ideas are extended to the problem of testing whether there is a statistically significant difference between two samples of measurements.

The scenario

We return to the hypothetical experiment described in Statnote 2 to investigate the efficacy of a novel media supplement in promoting the development of cell biomass. To recapitulate, two 10-litre fermentation vessels were filled with identical growth media with the exception that the media in one of the vessels was supplemented with 10ml of a novel compound. Both vessels were allowed to equilibrate and were subject to identical environmental / incubation conditions. The vessels were then inoculated with a culture of Bacterium x at an equal culture density and the fermentation allowed to proceed until all the available nutrients had been exhausted and bacterial growth had ceased. The entire volume of culture media in each fermentation vessel was then removed and filtered to recover the bacterial biomass. This experiment was repeated 25 times and the biomass measurements are given in Statnote 2.

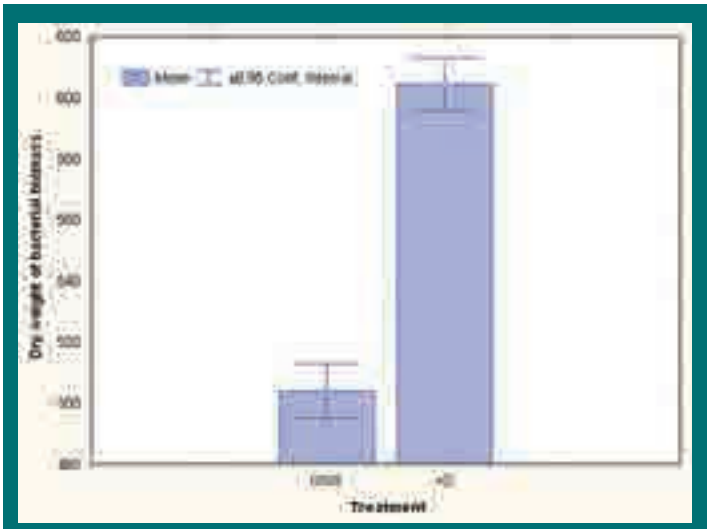


Fig. 1. *Mean bacterial biomass (with 95% confidence intervals) using unsupplemented (UNS) and supplemented (+S) media (t-test for comparing the means of both groups, t = 16.59, P < 0.001)*

How are the calculations done?

To determine whether an individual measurement is typical of a population requires knowledge of how individual measurements vary; i.e., the SD of the population (Statnote 2). Similarly, to determine the degree of error associated with a sample mean requires knowledge of how means vary; i.e., the SE of the mean (Statnote 2). Extrapolating this principle further, to determine whether there is a significant difference between the means of two samples, knowledge is required of how the differences between two sample means would vary. Hence, for each of our two samples, the mean is calculated and the difference between the mean of the unsupplemented (U*) group subtracted from the mean of the supplemented (S*) group. This difference represents the treatment effect of the experiment; i.e., the degree to which the media supplement may have increased bacterial biomass. If this experiment was repeated many times, several estimates of U* - S* would be obtained and we could construct a frequency distribution of the differences between the means. However, if the distribution of the means from the supplemented and unsupplemented groups are normally distributed, then the distribution of the differences between pairs of means taken from these two populations will also be normally distributed. As a result, the standard normal distribution (SND) can be used to test whether there is a true difference between the means.

The group means of the two treatments differ by 100.16 units (Fig 1) and the two samples exhibit little overlap suggesting a real effect of the supplement (Fig 2). There is, however, variation in microbial biomass between replicate flasks within each group. Hence, is the difference between the means actually attributable to the effect of the supplement or could it be accounted for by random variation between the flasks? To decide between these two alternatives, the treatment effect (U* - S*) is compared with the degree of variation pooled from both groups by carrying out a 't' test. The statistic 't' is the ratio of the difference between the two means to the SE of the difference between the means:

$t = U^* - S^* / \sqrt{s2\ (1/n_1 + 1/n_2)}$

where 's2' is the average variance of both groups and n1 and n2 are the number of observations within each group ($n_1 = n_2 = 25$). Note that this calculation is similar to that carried out in Statnote 2 when an individual value 'x' was converted so that it became a member of the SND. In the present example, the 't' distribution is used instead of the SND because 't' describes the variation of means calculated from small numbers of observations more accurately. Hence, when 't' is calculated, the difference between the means (U* - S*) becomes a member of the 't' distribution. The probability of obtaining a value of 't' of this magnitude by chance from two groups of samples when each comprises 25 observations is obtained either from statistical software or by consulting statistical tables (Fisher and Yates, 1963). In the present example, a value of t = 16.59 was obtained and is taken to a table of the 't' distribution, entering the table for n1 + n2 – 2 DF (DF = 48). When 't' is equal to or

greater than 2.01 (the value at P = 0.05 for 48 DF), the value is in a region of the distribution which includes the 5% most extreme values. Hence, t = 16.59 is an unlikely value to occur by chance and therefore, there is a real difference between the two means.

One-tail and two-tail tests

It is possible to propose two different null hypotheses. First, that the addition of the supplement (S) would have no effect on bacterial biomass. This hypothesis does not specify whether a significant *increase* or a *decrease* in biomass would be necessary to refute the null hypothesis. In this case, a *two-tailed* test would be appropriate, i.e., both tails of the 't' distribution are used to test the hypothesis. Second, that the supplement would only increase biomass since it may be known in advance that it could not significantly decrease biomass. If the hypothesis specifies whether a positive or a negative effect is necessary to refute the hypothesis, a *one-tail test* would be appropriate. Some statistical tables indicate both the one-tail and two-tail probabilities corresponding to particular columns. Most statistical tables, with some notable exceptions, only indicate the two-tail probabilities. To find the one-tail probabilities in a two-tail table, halve the probabilities; i.e., the 5% one-tail probabilities are found in the 10% two-tail column.

Paired and unpaired 't' tests

An experiment involving two treatments or groups can be carried out in two different ways, *viz.*, the *unpaired* and the *paired* methods. The experiment described in our scenario was carried out using an unpaired design; *i.e.*, the media supplement was allocated at random and without restriction to half of the 50 original flasks. In a paired design, however, the 50 flasks are first divided into 25 pairs with the intention of processing a single pair (one supplemented, the other unsupplemented) on each of 25 days and second, the supplement is then allocated to one flask of each pair independently and at random. Hence, there is a restriction in the allocation of the treatments to the flasks and a different analysis is required. In a paired design, the 't' test is made as follows:

$t = d^*/ (sd/ \sqrt{n})$

In this case, 'd*' is the mean of the differences between each of the 25 pairs of observations and 'sd' is the standard deviation of these differences. The same 't' table is used for determining the significance of 't'. In a paired 't' test, however, a different rule is used for entering the 't' table; *viz.*, 't' has n-1 degrees of freedom, where 'n' is the number of pairs of subjects. Again, one-tail or two-tail tests may be made as appropriate.

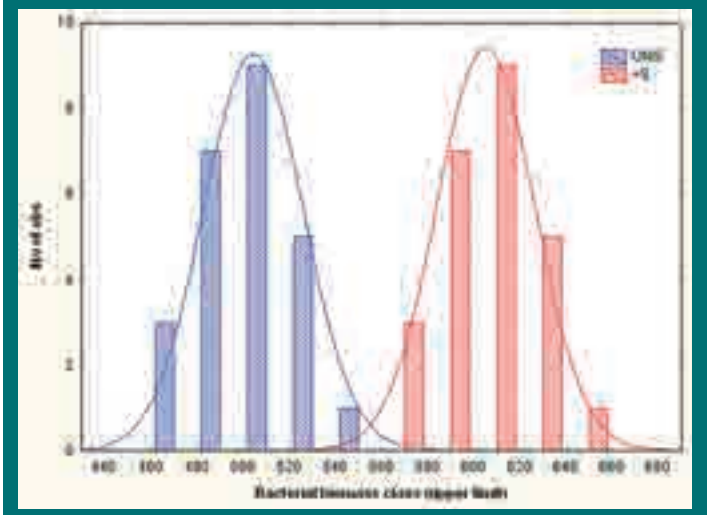


Fig. 2. *Frequency distribution of the individual biomass measurements using unsupplemented (UNS) and supplemented (+S) media. Curves represent the normal distribution fitted to each sample.*

Advantages of pairing

Is a paired or an unpaired design the best method of carrying out the experiment? Each type of design has advantages and disadvantages. A paired design is often employed to reduce the effect of the natural variation that always exists between flasks or replicates. How this may be achieved can be seen by examination of the formula for the unpaired 't' test. A value of 't' is the difference between the two treatment means divided by the SE of this difference. If variation among flasks is large, say from processing them at different times of the day or on different days, it will increase the SE of the difference and lower the value of 't' even if the difference between means is large. Notice, however, that in an unpaired design, the 't' table is entered for 48 DF. Pairing the flasks may reduce the SE because paired 't' is calculated from the differences between pairs of observations. In other words, the effect of the experimental treatment is being determined within a matched pair of flasks. Pairing should only be considered, however, if there is evidence that it actually reduces the variability, e.g., pairing supplemented and unsupplemented flasks on the same day when the day of assay does significantly affect the measurement. If there is no reduction in the SE by pairing, i.e., it does not matter which day the samples are measured, then there is a disadvantage of the paired design because the 't' table is entered with only 24 DF (1 less than the number of groups). Entering the 't' table with a smaller number of degrees of freedom means that a larger value of 't' will be required to demonstrate a significant difference between the means.

The 't' test is a useful method of comparing two groups when the data approximate to a normal distribution. In many cases in microbiology, however, data may not conform to this distribution and different methods may be required to test differences between the groups and these methods will be reviewed in Statnote 4.

References

- Fisher RA & Yates F (1963) Statistical Tables for Biological, Agricultural, and Medical Research. Sixth Ed, Longman, Bristol.
- Hilton A & Armstrong RA (2005) Are the data normal? *Microbiologist* June 2005, **Vol 6** No 2, 34-35
- Hilton A & Armstrong RA (2005) Describing the normal. *Microbiologist* September 2005 **Vol 6** No 3, 30-32

Dr Anthony* Hilton and Dr Richard Armstrong**
*Pharmaceutical Sciences and **Vision Sciences, Aston University, Birmingham, UK

Clostridium difficile:
Return of the old enemy

Laura Wheeldon gives us an overview of another hospital-acquired pathogen

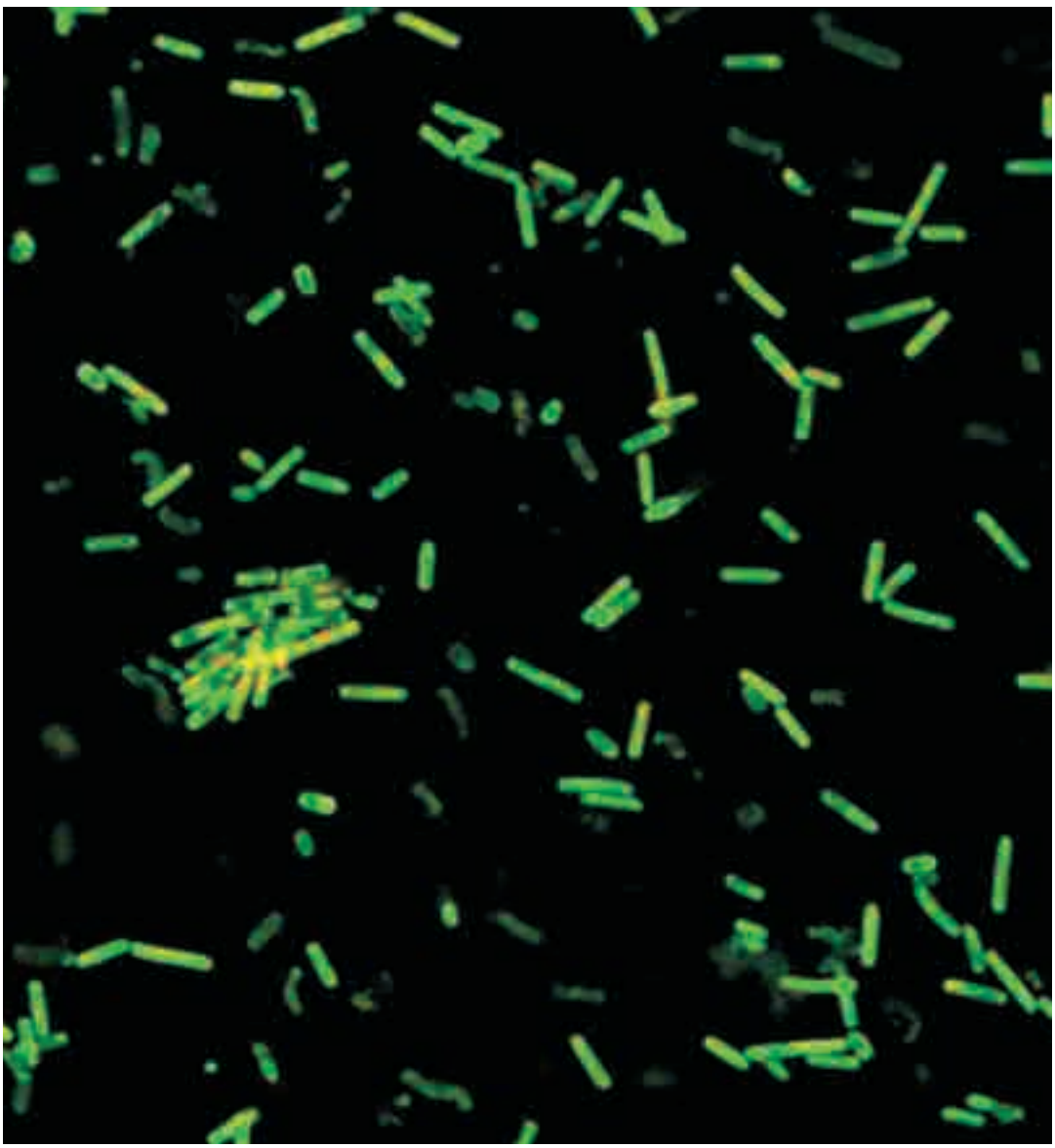


Fig. 1. *C. difficile cells fluorescently stained with LIVE/DEAD ® BacLight stain and observed under a confocal microscope*

Clostridium difficile is an anaerobic, spore-forming, Gram-positive bacillus (see figure 1). It was first described in 1935, as a component of the faecal flora in healthy babies and was not recognised as a cause of toxin-associated disease, which ranges from diarrhoea to pseudomembranous colitis, until 1978. *C. difficile* has now been identified as the leading cause of hospital acquired infectious diarrhoea in adults, with 43,672 reports in the UK in 2004 (Anon, 2005).

Acquisition of disease and risk factors

C. difficile has been cultured from all over hospital wards and acquisition of the organism occurs primarily in the hospital setting. Organisms have been recovered from beds, toilets, floors, mops and furniture and objects have been found to be contaminated where infected patients were not known to have visited. *C. difficile* has also been isolated from the stools and hands of hospital staff that were asymptomatic for the disease (Fekety *et al.*, 1981). Spores have been found to survive up to 56 days, in temperatures of 4ºC and -20ºC (Freeman and Wilcox, 2003) and are fairly resistant to many common cleaning agents. Patients most at risk of developing infection are those which have undergone treatment that may impair or disrupt the microflora of the intestine, for example; antibiotics, surgery, immunosuppressive therapy and antacids. Other risk factors include old age, multiple and severe underlying diseases and prolonged hospital stay. Figure 2 shows how risk factors that cause disruption of the flora can enable *C. difficile* to colonise the gut and possibly cause disease.

Pathogenesis of disease

The pathogenesis of disease can be considered in six stages; entry, adhesion, multiplication, avoidance of host defences, damage to the host and release (Poxton, 2005).

Entry. *C. difficile* almost always comes from an exogenous source as it is not detected in the majority of patients and a single strain is often responsible for an outbreak. It is transmitted by

the faecal-oral route, by the ingestion of spores.

Adherence. Many adhesins have been described for *C. difficile*, including surface layer proteins (SLPs) and flagella. SLPs are highly immunogenic and patients produce antibody directed against SLPs. It is thought that the flagella may aid in the penetration of the mucus barrier.

Multiplication. In order to cause disease there must be sufficient numbers of the organism to produce toxin. As the use of broad spectrum antibiotics eliminate much of the normal flora of the intestine, *C. difficile* is able to multiply without much interference by competing organisms. Some studies have also shown that more toxin is produced by *C. difficile* when it is exposed to sub-lethal concentrations of antibiotic (Drummond *et al.*, 2003).

Evasion. It is thought that *C. difficile* uses its wide array of SLPs to evade the immune response of the host along with degradative enzymes and capsules, however none of these have been proven to be involved in evasion.

Damage to the Host. *C. difficile* is thought to produce at least two toxins. The two major toxins; toxin A and toxin B are similar in action, causing cell death by disrupting the actin cytoskeleton, after being endocytosed by the host cell. The toxins also induce host inflammatory responses and pseudomembrane formation, (figure 3).

After entrance to the cytosol via passage through an intracellular compartment, the toxins act on the actin cytoskeleton. They cause cell rounding, as cell processes retract due to the disassembly of filamentous F-actin and an increase in G-actin. Before cell rounding occurs, the toxins act enzymatically to modify Rho proteins which regulate

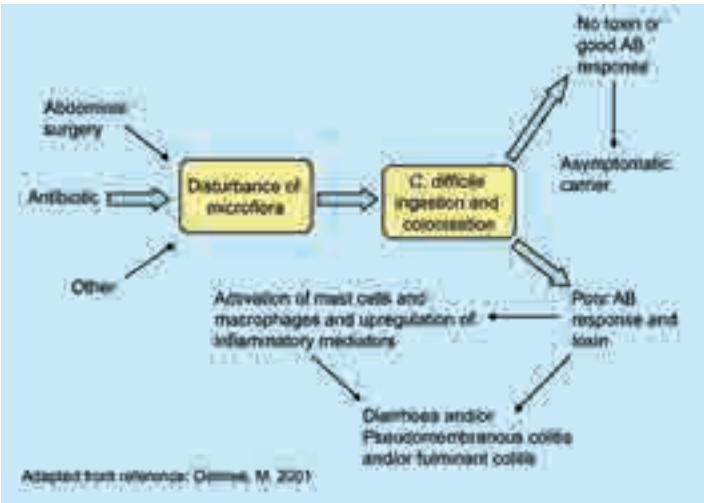


Fig. 2. *Adapted from reference: Delmee, M. 2001*

fiber assembly and actin polymerization. The loss of functional Rho proteins and the breakdown of actin filaments causes a disruption of the barrier function, by opening the tight junctions between intestinal epithelial cells. This increases permeability in the intestine and causes watery stools, which is a characteristic feature of *C. difficile* antibiotic-associated diarrhoea (Dillon *et al.*,1995).

Although both toxins have similar structures and mechanisms of action, they differ largely in their potencies, with toxin B approximately 1000 times more potent than toxin A.

Both toxins induce apoptosis of enterocytes and activate the immune system by stimulating the release of TNFα and activating macrophages and monocytes to release IL-8, which causes migration of neutrophils to the site of mucosal inflammation. Histological changes that may follow include diffuse cell death and ulceration (colitis) and development of a pseudomembrane consisting of mucin, fibrin, leukocytes and cell debris (figure 4).

Some strains produce an additional toxin, a binary toxin, termed CDT. It is thought that this toxin could be an additional virulence factor as it has been shown to

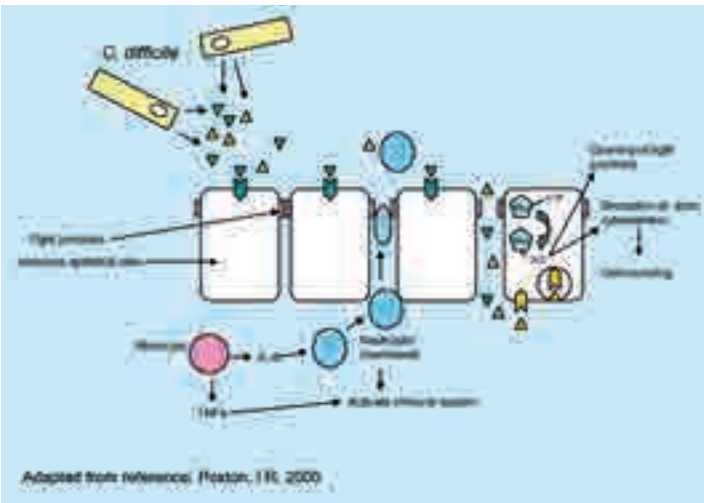


Fig. 3. *Adapted from reference: Poxton, I.R. 2005*

have cytopathic effects on cell lines that are similar to the other toxins.

Release. Release of spores is easily accomplished as *C. difficile* causes diarrhoea, which is often explosive. The spores that *C. difficile* produce act as a reservoir of infection and make it possible for the organism to survive in aerobic conditions. They are extremely hardy and resistant, making them hard to eradicate. Spores are able to survive passage through the highly acidic environment of the stomach and eventually enter the colon, where they may germinate into *C. difficile* bacterial cells. Contamination of the environment is a major factor in the spread of the organism and it has been estimated that infected patients excrete over 100 *C. difficile* per gram of faeces (Wilcox, 2003).

Clinical Manifestations of disease

C. difficile causes antibiotic associated diarrhoea (CDAD) and colitis; the severity of which ranges from asymptomatic carriage, mild to moderate diarrhoea and pseudomembranous colitis. The mild form of the disease is associated with lower abdominal cramps, but no systemic symptoms. Sparse or diffuse colitis may be evident from endoscopy. A moderate form of the disease is characterized by abdominal pain, profuse diarrhoea and occasionally a small amount of colonic bleeding.

Other symptoms include fever, malaise, nausea and anorexia. There may also be evidence of patchy or diffuse colitis and fecal leukocytes. Approximately 1 - 3% patients develop fulminant colitis, along with toxic megacolon and perforation. In a minority of patients, a reactive form of arthritis may develop, one to four weeks after developing colitis.

Laboratory Diagnosis

Laboratory diagnosis of *C. difficile* in the stools of a patient is based on two kinds of tests; toxin detection and faecal culture. Unfortunately laboratories differ throughout the UK in that some only use a single toxin detection test or a single immunoassay, where as others use both toxin detection and culture. Samples are cultured on selective CCFA (cycloserine cefoxitin fructose agar) plates. Sodium taurocholate may also be added to the agar to enhance germination of spores. Culture is a very sensitive method, however it lacks specificity due to the possibility of isolation of non-toxigenic strains. Toxin testing is more specific and involves inoculating a cell culture (usually HeLa cells) with filtrate of a stool suspension and then observing any cytopathic effects. Enzyme immunoassays are also used widely as they produce results very rapidly; however they have slightly lower sensitivity and specificity.

Prevention and Treatment

The most important and effective measure to reduce spread of *C. difficile* is a strict cleaning regime of the clinical environment. There is conflicting evidence on best the choice of cleaning agent, but most agree that soap and water is the most effective (Anon, 1994). There should also be strict control over the antibiotics that precipitate disease more readily than others (Schroeder, 2005).

Treatment of *C. difficile* has not advanced much over the past decade; however, some promising new options are currently being explored. The need to find new, alternative treatments for CDAD has arisen from the high relapse rate when initial, conventional therapy is discontinued. Relapse usually occurs in around 5% - 24% of patients between 1- 3 weeks after termination of initial treatment (Buckley, 1996, Pepin *et al.*, 2005). Most relapses are due to infection with new strains of *C. difficile*. The elderly and those who have recently undergone abdominal surgery are more likely to suffer relapse (Young *et al.*, 1986).

The initial treatment of CDAD is termination of the inciting antibiotic whenever possible or the switch to a narrow spectrum antibiotic. In cases of mild diarrhoea this may be adequate to resolve symptoms; however, most require further treatment with one of two antibiotics; metronidazole or vancomycin. Hydration and electrolyte replacement therapy may also be required in conjunction with antibiotics in severe cases and in the young and elderly.

Metronidazole and vancomycin are the main antibiotics used to treat CDAD. Metronidazole is usually the treatment of choice as it is as effective, but not as costly as vancomycin and vancomycin should really be reserved for treatment of MRSA.

Alternative treatments options are very important in pursuing as most antibiotics only target vegetative cells. Other possible therapy options for treatment of CDAD include: probiotics, adsorbents, immune products, faecal enemas and bowel irrigation and a series of new compounds. Identifying which strain of *C. difficile* is causing disease may also aid in deciding which treatment will be most effective (McFarland, 2005).

Fig. 4. *Reprinted with permission: Biomedical Scientist 2005; 49: 1034–40. © Institute of Biomedical Science. I Poxton*

Probiotics are living organisms, which have specific therapeutic properties and inhibit growth of pathogenic bacteria. The normal healthy gut has colonization resistance, which prevents pathogenic bacteria from attaching to the gut and causing disease. When the balance is disrupted due to antibiotics or other disruptive factors the luminal wall is susceptible to infection by pathogenic bacteria. Probiotics help to re-colonise the gut with non-pathogenic bacteria, thus preventing the attachment of harmful bacteria.

C. difficile has been a major cause of nosocomial infection for many years, but until now it had not gained the attention of the media. With the organism now being hailed as 'the new superbug' will awareness now increase and will more be done to decrease infection rates in our hospitals?

Laura Wheeldon
Aston University

References

- Anon (1994) Public Health Laboratory Service / Department of Health.
- Anon (2005).
- Buckley, P. (1996) *Pharmacy in Practice*, **2**, 48-49.
- Delmee, M. (2001) *Clin Microbiol Infect,* **7**, 411-416.
- Dillon, S. T., Rubin, E. J., Yakubovich, M., Pothoulakis, C., LaMont, J. T., Feig, L. A. and Gilbert, R. J. (1995) *Infect Immun*, **63**, 1421-6.
- Drummond, L. J., Smith, D. G. and Poxton, I. R. (2003) *J Med Microbiol*, **52**, 1033-8.
- Fekety, R., Kim, K. H., Brown, D., Batts, D. H., Cudmore, M. and Silva, J., Jr. (1981) *Am J Med*, **70**, 906-8.
- Freeman, J. and Wilcox, M. H. (2003) *J Clin Pathol*, **56**, 126-8.
- McFarland, L. V. (2005) *J Med Microbiol*, **54**, 101-11.
- Pepin, J., Alary, M. E., Valiquette, L., Raiche, E., Ruel, J., Fulop, K., Godin, D. and Bourassa, C. (2005) *Clin Infect Dis*, **40**, 1591-7.
- Poxton, I. R. (2005) *The Biomedical Scientist*, **49**, 1034-1040.
- Schroeder, M. D. (2005) *American Academy of Family Physicians*, **71**.
- Wilcox, M. H. (2003) Best *Pract Res Clin Gastroenterol*, **17**, 475-93.
- Young, G. P., Bayley, N., Ward, P., St John, D. J. and McDonald, M. I. (1986) *Med J Aust*, **144**, 303-6.

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

Antimicrobial properties of mineral silicate.

Lewis Coates reports on his project

I AM CURRENTLY STUDYING Applied and Human Biology at Aston University. My course offers me the opportunity to do a Year in Industry placement which involves taking a year to work in a field of Biology to earn experience, but due to lack of time on my part I could not do this. I opted to ask my lecturers if I could work over the summer in one of the Labs, preferably in the microbiology lab as this is my favourite subject and something I would like to do in the future. One of my Lecturers agreed to take me on over the summer so I worked in the Microbiology lab at Aston University.

Over the eight weeks I was working with a mineral silicate compound to see if it could be used as an antimicrobial substance. The test and experiments that I have performed seem to show that it can kill a wide range of bacteria including, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus megaterium*, *Bacillus subtilis*, *Staphylococcuse epidermidis*, and *Enterococcus faecalis*. More interestingly it can also kill MRSA. On average, the compound seems to kill off these bacteria after approximately 5 minutes of exposure. Unfortunately the compound is toxic to humans and thus can't be given orally or intravenously. Perhaps with further development the compound could be used in detergents and in cleaning hospital wards to try and minimize the spread of infection, especially MRSA.

One theory of how the compound was acting on the bacteria was that it was just toxic to the bacteria and that is why it was killing them in such large numbers, but the same experiments on *Candida Albicans* and *Aspergillus Niger* showed that the compound was not active against these two strains of fungus and thus the compound must have some specific way of effecting only bacteria. Unfortunately my time in the lab ran out and so I didn't get the chance to figure out how the compound actually works. This will have to be left for further study.

During my time working in the Microbiology lab I have developed my scientific skills greatly and I believe this experience has given me a great advantage for my further study at Aston and in the future for when I start working full time. As well as developing a better scientific skill in conducting experiments, I have also learned to develop a better understanding of science and to know what experiments I should perform next to obtain more relevant results, and also understand why I am performing such tasks.

Whilst working in the Microbiology Lab, my enthusiasm for science has dramatically increased, especially for microbiology. I now know that after I finish my studies at Aston I would like to work in the field of microbiology or perhaps even study for a PhD.

The only complaint I have about working in the Microbiology Lab is that I did not have enough time, I would have liked to spend more time working there as it has been one of the best experiences at University so far.

Lewis Coates
Aston University, Birmingham

Nanosized Magnetic Particles created by Bacterial Respiration

James Byrne reports on his project

DURING MY SUMMER vacation I was given the opportunity to carry out practical research at the University of Manchester's, *'Williamson Research Centre for Molecular Environmental Science,'* as part of a collaboration between the work of Professor Peter Gilbert and Professor Jon Lloyd. The placement also included a week at Daresbury Laboratories, *'Synchrotron Radiation Source, (SRS),'* in Warrington, Cheshire. As a physics student from the University of St.Andrews this gave me the chance to work between disciplines which required both the knowledge of my own degree work and of the biological sciences. I was supervised in my work by PhD student Vicky Coker.

For ten weeks I researched the reduction of Iron (III) containing compounds by the anaerobic respiring bacterium *Geobacter sulfurreducens*. This bacteria uses Iron (III) as an electron acceptor and reduces it to Iron (II), leading to the production of nanosized particles of Magnetite (Fe_3O_4). This nano-scale magnetite has potential commercial uses for computer manufacturers in the design of data storage devices.

The principle focus of my research was the conversion of an iron containing material, which we collected from a water-works near Birmingham, to nano-scale biomagnetite. Water stored in reservoirs requires treatment to remove impurities before being pumped into the national water supply. During this process iron sulphate is added to coagulate loose material to form flocs. These flocs are less dense than the water, float and can be subsequently removed from the surface. The flocs are iron-rich due to the addition of the coagulant and could be used by anaerobic bacteria as an electron dump. After removing the flocs from the water they are dried in large presses and dumped in landfill sites. This is costly and potentially harmful to the environment. My studies centred on finding possible methods of recycling the material to create a useful end-product.

First the flocs were tested for elemental composition. The results showed high concentrations of some transition metals other than iron, which included titanium, vanadium, chromium and manganese. The presence of some of these metals could have caused problems for my proposed project, because they could inhibit the activity of the Iron (III)-reducing bacteria. For example, they could be toxic to the cells (in the case of chromium), or act as electron sinks (in the case of manganese, as Mn(IV)).

My results showed that when I added *G. sulfurreducens* and a few key nutrients to the test materials, Iron (III) reduction took place. Seven weeks after inoculation of the bacteria, magnetite was visibly produced and detected using a magnet. Previous work had already demonstrated reduction of synthetic Iron (III) oxides, and the time period for magnetite formation in these experiments was much quicker than with the waste materials that I was testing. Some samples took little over a day to completely convert to magnetite. The extended duration required to form the magnetite by floc reduction may be due to the concentrations of other metals e.g. manganese, which are also used by the bacteria for respiration, and would have to be reduced before the Iron (III).

As well as working at the University of Manchester, my summer placement included a week at Daresbury Laboratories. I observed and learned about the investigations of post doctorial researcher Carolyn Pearce who is working on the reduction of selenium by anaerobic bacteria. The Synchrotron Radiation Source at Daresbury is designed to produce varying wavelengths of electromagnetic radiation ranging from Infrared to Hard X-rays. Electrons are accelerated and then fed into a large storage ring which keeps the electrons travelling in a circular path using very large magnets. As the velocity of the electrons approaches the speed of light they emit radiation at tangents to the ring. This radiation can be used for fundamental research including the techniques employed by Carolyn for her work on selenium.

The synchrotron was able to provide a real time assessment of the selenium as it changed between oxidation states. This involved using a technique called EXAFS *(Extended X-ray Absorption Fine Structure)* which works by firing X-Rays at a specially designed cell which held the selenium containing solution and anaerobic bacteria. The beam excited atoms in the chamber, causing electrons to move to different orbital valences which induced the emission of photons. These photons were detected and processed by a computer. The energy of the photon emitted changed according to the oxidation state of the selenium and resulted in a shift in the data points which were displayed graphically on the computer. The information collected showed that the bacteria had reduced the selenium through several different oxidation states.

The experience which I have obtained at the University of Manchester has been very valuable and has given me a very detailed insight into carrying out research. I will continue with my studies at the University of St. Andrews and look forward to continuing in academic work, preferably within a discipline that bridges physical and biological sciences, after completing my undergraduate degree.

James Byrne
University of St Andrews

Production of phosphatidylinositol specific phospholypase C (PI-PLC) by *Listeria monocytogenes.* **Sonia Chohan** reports on his project

DURING SUMMER 2005, I had the opportunity to work in a Research and Development laboratory of Technical Service Consultants Ltd. (TSC) based in Heywood, Lancashire. I am currently a student from the University of Salford. On completion of my first year I undertook a 4-week placement funded by SfAM Student into Work grant. I searched for a short placement, so that I could learn new skills to allow me a taste of the future job market, which I hope will help me to make a final decision when I choose my future specialty.

TSC is a microbiological company—a manufacturer of laboratory consumables, transport and diagnostic swabs, prepared culture, media and a bacterial preservation system. During my placement I was involved in a development project and carried out investigations into the virulence factors of *Listeria monocytogenes*, which is probably one of the most intensively studied microorganisms over the last 20 years. Acceptance of a zero-tolerance policy by the food industry stimulated growing interest in rapid methods for *Listeria* detection to cut down on time and to get accurate results. Some recently developed methods for *L.monocytogenes* detection are based on the selective growth and chromogenic detection of *Listeria* virulence factor; phosphatidylinositol-specific phospholypase C (PI-PLC). This is an enzyme, specific for pathogenic *Listeria* and it acts as a marker to distinguish between pathogenic and non-pathogenic *Listeria* species.

The aim of the project was to evaluate the possibility of using PI-PLC production as a method for *L.monocytogenes* diagnosis. All my tests were performed using 96-well microtitre plates. Different bacteria were tested for their ability to synthesize PI-PLC in pure and mixed culture. Moreover, the effect of bacteria concentration on enzyme detection was evaluated. In the frame of this work I gained experience in different methods and approaches of general microbiology, including, aseptic work techniques, media preparation, sterilization methods, bacteria isolation, cultivation and analysis. Firstly, I investigated the ability of *L. monocytogenes* and other Gram-positive strains from TSCs collection of microorganisms to synthesize PI-PLC. A simple chromogenic reaction was utilized to detect enzyme production: Test bacteria were grown in non-selective media in the presence of a chromogenic compound which is a substrate for PI-PLC. Enzyme synthesized by bacteria reacts with substrate resulting in formation of a blue-coloured product. Only pathogenic *Listeria* species: *L.monocytogenes* and *L.ivanovii* were found to synthesize PI-PLC. Among other PI-PLC producers were strains of *Bacillus cereus* and *Staphylococcus aureus*. The sensitivity of this method for enzyme detection was evaluated using different concentrations of PI-PLC positive test organisms. Serial dilution method followed by plating on agarised medium was applied to calculate inoculum concentration. For *L.monocytogenes* the colour formation as a result of chromogenic substrate conversion by PI-PLC was visibly detectable in 24-48 h at initial bacterial concentration of 300 colony forming units per ml.

In the final experiment *Listeria monocytogenes* was incubated together with another organism known to be negative for PI-PLC synthesis in the presence of chromogenic substrate to look for the effect of bacteria growth competition on PI-PLC synthesis and, therefore, enzyme detection. The result of this test was not conclusive; therefore, the experiment will be repeated. These results showed a series of outcomes, which confirmed the suitability of PI-PLC analysis by means of chromogenic reaction for detection of *L.monocytogenes.* Further investigations will be required to improve specificity and sensitivity of this method for future applications.

The summer placement has allowed me to gain an insight into research work, as well as acquire valuable experience and laboratory skills, which I am sure I will find useful in both my education and future career. I had the opportunity to use and apply skills already taught and understood. In particular I was pleased with the outcome of my research project, which allowed me to generate further ideas, being both enjoyable and challenging. I would like thank SfAM for affording me this valuable opportunity and my project supervisor, Dr Irina Barbolina and the entire staff at TSC for their support, especially Rachael Greig for helping me throughout and putting up with my countless queries.

Sonia Chohan
University of Salford

Am I eligible - can I apply?

The President's Fund provides limited grants to ALL members to assist them to attend scientific meetings or workshops related to their area of work. Awards are made at the sole discretion of the Honorary President. Please note that this Fund is open to members of all ages! Why not apply to the Fund? The maximum grant available is normally £1,000.

To apply, visit **www.sfam.org.uk/members/prizes.php**

TERMS & CONDITIONS

1. The applicant must have been a member for at least a full subscription year before the event to be attended and must be a fully paid-up member at the time of application.

2. A successful applicant cannot re-apply to the Fund for three years from the date of the award.

3. Preference will be given to applicants who are contributing to the meeting they wish to attend and/or are unable to obtain funds elsewhere.

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.

6. The maximum grant available is normally £1,000.

7. Under exceptional circumstances this maximum may be exceeded.

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.

Investigating microbe-mineral interactions

MICROORGANISMS HAVE BEEN agents of geochemical change for much of the Earth's history, and it is now widely accepted that they play a key role in mineral weathering processes such as the formation of soils and sediments, the determination of the chemical composition of aquatic environments, and in the long-term regulation of the Earth's climate.

They play an important role in biogeochemical cycling of nutrients (Gadd, 2004) and can affect speciation and distribution of elements and metals through redox reactions, by inducing mineral precipitation, through release of organic and inorganic by-products, and by directly or indirectly modifying the rates and mechanisms of mineral degradation (Banfield, 1998). Silicate minerals (such as feldspar, quartz etc.) are among the most abundant in the Earth's crust, and key questions concerning microbial activity in surface weathering processes of silicates are the degree to which microbial populations target specific silicate minerals and the extent to which microbes derive nutrition from silicate weathering. Estimates of microbial community diversity are generally problematic due to the inherent biases of culture-based microbiology, with media selecting for copiotrophic microbial populations; it is thought that less than 5% of microbial species can be cultured.

In recent years reliable DNA-based culture-independent techniques to characterise microbial populations on weathering surfaces have, at one level, circumvented the difficulty of non-culturability of environmental microbes. The Microbial Ecology Group at UCD in collaboration with UCD geologists has been at the forefront in exploiting these techniques, and our inter-disciplinary approach has resulted in significant recent progress in characterising the bacterial and fungal populations hosted by mineral weathering surfaces. A striking recent discovery from these DNA-based studies (Gleeson *et al.*, 2005a; 2005b) has been that the surfaces of different mineral types host distinctive microbial communities, with many ribotypes associated exclusively with particular mineral types. This mineral-specific selectivity by mineral type on a centimetre scale was found within a single pegmatitic granite outcrop in which adjacent crystals of K-feldspar, plagioclase feldspar, muscovite and quartz strongly discriminated with respect to their surficial microbial populations. This is a critically important finding because it offers tantalising new insights into the possible relationship between mineral substrate major-element chemistry and particular bacterial and fungal ribotypes with implications for the functional role of such populations.

Although the advent of molecular methods based upon cloning or genetic fingerprinting has allowed much more detailed analysis of microbial communities in natural environments, it has become increasingly important to develop an understanding of how microorganisms interact with their environment *in-situ*. Microscopic imaging techniques provide a direct way to characterize the distribution of microorganisms *in-situ*. Some of the more established microscopic methods that have been used include transmission and scanning electron microscopy (TEM and SEM), both of which provide high resolution images. However, sample preparation required for both SEM and TEM may introduce artifacts due to shrinking or tearing of biological tissues. Advances in microscopic techniques include the development of environmental scanning electron microscopy (ESEM) and confocal scanning laser microscopy (CSLM). ESEM permits the same type of high resolution, high magnification and large depth of field that is obtained with

standard SEM, but has a variable pressure chamber allowing for imaging of biological specimens without sample preparation, thus eliminating the introduction of artifacts and reduction of sample dehydration. Information that can be obtained from ESEM and CSLM includes morphological data, identification of cell surface components (through labeling), identification of specific species within microbial consortia, distinctions between viable and non viable cells and spatial organization of microorganisms occurring within different microenvironments.

CSLM is currently providing new opportunities for studying microbial communities on mineral surfaces. Since the illumination source for fluorescence microscopy and CSLM is reflected instead of transmitted, the thickness of the sample has less effect on the ability to obtain high resolution images. Confocal imaging produces sectioned images from the upper layers of solid samples, allowing 3-D analysis. This sectional data can then be further analyzed using stereological approaches to give estimates of bacterial numbers and volumes, allowing the 3-D dimensional structure of the samples to be examined. CSLM may be performed with one photon or two (multi) photon (MP) excitation. MP excitation has several major advances: excitation in the focal plane only, deeper penetration of infrared light into scattering samples, less light scattering, higher resolution in deep areas of the sample and no need for UV excitation and UV optics. As a consequence two photon systems are suitable for examination of thick, scattering biological samples such as microbial communities (see Neu and Lawrence 2005 for a comprehensive review).

To determine how individual populations are distributed within communities on weathering surfaces and contribute to biomass, species specific fluorescent oligonucleotide probes can now be designed. These probes can then be hybridized to surface communities using fluorescent *in-situ* hybridisation (FISH), and visually analyzed by CSLM. Probes for cell identity, viability, as well as cell and enzyme activity can also be designed, as well as probes for microenvironment analysis. Of particular interest are acidification processes resulting from microbial activity, together with ion contents of solutions surrounding colonies and biofilms. Microspatial pH and K concentrations surrounding microbial colonies can be assessed using the fluorescent fluorophores CL-NERF and Lucifer Yellow for pH and PBFI for K (available from Molecular Probes Inc.). Although microbial colonies on mineral grains have previously been examined by cryo-SEM (cryo-scanning electron microscopy), epifluorescence microscopy and CSLM (Barker et al. 1998, American Mineralogist), this work was performed using cultured microbes in a laboratory setting.

A recent visit to the University of Western Australia (UWA) has lead to a new collaboration for the Microbial Ecology Group at University College Dublin. A Leica TCS SP2 multi photon confocal microscope located in the Centre for Microscopy and Microanalysis (CMM) at UWA was used in an initial study to investigate the possibility of using CSLM to image microbial populations on granitic rocks *in-situ*. Auto-fluorescence is a major problem when attempting to image microbial communities on geological samples, as it interferes with signal from probed microorganisms. We imaged different mineral surfaces using the available laser lines to determine the level of auto-fluorescence exhibited by different samples. Mineral samples exhibited auto-fluorescence when viewed using traditional red and green lines, however, the same sample when viewed in MP-UV mode exhibited a much reduced level of auto-fluorescence. This finding allows us to now assess the types of probes that may be used in conjunction with UV excitation to image microbial communities, in turn should lead to increased use of CSLM in the visualization of microbe-mineral interactions *in situ*.

Deirdre Gleeson
University College Dublin

Acknowledgements

The author would like to thank staff at the Centre for Microscopy and Microanalysis (CMM) at the University of Western Australia (UWA) for access to their equipment. We also gratefully acknowledge the assistance of John Murphy and Peta Clode at the CMM for technical assistance. Staff at the School of Earth and Geographical Sciences are also thanked for their assistance, in particular Lyn Abbott, Bob Gilkes and Dan Murphy. We would also like to thank the Irish Research Council for Science, Engineering and Technology (IRCSET) for funding this project.

References

- Barker, W.W., Welch, S.A., Chu, S., and Banfield, J.F. (1998) Experimental observations of the effects of bacteria on aluminosilicate weathering. *American Mineralogist* **83**, 1551-1563.
- Gadd, G.M. (2004) Microbial influence on metal mobility and application for bioremediation. *Geoderma*. **112**, 109-119.
- Gleeson, D.B., McDermott, F., Kennedy, N., Melville, K., Gadd, G.M., and Clipson, N. (2005a) Characterization of bacterial community structure on a weathered pegmatitic granite. *Microbial Ecology*, In press (MECO-2005-0009).
- Gleeson, D.B., Clipson, N., Melville, K., Gadd, G.M. and McDermott, F.P. (2005b) Characterization of fungal community structure on a weathered pegmatitic granite. *Microbial Ecology*, In press (MECO-2004-0198).
- Neu, T.R. and Lawrence, J.R. (2005) One-photon versus two photon laser scanning microscopy and digital image analysis of microbial biofilms. *Methods in Microbiology.* **34**, 89-136.

Host defence peptides in the control of oral and respiratory bacteria

HOST DEFENCE PEPTIDES (HDPs) are highly diverse components of the innate immune systems of most living organisms, acting synergistically *in vivo* with other innate defence molecules to combat infection and control resident microbial populations (Devine, 2004). These low molecular weight, cationic, single gene products are synthesised within phagocytic cell granules or secreted by epithelial cells throughout the body (Devine and Hancock, 2002). Expression of epithelial cell HDPs such as human b-defensins and cathelicidin LL-37, may be constitutive or induced in response to infection or inflammatory mediators. These HDPs are directly antimicrobial and bind lipopolysaccharide (LPS) and lipoteichoic acid, damping down normal immune responses to these molecules. They are also immunomodulatory signals that help regulate both innate and adaptive immune responses (Yang *et al.*, 2002). HDPs have evolved in response to positive selection pressures exerted by colonising microorganisms.

The diversity and breadth of antimicrobial cover and immunomodulatory function they provide, is central to the ability of a host to respond to the diverse and highly adaptable organisms colonising mucosa.

Pathogens and commensals alike have developed strategies for surviving the activities of HDPs. Resistance mechanisms are related to structure of cell wall components (especially LPS) and cytoplasmic membranes, and peptide breakdown mechanisms (Devine & Hancock, 2002). The importance of LPS in determining resistance to HDPs is best characterised in *S. enterica* serovar Typhimurium, in which environmentally regulated two component signal transduction pathways (*phoPQ*, which in turn regulates *pmrAB*) cause LPS modifications that decrease binding and killing by HDPs. These modifications include the addition of 4-deoxy-4-aminoarabinose (Ara4N) or phosphoethanolamine to the phosphate residues in lipid A and core, which result in partial charge neutralisation of lipid A and reduced binding of HDPs. Also, the addition of palmitate to lipid A results in alterations to membrane fluidity and self-promoted uptake of HDPs.

The mouth, upper and lower respiratory tract are defended by a variety of HDPs, including beta-defensins (secreted by epithelial cells and macrophages), LL-37 (secreted by epithelial cells and released from neutrophils) and neutrophil alpha-defensins. Increasing attention has been paid to the roles of HDPs at these sites in defence against conditions such as periodontal disease and cystic fibrosis (CF) associated lung disease. HDPs are additionally important as potential novel anti-infective agents. We are examining the roles of environmental cues in determining interactions with HDPs and other innate defences of oral and respiratory bacteria, particularly the CF lung pathogen *Burkholderia cenocepacia* (formerly *B. cepacia* genomovar III) and *Porphyromonas gingivalis*, an obligately anaerobic periodontal pathogen. Both persist at infected sites for long periods and cause chronic diseases and both have become paradigm organisms with respect to their LPS.

B. cenocepacia is innately resistant to HDPs largely because of the structure of its LPS, the polysaccharide core of which consists of Kdo linked to Ko (D-*glycero*-α-D-talo-oct-2-ulopyranosonic acid), rather than Kdo-Kdo typical of enteric bacteria. *B. cenocepacia* LPS is relatively under-phosphorylated and is highly substituted with Ara4N. This structure results in poor binding and the lack of a self-promoted uptake pathway for HDPs. However we have identified two peptides with activity against *B. cenocepacia* and other genomovars of the *B. cepacia* complex: ovine cathelicidin SMAP-29 and the designed linear peptide D2A-22 (Devine, 2003). We have isolated mutants of *B. cenocepacia* that display an increased sensitivity to HDPs and have also assessed effects of environmental conditions on strains grown in complex and defined media with varying ionic compositions. Negative electrospray ionisation mass spectrometry (MS) and tandem MS showed that mutants and wild type organisms grown under altered medium conditions produced lipid A with variations in the degree of phosphorylation and acylation of the diglucosamine. So, although it has been stated that *B. cenocepacia* is constitutively resistant to HDPs, this organism does display some environmental regulation of lipid A structure resulting in altered HDP susceptibility. It is possible that these changes to lipid A acylation may also affect host cell recognition, as has been described for other Gram-negatives (Dixon & Darveau, 2005) and this is being investigated.

P. gingivalis is sensitive to a number of HDPs but resistance has been reported. Its LPS is structurally unusual compared with classical enterobacterial LPS and differs significantly in its biological activity. It was recently shown to produce a mixture of lipid A chemotypes that may activate human cells through either TLR2 or TLR4 (Dixon & Darveau, 2005). We have also provided evidence that *P. gingivalis* alters its cell surface in response to environmental cues that alter with the establishment of inflammation in the periodontal pocket, and we are determining the role of LPS alterations in this. *P. gingivalis* also produces potent extracellular and membrane bound proteases that cleave HDPs and play an important role in determining interactions with other host defences and receptors. We have shown that *P. gingivalis* proteases degrade HDPs, and protease production is also regulated by environmental conditions relevant to inflammation.

Oral and respiratory HDPs interact with large numbers of colonising bacteria. The diversity of these populations can be immense and, in addition to the species diversity evident in resident populations, single species exhibit substantial genetic and phenotypic diversity. The innate immune system must respond appropriately to commensal populations while retaining the ability to defend against pathogens. The importance to this of HDPs is becoming apparent, in protecting against specific pathogens, modulating resident populations and in regulating host responses to bacteria and their products, especially LPS. It is also apparent that pathogens such as *B. cenocepacia* and *P. gingivalis* have evolved mechanisms to rapidly adapt to changing environmental conditions, in ways that alter HDP sensitivity and binding to host receptors, thereby modulating host immune responses and increasing their survival.

An award from the President's Fund, which allowed studies of the environmental regulation of HDP resistance in *B. cenocepacia* to be presented at the 4th Gordon Research Conference on Antimicrobial Peptides, is gratefully acknowledged.

References

- Devine, D.A. (2003) Antimicrobial peptides in defence of the oral and respiratory tracts. *Molecular Immunology* **40**:431-443.
- Devine, D.A. (2004) Cationic antimicrobial peptides in regulation of commensal and pathogenic microbial populations. pp.9-37. In *Mammalian Host Defence Peptides*, ed. D.A. Devine and R.E.W. Hancock. Cambridge University Press: Cambridge.
- Devine, D.A. and Hancock, R.E.W. (2002) Cationic peptides: distribution and mechanisms of resistance. *Current Pharmaceutical Design* **8**:703-714.
- Dixon, D.R. and Darveau, R.P. (2005). Lipopolysaccharide heterogeneity: innate host responses to bacterial modification of lipid A structure. *Critical Reviews in Oral Biology & Medicine* **84:**584-595.
- Yang, D., Biragyn, A., Kwak, L.W., Oppenheim, J.J., 2002. Mammalian defensins in immunity: more than just microbicidal. *Trends in Immunology* **23:**291-296.

Deirdre Devine

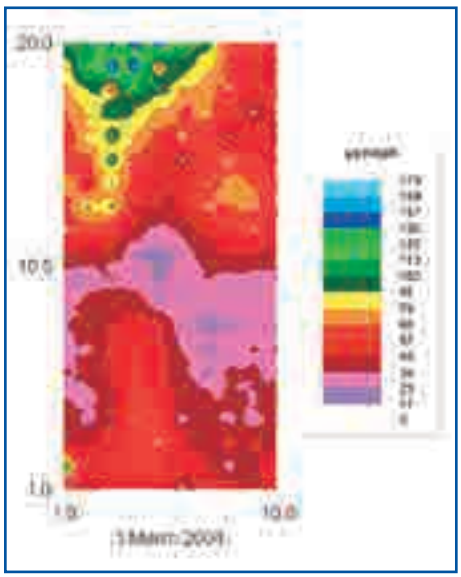
Spatio-temporal variations in nitrification in soil

NITRIFICATION IS A TWO STEP process performed by two distinct groups of autotrophic bacteria, the nitrifiers. In the first step the ammonium oxidisers, aerobic obligate chemoautotrophs or lithoautotrophs that use ammonium-nitrogen as their sole energy source and carbon dioxide as their main carbon source, convert ammonium-nitrogen to nitrite –nitrogen. The nitrite oxidisers complete the conversion to nitrate-nitrogen. The nitrifiers are relatively slow growing and form a very small proportion of the total bacterial population in soils, but have a large functional input into the plant-soil system.

Nitrification is of importance in crop production, converting an immobile form of inorganic nitrogen into a mobile form that may be more readily available to the plants. However this conversion can have environmentally undesirable affects, such as the eutrophication of water systems, when the soluble nitrate-nitrogen is washed out of the soil. Also gaseous nitrogen oxides, that are greenhouse gasses, are produced during nitrification, and a subsequent nitrogen transformation process, denitrification results.

Many studies have shown that large temporal variation in both actual and potential nitrification rates is a common characteristic of mineral soils. We have examined this, and looked for the drivers of these changes, for many years. Examining the effects of carbon inputs on nitrification and denitrification showed that microbial activity in soils is carbon limited. Inputs from plants, such as potatoes, at best provide probably only a quarter of the carbon, for energy, required for maximum microbial activity. The addition of large amounts of readily utilisable carbon, such as sucrose, stimulated denitrification rates, up to fourfold, but could halve potential nitrification rates. Both of these functions showed temporal variations of four to five times through the growing season (Wheatley *et al.*, 1991), and total microbial biomass increased for the first 25 days after amendment, but then returned to pre amendment rates.

Using a method that removed limiting factors such as soil ammonium-nitrogen availability, water, spatial relationships and aeration in laboratory incubations revealed even greater temporal variations, of up to tenfold, in organically fertilised field soils. Similar temporal patterns also occurred, with periodic abrupt positive and negative changes of up to fourfold in function rates over a 10 day period. Nitrification rates were significantly affected by inputs from barley plants, being increased early in the season and inhibited at the end. This and the fact that the addition of manures stimulated nitrification rates, together with the abruptness of the changes in dynamics, implicates interactions between parts of the microbial population as possible drivers of these temporal variations (Wheatley *et al.*, 1997).



Potential nitrification rates in a barley field over a 4 month sampling period (Fig 1 of 4 shown)

Such interactions could be driven by substrate inputs. We have investigated this using various substrate amendments, such as sucrose and amino acids at rates ranging between that representative of root exudate input to the amounts input in plant residues. When these were added to a field soil, in which barley was growing, nitrification rates were significantly altered. Such changes ranged from an 88% decrease to a 654% increase, at various times during the growing season. Generally when nitrification rates in the field soil were low, additions of the amino acids increased rates, and conversely additions of glucose, even when amended with inorganic-nitrogen, tended to depress them. This again supports the possibility that interactions between general soil heterotrophs and the autotrophic nitrifiers may occur in arable soils (Wheatley *et al.*, 2001).

The effects of the size, structure and activity of both the eubacterial and nitrifier populations on nitrification functional dynamics was examined by comparing eubacterial community structure with the subcomponents and dynamics of the ammonium- oxidiser population within and between three arable fields. Nitrification rates were significantly different between these three fields, and had distinct temporal patterns with changes in rates of between two and five fold in each. In all three, rates could occasionally double or triple in as little as ten days. Polymerase chain reaction followed by denaturing gradient gel electrophoresis (PCR-DGGE) with eubacterial primers showed that each field had its own significantly different community structure, and that these communities changed with time over the season. In contrast ammonium-oxidiser specific phylogenetic PCR primers suggested that the nitrifier populations were similar in types between the fields, and placed the ammonium-oxidising nitrifiers in *Nitrosospira* spp. clusters. Competitive PCR suggested nitrifying population sizes were different between, but did not change with time within, each field, with no relationship between population size and nitrification rates. Again there was no relationship between nitrifier population size and nitrification rates (Wheatley *et al.*, 2003).

Spatial aspects of these studies were intensified by comparing changes in rates in the most active of the three fields in soil samples taken on a 6m grid, at 91 points over the growing season. This showed spatially consistent temporal dynamics in nitrification rates. Nitrification rates changed five fold over the growing season, again with some very abrupt changes in rates on occasions. This has been followed by the present study, using 200 sampling points on a 4m grid in the same barley field. Nitrification rates, together with other parameters such as denitrification rates, soil ammonium and nitrate-nitrogen

concentrations, soil moisture, pH, etc are being assessed. Again there are dynamic spatial and temporal changes in nitrification rates in the field (Wheatley *et al.*, 2004). Activity levels in some areas of the field are consistently relatively greater than in others, but all show large temporal variation. Sample points covering a range of degrees of change with time have been selected for population analyses. Preliminary results from sequencing with ammonium-oxidiser functional primers, has revealed many *Nitrosospira* spp. sequence types. Work is continuing on studying their occurrence over space and time in the field, and in examining their functional expression.

I am grateful to the SfAM for awarding me a President's Fund Grant that allowed me to present some of my latest research.

References

- Wheatley R.E., Griffiths B.S. & Ritz K. (1991) Variations in the rates of nitrification and denitrification during the growth of potatoes (*Solanum tuberosum* L.) in soil with different carbon inputs and the effect of these inputs on soil nitrogen and plant yield. *Biology and Fertility of Soils* **11**, 157-162.

- Wheatley R.E., Ritz K. & Griffiths B.S. (1997) Application of an augmented nitrification assay to elucidate the effects of a spring barley crop and manures on temporal variations in rates. *Biology and Fertility of Soils* **24**, 378-383

- Wheatley R.E., Ritz K., Crabb D. & Caul S. (2001) Temporal variations in potential nitrification dynamics related to differences in rates and types of carbon inputs. Soil *Biology & Biochemistry*, **33**, 2135-2.

- Wheatley R.E., (2003) Caul S., Ritz K., Daniell T., Crabb D. & Griffiths B.S. Microbial population dynamics related to temporal variations in nitrification function in a field soil. *European Journal of Soil Science*, **54**, 707-714.

- Wheatley R.E., Daniell T., Banks G., Mitchell S., Davidson J., Ritz K. & McNichol J. (2004) Spatio-temporal variations in nitrogen transformation rates in soil. *Proceedings of 10th International Symposium on Microbial Ecology*, Cancun Mexico, August 2004

Ron Wheatley

Antimicobial Effect of Essential Oil of Egyptian Marjoram on Fungus and Bacteria

MARJORAM (*Origanum majorana L.*) is a hardy perennial and herbaceous plant which grows wild in its natural areas: Egypt and eastern Mediterranean countries. It belongs to the mint family (*Lamiaceae*) and has dark green oval leaves and small greyish white flowers in cluster (Furia & Bellanca, 1971).

Commercial *Origanum majorana L.* oil is used as a spice and condiment. This volatile aromatic compound is employed in the food industry as a spice in sausages. It is also used in baked goods, processed vegetables, condiments soups, snack foods and gravies. The oil is used in perfumery, herbalism, pharmacology and medical and clinical microbiology, as it is a good antispasmodic, antiviral, analgesic and antiseptic. Marjoram is also used in phytopathology and food preservation (Reineccius, 1994 and Circella *et al.*, 1995). Among the approaches employed in food preservation by inhibiting growth of undesirable microorganisms, is the use of chemical agents exhibiting antimicrobial activity. These chemicals may be either synthetic compounds intentionally added to foods or naturally occurring, biologically derived substances, *i.e.*, naturally occurring antimicrobials. These substances may exhibit antimicrobial properties in the foods in which they normally are found or may be used commercially as additives to other foods requiring preservation. The essential oil of marjoram has been shown to exhibit antifungal properties against *Candida albicans*, the yeast that is responsible for candidasis. It has also been shown to inhibit mycelial growth and aflatoxin production in *Aspergillus parasites* and *Aspergillus flavus*—both fungi which grow on stored grains and produce liver damaging aflatoxins (Paster *et al.*, 1995). These investigators have proposed using marjoram, cinnamon, cloves and sage as an alternative to chemical compounds for preserving stored grains. Marjoram is also a powerful antibacterial agent and is effective against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* sp. (Dorman & Deans, 2000). A recent study investigated the use of marjoram oil in the treatment of enteric parasites in 14 adult patients. After six weeks of treatment, there was a complete disappearance of *Entamoeba hartmanni* in four cases and *Blastocystis hominis* in eight cases. Furthermore, Gastrointestinal symptoms improved in seven of the eleven patients infected with *Blastocystis hominis.*

Chemical Composition of Egyptian Marjoram essential oil

The essential oil of Egyptian Marjoram (*Origanum majorana L.*) was extracted by steam distillation from the whole plant and analyzed by gas chromatography. The average yield of essential oil was 0.3 gm / 100 gm (fresh plant). Out of the 15 constituents separated by GC, ten were identified. The identified compounds represent (83.42%) of the total essential oil. The gas chromatography data showed that the tested essential oil sample of (*Origanum majorana L.*) was rich in linalool (20.98%), limonene (16.78%), β-pinene (12.49%), P-cymene (10.88%), α-pinene (9.69%) and 1,8 cineol (6.84%).

The essential oil also contained smaller quantities of terpinene-4-ol (1.92%), linalyl acetate (1.82%), α-terpinene (1.03%) and eugenol (0.99%). The identified compounds were classified into two groups: Monoterpenes (M) and Light–Oxygenated Compounds (LOC). Monoterpenes constitute the major class (50.87%) in steam distilled essential oil of Egyptian marjoram followed by Light–Oxygenated Compounds which constitute the second most predominant group (30.73%) in Egyptian marjoram oil.

Antifungal activity

The effect of Egyptian Marjoram essential oil on radial fungal growth was determined by the disc agar method as stated by Bauer *et al.*, (1996), in the appropriate culture medium (potatoes dextrose agar). The growth of *Aspergillus niger*, *Aspergillus flavus*, *Fusarium moniliform*, and *Penicillium expansum*, was inhibited by marjoram essential oil. The growth of the tested molds was decreased by increasing oil concentration.

The minimum inhibitory concentration (MIC) values for essential oil against the tested molds varied from 300µl/100ml for *Aspergillus niger* and *Penicillium expansum* to 350µl/100ml for *Fusarium*

moniliform, while the MIC value for *Aspergillus flavus* was found to be 400µl/100ml. However, the sensitivity of mold growth to the essential oil at each of the applied concentrations was different. Although *Aspergillus flavus* showed the highest sensitivity at low concentration (100µl/100ml), its total inhibition occurred at high concentration (400µl/100ml). Sensitivity of mold growth to the essential oil followed the sequence Penicillium expansum > *Aspergillus niger* > *Fusarium moniliform* > *Aspergillus flavus*

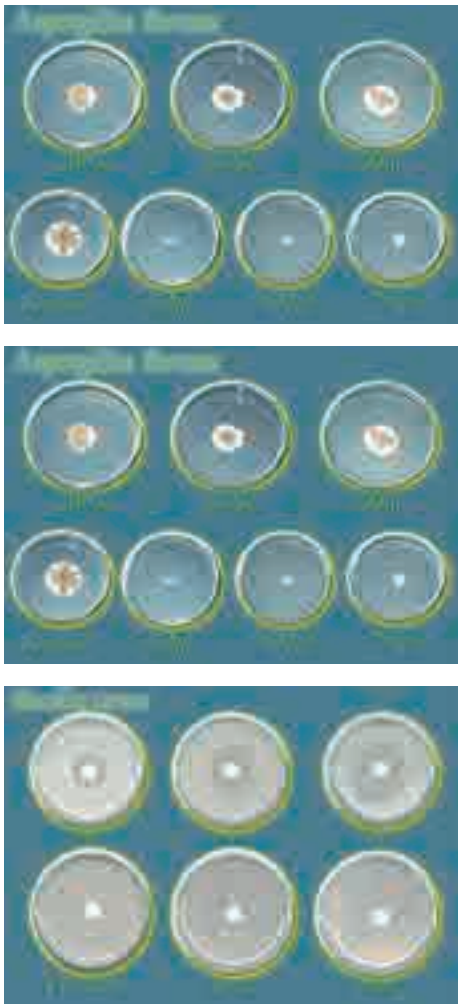
A total inhibition of fungal growth was observed in the presence of the highest concentration of oil. However, at concentrations up to 250µl/100ml, inhibition of growth was less pronounced. At a concentration of 300 to 400µl/100ml, the growth of mycelia of tested molds was completely inhibited. The antifungal activity of marjoram essential oil obtained from whole plant was due to the presence of some effective components such as linalool, limonene, β-pinene, P-cymene, α-pinene and 1,8 cineol, and their antimicrobial activity has been reported in literature (Paster *et al.*, 1990 and Yadava & Saini, 1991).

Antibacterial activity

The effect of marjoram essential oil on the growth of bacteria was determined according to the disc diffusion method (Deans & Ritchie, 1987). The essential oil of Egyptian marjoram has a significant activity against *Bacillus cereus* and *Escherichia coli* O157:H7. The diameter of inhibition zones increased with increasing concentration of marjoram oil.

A total inhibition of bacterial growth was observed in the presence of the highest concentration of oil. At concentration of 100 and 160µl/disc, the growth of *Bacillus cereus* and *Escherichia coli* O157:H7 was completely inhibited, respectively, as seen in Fig. 2. Zaika (1988) and Smith-Palmer *et al.*, (1998) proposed that Gram-positive bacteria are more resistant than Gram-negative bacteria to the antimicrobial properties of volatile plant oils, which is in contrast to the hypothesis proposed by Deans & Ritchie (1987). They state that the susceptibility of bacteria to volatile plant oils and the Gram reaction appears to have little influence on growth inhibition. Although, the volatile oil of *Origanum vulgare ssp. Hirtum*, *Myristica fragrans*, *Syzygium aromaticum* and *Piper nigrum* did appear to be equally effective against both Gram-positive and Gram-negative microorganisms, as reported by (Dorman and Deans, 2000). However, Egyptian Marjoram essential oil appeared preferentially more active with respect to Gram-reaction, exerting greater inhibitory activity against Gram–positive bacteria.

The antimicrobial activity of marjoram oil could be due to the action of phenolic



Effect of marjoram essential oil on growth inhibition at different concentrations

compound of the oil on cellular membrane, destroying its permeability, releasing intercellular constituents and causing membrane malfunction in respect to electron transport, nutrient uptake, nucleic acid synthesis and ATPase activity (Skandamis *et al.*, 1999 and Fisher, 2002). The use of industrially synthesised food antimicrobial compound may be associated with potential toxicological problems and this has generated interest in the use of naturally antimicrobial compounds for the preservation of so-called Natural foods.

References

- Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C. and Turck, M.(1996). Antibiotic susceptibility testing by Standardized Singl Disc Method. *Am. J. Clin. Path.*, **45:** 493-496.
- Circella, G.; Franz, C.; Novak, J. and Resh, H. (1995).Influence of day length and leaf insertion on the composition of Marjoram essential oil. *Flavour Fragr. J.*,**10:**371-374.
- Deans, S.G. and Ritchie, G . (1987). Antibacterial properties of plant essential oils. *Int. J. food Microbiology.*, **5:** 165-180.
- Dorman, H.J.D. and Deans, S.G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Applied Microbiology.*, **88:** 308 -316
- Fisher, C. (2002). Spices of life. www.Chemsoc.org/Chemytes/ezine/2002/fisher_jano2.htm
- Furia, T. E. and Bellanca, N. (1971). Fenaroli's handbook of flavor ingredients. The Chemical Rubber Co., Cleveland, OH.
- Paster, N.; Juven, B.; Shaaya, E.; Menasherov, M., Nitzan, R.; Qweisslowicz, H. and Ravid; N. (1990) Inhibitory effect of oregano and thyme essential oils on molds and food borne bacteria. *Letters in Applied Microbiol.*, **11:**33 - 37.
- Paster, N.; Menasherov, M.; Ravid, U. and Juven, B. (1995). Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attaching stored grains. *J. of food protection.*, **58:** 81-85.
- Reineccius, G. (1994). Source book of flavours. Chapman and Hall : New York, pp.300-301.
- Skandamis,P,N.; Michailidou, E. and Nychas G-J,E. (1999). Modeling the effect of oregano (*Origanum vulgare*) on the growth – survival of *Escherichia coli* O157:H7 in broth and traditional Mediterranean foods . International *Congress on improved traditional foods for the next century,* Valencia 28 – 29/1999,Spain, pp.270 -273.
- Smith–Palmer, A.; Stewart, J. and Fyfe, L. (1998). Antimicrobial properties of plant essential oils and essences against five important food – borne pathogens. *Letters in Applied Microbiology.*, **26:** 118-122.
- Yadava, R. and Saini, V. (1991). Antimicrobial efficiacy of essential oils of (*Majorana hortensis* Moench.) and *Anisomeles indica* (linn.) Kuntze. *Indian Perfum.*, **35:** 58 - 63.
- Zaika, L.L. (1988). Spices and herbs: their antimicrobial activity and its determination. *J. food nutrition*, **9:** 97-118.

Professor Gibriel Ahmed

Mucosal Bacterial Communities and Inflammatory Bowel Disease

THE HUMAN LARGE BOWEL harbours a complex microbiota comprising several hundred different bacterial species and strains. Studies have shown that there are marked variations in bacterial populations in the gastrointestinal (GI) tract along its length, and that bacterial species composition varies from individual to individual.

This is due to a number of factors, such as host genetics, environment, substrate availability, pO2, pH, diet and lifestyle. Although the dominant bacterial communities in an individual remain stable over time, the microbiota can be adversely affected by age, changes in diet, antibiotic use, immunosuppressive therapy and irradiation. Commensal bacteria colonising the large bowel are important in human health, and are involved in modulation of the host immune system, protection of the host against invading bacteria and viruses and vitamin production. They also take part in the final processes of digestion. Most of us tolerate this complex metabolically active and antigenic biota, but in approximately two per 1000 of adults living in the UK, an intense inflammation develops in the mucosa, that is not caused by known pathogens, but which is often associated with bloody diarrhoea, urgency to defecate and general morbidity (Macfarlane & Cummings 2003).

These inflammatory bowel diseases (IBD) are acute and chronic idiopathic disorders of the gut. The principal forms of IBD are ulcerative colitis (UC) and Crohn's disease (CD). UC is an inflammatory disease principally affecting the superficial epithelial layer of the rectum, distal colon, and occasionally, the proximal large bowel. In contrast, CD can involve any part of the gastrointestinal tract, and unlike UC, is manifested by chronic inflammation in all layers of the bowel wall. The aetiologies of UC and CD are poorly understood, but it is known that environmental, microbiological, immunological and genetic factors are involved. The recurring inflammation observed in both diseases points to the cause of these disorders lying in regulatory dysfunction of the mucosal immune system.

Animal models show that commensal bacteria are essential for colitis to develop, however, while there is increased antibody production against anaerobes in UC (Furrie *et al.*, 2004), evidence for a specific transmissible agent in this disease is weak. Mucosal bacteria growing in biofilms on the gut wall are increasingly being linked to IBD, either through pathogens colonising the epithelial surface, non-pathogenic commensal species occupying adhesion sites on the mucosa and preventing invasion by harmful bacteria, or by inappropriate host immune responses to members of the normal microbiota. Studies on bacterial communities in biopsies from normal and inflamed colonic mucosa in healthy controls, and in patients with acute UC, have demonstrated that large reductions in bifidobacterial numbers occur in IBD, while there are significantly increased levels of enterobacteria, together with highly immunogenic enterococci and peptostreptococci (Macfarlane et al. 2004). Studies by Duchmann *et al.*, (1999) indicated the role played by gut commensals as modulators of inflammatory processes. These workers demonstrated that when exposed to their own intestinal bacteria, mucosal, but not peripheral blood mononuclear cells from patients with IBD proliferated. Autologous bacteria failed to induce proliferation in cells from uninvolved mucosa of the same patients, and from patients in remission. However, they did proliferate in response to sonicates of bacteria from a heterologous intestine. Further evidence of a relationship between the normal colonic microbiota and the pathogenesis of UC can be seen in experiments on genetically modified rats. Here, the animals do not develop IBD when housed in a germ-free environment, whereas those harbouring a normal faecal microflora, or given bacterial cell wall products, develop chronic colitis. Taken together, these results support the hypothesis that without the initiating bacterial antigenic epitopes, genetic susceptibility is not sufficient by itself to cause clinical symptoms associated with IBD.

It is believed that in IBD, immune tolerance to endogenous bacteria can be lost as a result of an imbalance between pro-inflammatory and anti-inflammatory cytokines, which can lead to an exaggerated/inappropriate immune response towards commensal bacteria or food antigens, thereby triggering inflammation. Because of this, many experimental and clinical investigations have focused on the role played by protective mucosal inhabitants in reducing the symptoms of IBD. A recent study by Furrie *et al.*, (2005) showed that a synbiotic comprising a probiotic strain of *Bifidobacterium longum* (originally isolated from a healthy colonic mucosa), together with oligofructose as the prebiotic component, strongly reduced inflammatory processes in the mucosa in UC patients. The treatment was given in a double-blinded randomised controlled trial with 18 patients, for a period of one month. The bifidobacterium was shown to colonise the rectal mucosa and reduce production of TNFα and IL-1β, which are proinflammatory cytokines that drive inflammation in UC. Human beta defensins 2, 3 and 4, which are only produced by epithelial cells in response to injury, and not by inflammatory infiltrates in the mucosa, were also lowered to normal levels by the synbiotic. Biopsies from patients receiving the synbiotic had macroscopic evidence of reduced inflammation, and regeneration of healthy epithelial tissue, while clinical indicators of disease severity, such as sigmoidoscopy scores and bowel habit index values, were reduced significantly. These studies showed that introducing a bacterium with known anti-inflammatory properties to the gut mucosa could result in significant benefits in colitis patients, and offer the prospect of effective and inexpensive therapies being developed for treating IBD in the future.

I would like to thank the Society for Applied Microbiology for awarding me a President's Fund Grant which allowed me to attend and present my works in the XIth International Union of Microbiological Societies 2005 in San Francisco, USA.

References

- Cummings J.H., Macfarlane G.T., Macfarlane S. (2003) Intestinal bacteria and ulcerative colitis. *Current Issues in Intestinal Microbiology* **4**, 9-20.
- Duchmann, R., May, E., Heike, M., Knoll, C.P., Neurath, K.H., and Meyer zum Buschenfelde, K.H. (1999) T cell specificity and cross reactivity towards enterobacteria, *Bacteroides, Bifidobacterium* and antigens from resident intestinal flora in humans. *Gut*, **44**, 812–818
- Furrie E, Macfarlane S, Cummings J.H, Macfarlane G.T. (2004) Systemic antibodies towards mucosal bacteria in ulcerative colitis and Crohn's disease differentially activate the innate immune response. *Gut* **53**, 92-99.
- Furrie E, Macfarlane S, Kennedy A, Cummings J.H., Walsh S.V., O'Neil D A., Macfarlane G.T. (2005) Synbiotic therapy (*Bifidobacterium longum*/Synergy 1TM) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut* **54**, 242-249.
- Macfarlane S, Furrie E, Cummings J.H., Macfarlane G.T. (2004) Chemotaxonomic analysis of bacterial populations colonizing the rectal mucosa in patients with ulcerative colitis. *Clinical Infectious Diseases* **38**, 1690-1699.

Bahrum Bahrumi

Structural and Biochemical analysis of a hyaluronan lyase from *Streptomyces coelicolor* A3(2)

THIS BACTERIUM PLAYS AN important role in the degradation of organic material. The entire genome of *Streptomyces coelicolor* was sequenced in July 2001. It is considered the largest bacterial genome discovered so far as it has about 8,667,507 base pairs long and contains 7,825 predicted genes. In addition, *Streptomyces coelicolor* is of great importance as it is used to produce the majority of antibiotics (over 50 different antibiotics have been isolated from this bacterium) and anticancer agents used in human and veterinary medicine and are consequently invaluable in the medical field (Bentley S.D., *et al.*, 2002).

This bacterium carries an open reading frame (sc1c2.15) encoding a putative hyaluronan lyase that is a member of polysaccharide lyase family 8 (Coutinho PM & Henrissat B,1999). Polysaccharide lyases cleave polysaccharide substrates through a â-elimination mechanism, while polysaccharide hydrolyases cleave glycosidic bond through a hydrolytic mechanism. An important characteristic of this elimination is the formation of unsaturated C4 -C5 bond on the non-reducing end of one of the products. This bond provides the basis for the collection of data, as it produces an absorbance at 232 nm. There are 15 families of polysaccharide lyases, compared to 91 families of glycoside hydrolyases. This is because of restricted substrate specificity by polysaccharide lyases, as these enzymes require a free carboxylate group at the C5 position of the sugar ring of their substrates (Charnock, S.J., *et al.*, 2002).

Hyaluronan lyase degrades hyaluronic acid (HA) a main polysaccharide component of the extra cellular matrix of body tissues of all vertebrates into unsaturated oligosaccharide units as the end product. Hyaluronan is composed of repeating units of glucuronic acid and N-acetylglucosamine connected by β 1,4-glycosidic linkage (Li, S. *et al.*, 2000). It plays an important role in a variety of biological processes such as cell migration, cell proliferation, inflammation, wound healing and growth and metastasis of tumour cells. Also HA keeps tissues hydrated and maintains osmotic balance and cartilage integrity (Lokeshwar, V.B. *et al.*, 2002). Another substrate that is highly similar to hyaluronan is chondroitin. This substrate is composed of repeating disaccharide units of glucuronic acid and N-acetyle galactoseamine. It has sulphate groups on either the fourth carbon or the sxith carbon of the N-acetyl galactosamine.

In order to gain an insight into the substrate specificity and catalytic mechanism of this enzyme, the open reading frame sc1c2.15 from *S.coelicolor* has been cloned and the N-terminally hexatidine tagged protein solubly expressed in *E.coli* BL21. The expressed soluble protein was purified using immobilised metal affinity chromatography column (IMAC) charged with nickel. The hexahistidine tag which was incorborated into the gene allows for purification by this method. Purified protein from IMAC can then be further purified using and gel filtration chromatogeaphy to provide an ultra pure sample of protein.

This enzyme was biochemically characterised and found to be active against sodium hualuronan and chondroitin 4 and 6- sulphate. Also using High Pressure Liquid Chromatography (HPLC), which allows for the visualization of saccharides produced from an enzyme digest we found that the enzyme is acting exolytically and produces disaccharides as the end product.

Native complex of SC1C2.15 crystallised in the P43212 tetragonal cell space group with unit cell dimensions of a=140.389 Å, b=140.389 Å, c=100.114 Å .The structure was solved using a multiple isomorphous replacement method to a resolution of 1.7 Å. A mutant (Y264A) complex of SC1C2.15 crystallised in the H3 tetragonal cell space group with unit cell dimensions of a= 317.100 Å, b= 317.100 Å, c= 82.974 Å. The structure was solved using a multiple isomorphous replacement method to a resolution of 2.0 Å. The enzyme consists of a N-terminal α -helical domain and a C-terminal β-sheet domain.

Zainab Elmabrouk

References

- Bentley S.D., Chater K.F., Cerdeno-Tarraga A.M., Challis G.L., Thomson N.R., James K.D., Harris D.E, Quail M.A., Kieser H, Harper D., *et al.,* Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). *Nature* **417**, 141-147.
- Charnock, S.J., Brown, I.E., Turkenburg, J.P., Black, G.W. and Davis, G.J. (2002). Convergent evolution sheds light on the anti-β-elimination mechanism common to family 1 and 10 polysaccharide lyases. *Proceeding of the National Academy of Sciences* (USA). **99:**12067-12072.
- Coutinho P.M., & Henrissat B (1999) Carbohydrate-Active Enzymes server at: http://afmb.cnrs-mrs.fr/~cazy/ CAZY/ index.html.
- Li, S., Kelly, S.J., Lamani, E., Ferraroni, M., and Jedrzejas, M.J. (2000). Structural basis of hyaluronan degradation by *Streptococcus pneumoniae* hyaluronate lyase. *EMBO Journal*. **19:**1228-1240.
- Lokeshwar, V.B., Schroeder, G.L., Carey, R.I., Soloway, M.S. and Iida, N. (2002). Regulation of hyaluronidase activity by alternative mRNA splicing. *The Journal of Biological Chemistry.* **277** (37):33654-33663.

BOOKS

FREE BOOKS!

Would you like to review a book for ***Microbiologist*** and get to keep it? The Society receives several new books every week from publishers around the world and are always looking for enthusiastic additional reviewers who have an interest in the subjects covered.

There is an up-to-date list of titles available for review on the Society's website at:

www.sfam.org.uk/pubs/books.php.

To make an offer to review any book simply click its title. This will send an email request to the Editor of ***Microbiologist.*** In return for your efforts you get to keep the book!

You can also read online versions of book reviews published in ***Microbiologist*** on the same web page.

www.sfam.org.uk/pubs/books.php

Food Microbiology, An Introduction, 2005

T. J. Montville and K.R. Matthews
ASM Press. Washington, D.C.
ISBN: 1-55581-308-9
Price: £44.50 ($79.95)
Reviewed by: Dr Evdoxios Psomas

This is an up-to-date basic textbook about the complex and challenging field of Food Microbiology. Based on the success of the earlier book *Food Microbiology: Fundamentals and Frontiers* (also published by ASM) the authors presented a classroom-friendly adaptation of this previous publication.

The book is divided into five sections and each section is subdivided into four to six chapters. A quite useful characteristic of the books organisation is that each chapter begins with a *'learning objectives'* box that contains all the critical points of the chapter. Furthermore, at the beginning of each chapter there is a flow diagram of the topics covered that will facilitate student's studying. More pedagogical tools that help students are included at the end of each chapter; these are a conclusion, a summary and several questions for critical thought.

The first section (I) introduces the reader to the basics of food microbiology such as factors that influence microbial growth in foods, spores and how significant they are, methods for detection and enumeration of microbes in food commodities, rapid and automated identification methods for several microorganisms and their usefuleness in the food industry. The section ends with a chapter about indicator microorganisms and microbiological criteria providing useful information about their purpose and the need to establish microbiological criteria but also on the indicators of microbiological quality and on foodborne pathogens and toxins. The highlight of the chapter is the discussion on certain applications and specific proposals for Microbiological criteria for food and food ingredients.

Section two (II) covers Gram-negative Foodborne pathogenic bacteria (*Salmonella*, *Campylobacter*, *Yersinia enterocolitica*, *Shigella*, *Vibrio*) and a very well written chapter about enterohemorrhagic *Escherichia coli*

which includes valuable information for *Escherichia coli* O157:H7.
The third (III) section of the book deals with the Gram-Positive pathogenic bacteria. The biochemical characteristics of the microorganisms (*Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium botulinum*, *Clostridium perfringens* and *Bacillus cereus*) are discussed assisting identification of the particular microorganism. Furthermore conditions in food that favour growth of the bacteria are also described.

Section four (IV) deals with other important microbes such as fermentatine microorganisms, microbes that cause spoilage, molds, viruses and prions. The first chapter of this section is about fermentative organisms and describes the biochemical basis of food fermentations, the similarities and differences among fermentations of dairy, vegetable and meat products. Furthermore, which bacteria are related to each specific fermentation and the process for making fermented food and its benefits, are also discussed. The succeeding chapter covers food spoilage, the intrinsic mechanisms that inhibit it, the impact of food processing and the sources of microorganisms responsible for spoilage of specific products. The objective of this well-written chapter is to discuss procedures that can be implemented to prevent spoilage. The last chapter of this section covers common food spoilage and mycotoxin producing molds. Conditions that contribute to the growth of molds and toxin production are described in full detail.

The last section of the book (Section V) describes the strategies for producing safe food with proper control of microorganisms. The chapter on antimicrobial chemicals contains a lot of useful information not only on chemical preservatives (such as organic acids, nitrites, parabenzoic acids) but also on naturally occuring antimicrobial compounds (lysozyme, lactoferrin, spices, garlic and onion). The following chapter helps the reader to understand how biological methods could be used to enhance food safety in a 'natural' way without changing the nature of the food. Furthermore it contains a comprehensive review of probiotic bacteria and the potential antimicrobial use of bacteriocins. Physical methods of preservation such as dehydration, cool, freezing and frozen storage, heat treatment and irradiation are also described in the following chapter. The last chapter of the book, deals with the implementation of industrial strategies to ensure production of safe food. HACCP and GMP's are widely described and measures for application of these in the food industry are proposed.
In general this book offers a clear and simple introduction to Food Microbiology. It is an advanced text not only for graduate students but also for anyone interested in the aspects of Food Microbiology such as research microbiologists and professors of Food Microbiology. Overall it is an excellent-high quality publication and I recommend it highly.

Clinical Laboratory Medicine

Volume Editor: Lynne Shore Garcia
ASM Press, Washington. 2004
ISBN 1-55581-279-1 pp. 864
List Price $149.95.
ASM member price $129.95
Reviewed by: Philip F Wheat

This substantial book is divided into ten sections:

1 Overview of laboratory management and current healthcare environment
2 Managerial leadership
3 Personnel management
4 Requirements for effective laboratory management
5 Financial management
6 Generation of revenue
7 Reimbursement processes
8 Outside marketing and expansion
9 Defining and measuring standards for success
10 The future of clinical laboratories

The sections are each then divided into different chapters totalling fifty in all. Each chapter follows a unified style. Firstly, the objectives of the chapter are highlighted at the beginning in a shaded box. Secondly, for ease of reference the main sections of the chapter are listed on page one of the chapters. Each chapter is completed by a summary section, key points of the chapter, a glossary of terms, references and finally some have a suggested further reading list. In addition, appendices are at the end. These consist sometimes of simple listings of websites with useful further advice/information. In comparison appendix 2.4 (end of chapter 2) is a useful table entitled "Consequences of neglecting basic management functions". Four management functions are listed (planning, organising, directing and controlling) each of these functions is then subdivided and for each one of these sub-divisions a type of neglect is described and the consequences of this neglect. It is a pity that such a useful informative table could not be accommodated in the body of the text.

The book provides an excellent starting point for anybody starting out their managerial career in clinical laboratory medicine. However, I must be critical. The book is solely written with the North American model of clinical laboratory medicine in mind. Whilst this was obviously the prime purpose of the book it does lessen its appeal to readers outside this healthcare model. The result of this is that anybody purchasing this book outside this market will find that approximately fifty percent of the text will not be relevant to their health care environment. Whilst it is interesting and informative to appreciate North American management of clinical laboratories it would have found a wider audience acceptance with a broader scope in the areas which are solely describing the North American systems.

My other criticism is that some of the Chapters are very brief. For instance Chapter 12 (Effective Meetings) and Chapter 14 (Managing Change) are only five and seven pages respectively. In particular there are multiple books which weigh down bookshelves in many Business School libraries on the subject of managing change so devoting just seven pages to this very important management topic is rather puzzling.

These criticisms apart I do recommend this book. In particular any laboratorian that is currently working or about to start as a manager in the North American clinical laboratory market the book should be essential as a personal copy. For anybody not in this situation my advice would be loan the book first to assess whether a personal copy would be a worthwhile investment.

society for applied sfam microbiology

For more information about the Society, Society meetings, the benefits of membership, *Microbiologist*, or to join us please visit the Society website at www sfam.org.uk

Benefits of sfam membership

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

- **Reduced rates at Society meetings**
- **Access to the members areas of the Society website**
- **Generous grants and awards**
- **FREE access to three acclaimed journals**

Detailed information about all these benefits and more can be found on the Society website.

WEBSITE:

www.sfam.org.uk

The website is the best source of detailed information on the Society and it's many activities. It has a lively discussion forum and fully interactive membership areas where you can book your place at Society meetings find and advertise jobs, display your CV and much more.

CONTACT:

Society for Applied Microbiology, The Blore Tower, The Harpur Centre, Bedford MK40 1TQ, UK
Tel: 01234 326661
Fax: 01234 326678
email: info@sfam.org.uk
www.sfam.org.uk

membership options

- **Full membership** gives online access to the ***Journal of Applied Microbiology***, ***Letters in Applied Microbiology*** and ***Environmental Microbiology***, copies of ***Microbiologist***, preferential registration rates at Society meetings and access to the members areas of the website.

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

- **Associate membership** this class of membership is open to all current and new Society members including existing Associate Student Members and Retired members and gives quarterly copies of ***Microbiologist*** and preferential registration rates at all Society meetings.

- **Honorary Membership** of the Society is by election only and this honour is conferred on persons of distinction in the field of applied microbiology.

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

  - Online access to the Society's three journals.
  - Half page advertisement in each quarterly issue of ***Microbiologist*** (which can be upgraded to a larger size at very attractive discounted rates).
  - Full page advertisement in the Members' Handbook.
  - FREE banner advert on the Society Website with a direct link to your company site.
  - Up to three members of company staff attending Society meetings at members' rate. (This means a 50% discount on non member registration rate).

- **Retirement Mambership**
Full Members can apply to go on the 'Retired Members List' once they have retired from their employment and have completed at least 20 years membership of the Society. Retired members are entitled to all the benefits of Full Membership except access to ***Journal of Applied Microbiology***, ***Letters in Applied Microbiology*** and ***Environmental Microbiology.***

Publications

The Society publishes two monthly journals: ***Journal of Applied Microbiology*** and ***Letters in Applied Microbiology.*** We also produce our own quarterly in-house colour magazine: ***Microbiologist***, which contains features, reports topical news stories and full details of our meetings. The Society is also a partner with Blackwell Publishing in the bi-monthly journal ***Environmental Microbiology.***

Online journals

Synergy is an online service provided by Blackwell Publishing that gives Full and Student Members **FREE** access to the online versions of the Society's three journals: ***Journal of Applied Microbiology***, ***Letters in Applied Microbiology*** and ***Environmental Microbiology***. Members can register for this service at http://www.blackwell-science.com. Members can also submit papers directly to our journals via an online submission service.

For more information about Synergy or online manuscript submission, please visit the website.

Grants & awards

Many awards and prizes are available to members including the **W H Pierce Memorial Prize** and Prizes for Student Oral Presentations and Posters at the Summer Conference. In addition to these substantial awards, the Society has funds to assist members in their careers as microbiologists. These include **The President's Fund**, Conference Studentships, Sponsored Lectures and the popular **Students into Work** Scheme.

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

Meetings

We hold two annual meetings. The January Meeting comprises discussion sessions with the opportunity to display posters on related work. The Summer Conference is held every July and comprises a main symposium, a poster session, the AGM and a lively social programme. We also hold occasional joint ventures with other organisations on topics of mutual interest.

BD Diagnostic Systems

Solutions for Industrial & Clinical Microbiology

BD

Helping all people live healthy lives

BD Diagnostics
21 Between Towns Road
Cowley
Oxford OX4 3LY
Tel: 01865 781677
Fax: 01865 781528
www.bd.com

TAKE A CLOSER LOOK AT

Prepared Media

Oxoid offers a comprehensive range of high quality prepared media products to laboratories:

- CONVENIENT AND EASY TO USE.
- SAVING TIME AND EFFORT.
- RELEASING STAFF FOR MORE IMPORTANT TASKS.
- QUALITY AND RELIABILITY ENSURED.

READY-POURED PLATES

- Extensive range of poured plate media.
- Prepared under Class 100 conditions.

NEW STOCK WATCH

Stock Watch is an easy-to-use device that provides laboratories with a portable and accurate way of monitoring and managing laboratory stock.

BOTTLES, BAGS, TUBES

- Wide variety of volumes and formats.
- Available for broths, diluents and agar slopes.

OUTSTANDING SERVICE

- BS EN ISO 9000 registered Prepared Media production facilities.
- Each batch performance tested prior to release.
- Quality control certificates supplied as required.
- Every product marked with product code, batch number and expiry date to meet regulatory requirements and to provide complete traceability.
- Regular supply through easy-to-arrange standing orders.
- Flexible standing orders to meet the changing needs of your laboratory.
- **NEW** Quality control certificates can now be easily downloaded from www.oxoid.com

To find out more about how the Oxoid Prepared Media Service can make a difference in your laboratory, contact:

OXOID

DEDICATED TO MICROBIOLOGY

www.oxoid.com
Oxoid Ltd, Wade Road, Basingstoke, Hants, RG24 8PW, UK. Tel: +44 (0) 1256 841144 Fax: +44 (0) 1256 463388 Email: val.kane@oxoid.com