

Microbiologist

The magazine of the Society for Applied Microbiology ■ December 2004 ■ Vol 5 No 4

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A Night in the arms of enus...

Syphilis - a disease with history

ALSO IN THIS ISSUE:

Gonorrhoea: an unlikely love affair

The use of Analysis of Variance in applied microbiology

Summer Conference 2005

Biodiversity conservation in Costa Rica

Education in Ethiopia

Peter Silley looks at Open Access Publishing

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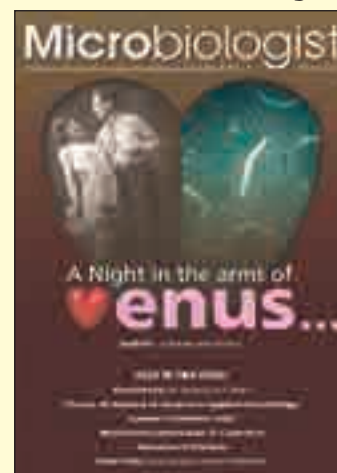
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The editor is always looking for enthusiastic writers who wish to contribute articles to *Microbiologist* on their chosen microbiological subject. Email: harperlv@aston.ac.uk

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Friday 11 March 2005

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Friday 8 July 2005

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Friday 16 Dec 2005

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Website: the society website is a timely source of up-to-date information on all Society matters and maintains a comprehensive archive of articles and reports on a variety of microbiological topics.

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WELL, CHRISTMAS IS NEARLY upon us again — how time flies! It only seems like yesterday that I was tucking into Turkey and singing *Jingle Bells* after some Gluhwein and Bratwurst (Birmingham has a lovely German Market that installs itself in Victoria Square over the festive season).

Anyway, we're currently planning our laboratory Christmas 'do' — an event that's always an afternoon and evening to remember (that is, if one *can* remember it afterwards!). Whilst I hope you, too, will enjoy the festivities this year, I'm sure you're only too familiar with the over-indulgence the seasons' jollities bring, along with their attendant dangers, many of them microbiological in nature. It's all too easy, after one-too-many eggnogs, to be lackadaisical in our approach to hygiene in the home. I'll never forget the year that my mother fell ill just before Christmas after eating under-cooked turkey one of her colleagues had prepared for her and her work-mates.

Unfortunately she was bed-ridden for several days. My mother's Christmas dinners are something to behold, but that year we had to smilingly endure dad's alternative. My father's never been renowned for his talent in the kitchen and I think the thought of preparing a full Christmas dinner was a little too daunting for a novice like himself. So it was beef stew and dumplings for us that year. Despite showing our sincere gratitude to Dad for his sterling efforts, even he conceded that it just wasn't quite the same! So, without wanting to put a downer on your jollities, the moral of my little tale is: *Cook it well, or there may be hell (to pay)*

In this issue of *Microbiologist*, there is a little 'light' reading, which those of you who didn't do a statistics degree, should find useful. They do say 'there's lies, damn lies and statistics', but I hope you'll agree that this article does nothing to support such a damning adage. Talking of statistics, here are a few Christmas facts you, and my mum's work-mate perhaps should have considered:

1. Nearly one third of Britons (32%) cook their stuffing inside the turkey. This method runs the risk that both the stuffing and the bird might not cook through fully. The safe and easy way to enjoy stuffing is by cooking it separately in a roasting tin.

2. Over four fifths of Britons wash the turkey before cooking it. In fact, washing



can splash harmful bacteria already on the bird around the kitchen leading to cross contamination. Thorough cooking will eliminate any pathogens so there is no need to wash the bird first.

3. 64% of people defrost their turkey by leaving it standing out in the kitchen whilst only 20% follow the ideal advice and defrost their turkey in the fridge. (<http://www.gnn.gov.uk>).

We also have a couple of entertaining articles that talk about other microbiological issues which may result from a little too much enjoyment of the festive season! Without wanting to go into too much detail, one article discusses syphilis and the other, gonorrhoea. Whilst researching this editorial, I came across a rather amusing adulterated version of the 'Twelve days of Christmas' which had a theme not dissimilar to the theme of this issue. I won't repeat it entirely here, but for those of you who know this Christmas song, imagine the 'five gold rings' line being replaced by the STI referred to in our second feature article and I'm sure you'll get the idea. On a more serious note, a study performed a few years ago by Wellings *et al.* (1999) revealed that sexual activity — including 'unsafe' sexual practices increases at Christmas and this correlates with a rise in the number of STIs recorded at this time of year. An unnecessary warning to us all I'm sure. However, this study also revealed that there was a marked increase in the number of births nine months afterwards. Hmm.. come to think of it, I thought I'd had to buy quite a few 'Congratulations on the birth of your baby' cards last September! Anyway, that's enough from me. I hope you enjoy this Christmas issue and that you all have a wonderful festive season, a Happy Christmas and a lovely New Year!

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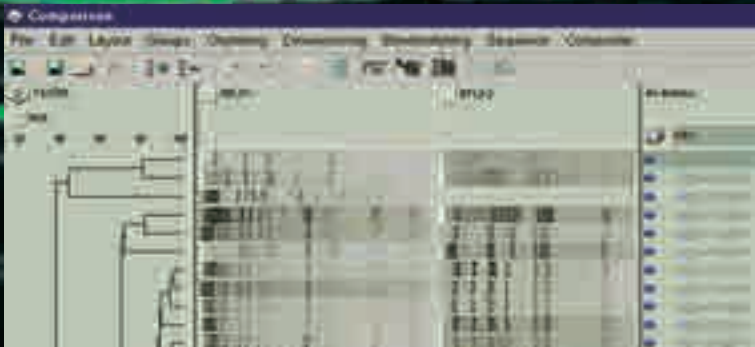
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Congratulations to **Michael Sloggins** who was one of the MANY correct competition entrants. The hidden phrase was: **'MICROBIOLOGISTS ARE ALWAYS CULTURED!'** Something I think we can all agree on! Michael will be the recipient of a £30 book token, as you could be, if you correctly complete the word search below. Please send your entries to the Society office by Friday 7th January 2005 to be in with a chance!

Word Search

- | | |
|---------------|-----------------|
| abscess | diphtheria |
| acetone | dna |
| | ebola |
| acquired | enteric |
| actinomycetes | enterobacter |
| acute | fermentation |
| adsorption | flagellum |
| aerobic | fleming |
| agglutination | folic |
| airborne | fomites |
| algae | foodborne |
| alimentary | fructose |
| amino | genetics |
| ampicillin | germs |
| anaerobic | globulins |
| antifungal | phosphorylation |
| antigen | protease |
| bacteriophage | rna |
| base | subcutaneous |
| benzoyl | taxonomy |
| biogenesis | tetracycline |
| bovine | vibrio |
| brucellosis | virulence |
| byssochlamys | virus |
| carbohydrates | |
| cultures | |
| dengue | |

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A £30 book token is waiting for the person whose entry we receive first! The closing date for entries is **Friday 7th January 2005**. The answers will appear in the March 2005 issue of **Microbiologist**.

Name: _____

Address: _____

Simply photocopy this page and send it to: 'Microbiologist Word Search', Society for Applied Microbiology, The Blore Tower, The Harpur Centre, Bedford MK40 1TQ, UK. **Remember, you could win a £30 Book Token!**

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

Ms M T Liong

Greece

Mr J Maniatis

India

Dr P K Jain

Ireland

Dr M Callanan; Dr A Coffey; Dr E Doyle;
Ms M Murphy; Ms L Scott

Israel

Ms V Kleitman

Malaysia

Mr C M Lew

Spain

Dr R Pagan Tomas

United Kingdom

Miss T Ali; Dr H Alloush; Dr G Couper;
Miss S L Cunningham; Dr P Freestone;
Miss V L Gray; Miss M Lyall;
Mrs R H Murphy; Miss N M Murphy;
Miss H Rajeb; Ms A Rajeb;
Miss M Rodriguez-Giraldez; Dr S A Wilks

USA

Mr A DeStephen; Dr J Mortensen;
Mr R Nuernberg; Dr K-H Seo;
Professor C Woolverton

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Dr Peter Silley looks at the thorny issue of open access publishing and concludes that it's not as simple as it may seem

How do you write a President's Column for December in the middle of October? It is difficult to think of Christmas when the sun is shining and so I will abandon any festive thoughts and focus on the thorny issue of open access publishing.

I wonder how many of us have really thought through this incredibly complex issue? Open access sounds wonderful, free at the point of access, 'haven't we heard that somewhere before?', but as in all things somebody has to pay.

Publishing material costs money, even electronic publishing has a cost despite many believing otherwise! If, as the proponents believe, open access is meant to increase circulation of knowledge, how will that square when you, as authors of a paper, knock on the door of the Head of Department and find there is no money left in the pot?

There is a more insidious issue which is particularly relevant to Learned Societies and is being driven by the National Institute of Health in the U.S. Earlier this year, a radical new policy concerning the publication of research funded by the National Institutes of Health (NIH) was proposed. The policy would require NIH grantees to place accepted, peer reviewed manuscripts of their work in *PubMedCentral*, an open repository, run by NIH. If this were successful it would not have a direct effect on most European Microbiologists but it will affect our US colleagues. Furthermore, if successful, this is likely to be seized on by the European proponents of Open Access as a model that we should follow.

Our journals are published by Blackwell Publishing, who also have the interests of many societies whose journals and books they publish at heart. I would like to quote from a recent open letter sent out by Blackwell Publishing in the US:

"...we are not suggesting a vote against 'Open Access', or a vote against effective dissemination of Government funded research information. We support the

mission of NIH and share their commitment to the scientific and medical community. We share their goal of ensuring the widest possible access to the results of NIH funded authors' research. Individual researchers who do all the work deserve to have the final say, not Government. We do not believe that the proposed NIH plan for all NIH grantees to place accepted, peer reviewed manuscripts of their work in *PubMedCentral* delivers. The fact is that it is an unfenced mandate that will not meet the needs of taxpayers, societies, and authors. The real problem is not one of access. It is one of support for academic societies and the scholarly communities they represent. We are asking you to consider the potential impact of this plan on your ability to represent your members effectively.

What will be the effect of this plan on society revenues?

Who will foot the bill for this plan?

What will be the impact of the plan on US jobs? Many of you employ significant numbers of staff who contribute to the health of the research community you serve.

What about the rights of authors? Surely authors should determine what happens to the results of their hard work. What happens to an author's right to assign copyright?

Will this approach compromise the integrity of the scientific record?"

Open access clearly threatens the future of all learned societies that rely on Journal Income to fund their activities. Clearly, current business models will need to change to meet the changing environment in which we now live and work. We do, however, need to think carefully as we move into a new era as to the wider implications for science and as microbiologists we cannot afford to leave these decisions to the politicians and bureaucrats.

Have a great Christmas!

Peter Silley

2004 sfam AGM

The 73rd annual general meeting of the Society for Applied Microbiology was held on Wednesday 14th July 2004 at 6.00pm in the Baltimore Room, Jury's Hotel, Cork. The Honorary President, Dr Peter Silley was in the chair and 41 members were present.

Present

Martin Adams; Tim Cogan; Karl A Bettelheim; Hilary Dodson; Janet Corry; Geoff Hanlon; Basil Jarvis; Cecil Phillips; Fred Skinner; Bernard Mackey; Sally Cutler; David McCleery; Anthony Hornby; Shona Nelson; Arthur Gilmour; Kunho Seo; Don Whitley; David Post; Guiseppe Spano; Carl Iversen; Valerie Marshall; Julius Mathara; K E Aidoo; Colin Harwood; Jane Sutherland; Alan Godfree; Ian Feavers; Irene Grant; Patrick J Naughton; R Compton; Peter N Green; J Mounil; Hakan Randahl; Rachel Jones; Julie Eastgate; John Rigalsford; Douglas Sheddon; Valerie Edward-Jones; Malcolm Dowson; Anthony C. Hilton; Margaret Patterson

1. Apologies for absence

Apologies for absence were received from Prof David Mossel, Dr Muriel Rhodes-Roberts, Prof Ron Board and Dr S Dinsdale.

2. 72nd Annual Meeting

The minutes of the 72nd AGM held at Surrey University were circulated to those present. The minutes were accepted as a true and accurate record of the meeting, proposed by Andrew Sails and seconded by Jeff Hanlon.

3. Matters arising

There were no matters arising.

4. Report of the Trustees

of the Society for the year 2003. Copies of the report of the trustees were previously distributed to all members attending the meeting.

(i) Report of the Honorary President:

Dr Silley reported on what he described as an eventful year in the life of the Society. Due to the increased workload of the Committee there will now be four Committee Meetings per year. In particular, Dr Silley highlighted the appointment of the Public Affairs Executive, a role shared by Professors Nigel Poole and Geraldine Schofield. He commented on their contribution to raising the profile of the Society and expressed his hope for the appointment to be made permanent. He also reported on the Society's invitation to be partner in the EU Framework Six, **MED-VET-NET**,

Network of Excellence. The Society has the responsibility of heading up one of the integrated work packages, namely, "Spreading Excellence", which aims to spread knowledge to member scientists, the general public, stakeholders and the international scientific community.

Dr Silley went on to express his disappointment in the Website developments and the loss of the Office and Events Manager. In response, the Committee has accepted the proposal to make an appointment of a Chief Executive Officer who will manage Society matters on a day-to-day basis to co-ordinate the voice of applied microbiology. In closing, the President encouraged the Society to rise to the challenge represented in its invitation to **MED-VET-NET**, which places SfAM into the spotlight of European Microbiology.

(ii) Report of the Honorary General Secretary:

Dr Margaret Patterson, Honorary General Secretary, began her report by highlighting Society Office staff changes, namely the loss Lynne Boshier and the resignation of Mavis Knight. She thanked staff for their work during these changes; in particular the valued efforts of Julie Wright, Membership Coordinator, and Michelle Dodd, Temporary Administrator. The Office structure is being reviewed and it was agreed that a full-time CEO is required. In addition, interviews for an Events Coordinator were to be held in the very near future.

Committee Matters – Mrs Margaret Harrison stepped down as Honorary Meetings Secretary and Professor Colin Harwood ended his term as Honorary Editor of Letters in Applied Microbiology. Dr Patterson thanked them for their hard work and support during their appointments. Professor Geraldine Schofield resigned as Honorary Treasurer. The post is now occupied by Dr Valerie Edward-Jones. Professor Geoff Hanlon was co-opted onto the Committee.

Membership – at the end of 2003 the Society had 1,393 members from 73 different countries. It was agreed that

the new CEO would be required to work alongside the Committee to increase membership numbers.

(iii) Report of the Honorary Treasurer:

This would be the final report of Dr Geraldine Schofield before Dr. Val Edward-Jones took over as Treasurer. The report was presented by Dr Peter Silley who referred the members to the published annual report which contained details of the Society's finances. It was noted that our journal income continued to increase and that our portfolio of shares continued to outperform the market average. This put the Society in a healthy position and enabled it to allocate funding to the new initiatives and staff outlined earlier.

(iv) Report of the Honorary Meetings Secretary:

Mrs Margaret Harrison, Honorary Meetings Secretary, commented upon the introduction of Society meetings held in hotels or conference centres rather than university accommodation. She reported that the change represented better value for money for delegates and that the venues were more conveniently situated for transport links. The Winter 2003 meeting saw record attendance, and addressed both current uses and technological developments in several areas of biosensor detection. However, she went on to report how the cancellation of the 2003 Summer Conference, due to poor attendance, had prompted a review of how joint meetings are handled and the scope of the Special Interest Groups. In closing, Mrs Harrison reported that Andy Davies would be stepping down as Convenor of the Food Safety & Technology Group to welcome Jane Sutherland. She went on to say that it would be her final report as Meetings Secretary, and that she wished her successor Professor Martin Adams and all the Interest Groups further success in promoting the activities and interests of the Society.

(v) Report of the Honorary Editors:

It was reported by the Honorary Editors, Mr Alan Godfree and Professor Colin Harwood, **(CONTINUED OVERPAGE)**

2004 sfam AGM continued..

that the introduction of the Electronic Editorial Office (online submission) had both simplified the process of submitting a manuscript and resulted in 29 % and 38 % increase in the number of submission to LAM and JAM respectively. The expansion of the online readership was also highlighted (doubling to one third of a million article downloads), as well as the increase in email alert subscription (15 % increase) and download of Online Early manuscripts (10,000 downloads). In closing, it was reported that Dr Jean-Yves Maillard would be taking over as Editor in Chief for LAM. The Editors also recorded their sincere thanks to editors, reviewers and the staff at Blackwell Publishing for their support in making the journals a success.

(vi) Report of the Honorary Microbiologist Editor:

Dr Anthony Hilton reported on the highlights of 2003, which saw the 'school friendly' September issue distributed to schools and colleges to raise awareness of microbiology and the Society. The issue resulted in several applications for the new Society grade of Associate membership, and its success has prompted the production of a similarly targeted issue to be launched in 2004 for high-school students. The website, although having suffered initial programming bugs, was anticipated to be valuable resource for the Society membership and its review was scheduled for January 2004.

5. Adoption of the Annual Report 2003

Dr Peter Silley asked for these reports to be officially adopted by those present. Professor Basil Jarvis proposed and Dr Karl Bettelheim seconded this proposal.

6. Proposed changes to the Constitution

Members were notified of a Committee proposal to introduce a new class of membership: Associate Membership. This will involve a change in the wording of the constitution. All members will be notified of this change in writing in advance of an Extraordinary General meeting which will be held during the winter meeting on 12th January 2005, where it is hoped the amendments will be adopted.

7. Additional Agenda Item: Custodian Trustees

The Society is required to have not more than four and not less than three Custodian Trustees, each of whom shall be appointed by the Society at an Annual General Meeting after hearing nominations from the Committee. During the year two of the Custodian Trustees (Dr Muriel Rhodes-Roberts and Dr Fred Skinner) resigned, leaving Professor Grahame Gould as the sole Custodian trustee. It is proposed to bring two names for new Custodian Trustees to the extraordinary General Meeting on 12th January 2005.

8. Election of New Officers

- (i) **Election of Vice President**
Dr Margaret Patterson. Proposed by the Committee and seconded Professor Arthur Gilmour.
- (ii) **Election of Honorary General Secretary**
Dr Anthony Hilton. Proposed by the Committee and seconded by Mr Don Whitley.
- (iii) **Election of Honorary Meetings Secretary**
Professor Martin Adams. Proposed by the Committee and seconded by Dr Kosi Aidoo.
- (iv) **Election of Honorary Treasurer**
Dr Valerie Edwards-Jones. Proposed by the Committee and seconded by Professor Basil Jarvis.
- (v) **Election of Honorary Editor (*Letters in Applied Microbiology*)**
Dr Jean-Yves Maillard. Proposed by the Committee and seconded by Mr David Post.
- (vi) **Election of Honorary Microbiologist Editor**
Dr Lucy Harper. Proposed by the Committee and seconded by Dr Fred Skinner.

9. Election of four new Committee members

Dr Margaret Patterson reported that this year there were four committee vacancies as Dr Hilary Dodson, Dr Peter Green and Professor Peter Gilbert were retiring by rotation. The fourth vacancy arose due to Dr Valerie Edwards-Jones becoming Honorary Treasurer. Four nominations were received from Society

members and the membership voted for Prof Geoff Hanlon (proposed by the Committee), Dr Susannah Walsh (nominated by Dr Jean-Yves Maillard), Dr John Coote (nominated by Professor Arthur Gilmour) and Dr Karen Stanley (proposed by Dr Valerie Edwards-Jones).

10. Election of New Members

A list of names of applicants for membership was tabled and all were accepted

11. Deaths and resignations

A list of names of members who had died or resigned was tabled and all were accepted

12. Any other business

There was no other business and the meeting closed at 6.40 pm

13. Date of next meeting

An extraordinary AGM will be held on January 12th at 18:00 in Norwich and the next ordinary AGM on 6th July at 18:00 in the Old Ship Hotel, Brighton.

'LOST' MEMBERS

The following members are noted on our records with 'address unknown' where we have had at least three communications returned to the Society. If you know of the whereabouts of anyone listed here, please contact the Society office:

Dr S M Avery, University of Wales

Professor L F L Clegg, Canada

Mr J C Dakin

Dr N DeLuca, Bioscience IT Services, Harpenden

Dr Z Khodaii, West Yorkshire

Professor J Liston, University of Washington, USA

Mrs K Strid, Sweden

Science Policy Review 2001-2004

SfAM Public Affairs executive, **Nigel Poole** reports on the Government's Science Policy Priorities for 2005 - 2009



In 2001, the Institute of Biology (IOB) and Affiliated Societies identified six priority areas of science policy that they believed the Government should focus on and published them in their 'Science Policy Priorities 2001' report. They were:

- The state and status of the UK research community
- Researchers' career structure and remuneration
- The post-genome challenge
- Public understanding of science
- Science underpinning sustainability
- Education

Before embarking on the next set of priorities, which will be published in 2005, the IOB has reviewed progress three years on. The IOB's report 'Science Policy Review 2001-2004' shows how steps forward have been taken in many areas. Others still require further work. On 28th April 2004, more than 100 participants from the science community came together to hear a line-up of eminent speakers give their views on progress in the IOB's six priority areas and discuss the challenges that will face science policy in 2005 and beyond. The full report can be viewed at the IOB website.

The Science Policy Priorities 2005-2009 will be published in April 2005. As the voice of Applied Microbiology SfAM will naturally be making its views heard in

government and will contribute to the proposals forwarded via the IOB and BSF.

Members are encouraged to contribute to the SfAM position paper. Please let me, (Nigel Poole, Public Affairs at SfAM) have your TOP six science policy priorities, with reasons. Some ideas are listed below, but these are not meant to be exclusive.

- Funding of scientific research
- Science in Government
- Science in society
- Science education and training (school and higher)
- Science and business
- Over arching priorities

Nigel Poole
SfAM Public Affairs Executive
Sekona@btopenworld.com

In Memoriam PROFESSOR DENVER RUSSELL

It with great sadness that we report the death of Professor Denver Russell, part-time professor at the Welsh school of Pharmacy. He passed away on the morning of October 13th after a short illness. Our sincere condolences go to his family and all who were close to him. A full obituary will follow in the March issue of *Microbiologist*.

Sponsor a new Member and win a £50 Book Token!

If you feel you could be our next winner for 2004, 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 Events Organiser appointed

We extend a warm welcome to **Marisa Ramsay**, our new **Events Organiser**,



who introduces herself and explains her role within the Society

As your new Events Organiser I have had a very busy and interesting first month at SfAM. The people have been warm, welcoming and eager to get me involved! I was originally born and raised in the beautiful city of Durban, in South Africa. My travels began in 1997, when I was chosen to represent South Africa as the Youth Leader of the Year and travelled to Denmark to study International Affairs at the International Peoples College. Whilst at the college, I met a young doctor from Kidderminster who told me lots of fascinating tales about England which I just had to see for myself. Now, some eight years later, I am still discovering the beauties that England and its people have to offer.

In my previous role, I worked as an events co-ordinator for the National College for School Leadership, organising and managing up to forty events a year. I am keen to be part of this dynamic Society and hope that my role will be effective and beneficial to you all. I would also like to take this opportunity to welcome any feedback from previous events, and any suggestions or ideas for future events e.g., topics of interest, key speaker suggestions, contacts of people in our field, locations you would like us to hold meetings at, other organisations that we could work with etc. Anything that you think might be useful to help to shape the future of our meetings and events!

I look forward to meeting you all at our January and July conferences in the New Year.

Marisa Ramsay
SfAM Events organiser
marisa@sfam.org.uk

In Memoriam: Ella May Barnes

O.B.E., D.Phil., Dip. Bact., M.A. (Cantab). 5 May 1916 - 23 August 2004

ELLA GREW UP IN LONDON, in a family that included an older sister and a brother. In 1934 she obtained a scholarship to Reading University where she studied until 1937, leaving with a General Honours Degree in Botany, Zoology and Chemistry and a Post-Graduate Diploma in Bacteriology.

In 1938 she became senior chemist at Messrs J.Lyons & Co. Ltd in London and worked for two years in the Analytical Chemistry Section and for a further four years in the Bacteriology Section. In 1944 she left Lyons to set up a new Bacteriology Department at Bengers Ltd, Cheshire, where she was concerned with factory inspections, the control of products, and some research. The products with which she was concerned mainly were bacteriological peptone and the antibiotic nisin, which was developed to the pilot plant stage.

Ella was awarded an MRC Studentship in 1948, which enabled her to work in the Sir William Dunn School of Pathology at the University of Oxford, in Sir Howard (later Lord) Florey's laboratory. At that time, staff in Florey's lab. were testing a range of microorganisms for the production of new antibiotics. Penicillin had been discovered by Fleming in 1928, but it was not until the early 1940s that Ernst Chain and Howard Florey, isolated and purified penicillin and produced sufficient quantities to show that it was clinically useful. Large scale production was then started in America. In 1948 Ella's project was to isolate and purify antibiotics from *Bacillus laterosporus* and *B.brevis*. For this work she obtained her doctorate.

In 1953 Ella joined the Low Temperature Research Station (LTRS), Cambridge, which had been built by the Government in 1922 (on a site made available by the University of Cambridge) in order to study the scientific principles of food preservation. Initially Ella worked with Dr Maurice Ingram and investigated the reason why clostridia in the deep muscle of animals did not multiply until the animal had passed through *rigor mortis*. She showed that the very low numbers of *Clostridium perfringens*



present in muscle tissue were unable to multiply until residual oxygen in the tissue had been used up, and the redox potential had fallen to about -50 mV. She also worked on the origin of faecal streptococci that caused spoilage of pasteurized canned hams, and developed the thallose acetate - tetrazolium - glucose agar to differentiate between *Streptococcus (Enterococcus) faecalis* (present in the human gut) and *Streptococcus (Enterococcus) faecium* (present normally in the pig gut). This was a most effective medium if prepared freshly with careful control of pH. At this stage Ella also became involved in microbiological problems relating to poultry.

In the early 1950's, following the development of the therapeutic use of antibiotics in humans, interest developed in the use of antibiotics as feed additives in the nutrition of young animals, particularly pigs and poultry, and to reduce spoilage of red meat, fish and poultry. In 1955 the USA passed legislation permitting the use of chlortetracycline, 10 ppm, in the ice/water chilling tanks for eviscerated poultry carcasses. Ella was sent to the USA by the LTRS to report on work there on the use of antibiotics in food preservation. It became evident that the development of antibiotic resistance in food poisoning bacteria was a likely

problem. Ella's group showed that feeding of chlortetracycline to poultry resulted in the development of chlortetracycline-resistant streptococci, and she also contributed to work on chlortetracycline-resistant *Salmonella Typhimurium*. Work in Japan and in the Public Health Laboratory Service Enteric Reference Laboratory in London, showed that antibiotic resistance was transferred between bacteria. An expert committee, set up to consider these problems, concluded (the Swann Report, 1969) that the medically important antibiotics should be used only for the treatment of diseases in man and animals, and not for food preservation.

In the 1960s the lease of the LTRS was terminated by the University of Cambridge and two new Food Research Institutes were established, one in Bristol for meat research and one in Norwich for work on fruit, vegetables, eggs and poultry. Both institutes were now under the control of the Agricultural Research Council. Ella moved to Norwich in 1966 and gave practical classes and lectures on food microbiology in the new University of East Anglia in the early years.

In the late 1960s Ella spent a sabbatical period at the Space Sciences Research Centre, Columbia, USA. Here she studied the caecal flora of the thirteen-lined ground squirrel and the effect of hibernation, the caecum being the site of the greatest microbial activity in the gut. Some bacteria in the caecal flora (anaerobic, Gram-positive rods and coccobacilli and aerobic atypical lactobacilli) disappeared or were reduced to very low numbers during hibernation. The organisms that survived after hibernation were unable to grow at 7°C, the hibernating temperature, and had probably been in a resting condition throughout this period. It was notable that the predominant organisms, strictly anaerobic, non-spore-forming anaerobes, survived well during hibernation.

Ella was joined in Norwich by Dr Geoff Mead who collaborated with her in much of the work on poultry. When regulations concerning the chilling of eviscerated poultry carcasses were being drafted in Europe, Ella and Geoff were the UK

members of a Commission Working Party set up to study the hygiene of chilling systems. This work led to tests in processing plants in several European countries and eventually to the introduction of a new chilling system, the counter-flow chiller, and to a change in the EC regulations in 1978-9.

Ella's work on poultry included work on the microbiological spoilage of unviscerated birds (because of a draft EC directive on meat inspection that would affect New York dressed birds) and of game birds. Experiments indicated that the diet of the wild bird is responsible largely for the control of the gut flora, and that pheasants fed on a turkey diet throughout life spoiled when they were shot and hung, whereas wild birds developed an acceptable flavour.

The work on poultry led Ella to develop an interest in the gut flora, particularly that of the caecum. The caeca of birds empty less frequently than the rest of the gut; in it non-spore-forming, strictly anaerobic bacteria gradually form the major population. The isolation of previously uncharacterized bacteria led to work on taxonomy and in 1966 Ella became a member of the IAMS Taxonomic Subcommittee on Gram-negative Anaerobic Rods.

One of the problems addressed by Ella and her group was the carriage of salmonellas in the gut of healthy poultry, with the consequent contamination of poultry carcasses and food poisoning in humans. Ella and her group pioneered use of the principle of "competitive exclusion" to improve the safety of poultry products; this entails using a mixture of bacteria from the caecum of adult poultry and adding this to live, day-old chicks via their drinking water, so that they would develop a gut flora that would inhibit salmonellas.

For her work for the poultry industry Ella was awarded the OBE, and the industry itself awarded her the BOCM-Silcock Trophy.

Ella's group found her fun to work with, they appreciated her high standards and her readiness to acknowledge their contribution. Her colleagues and collaborators enjoyed her enthusiasm and found her considerate and helpful both in the Institute and beyond the Institute. Ella was unfailingly generous in her exchange of information, in discussions about research and in advisory roles. She held strong views and was never afraid to voice them.

In addition to her scientific work, Ella served as Honorary Secretary for the Society of Applied Bacteriology (now the Society for Applied Microbiology) from 1959 to 1964 and as Hon. Meetings Secretary from 1965 to 1968. Between 1977 and 1979 she served as Hon. President of this Society.

Ella was a strong supporter of Chatterbox, the talking newspaper for the blind in Norwich, and on occasion could be seen standing in the centre of Norwich with a collecting box for this cause.

Sadly, after retirement Ella's health deteriorated gradually and her later years were spent in a retirement home. We remember with affection a colleague who made a major contribution to the development and application of food microbiology.

Barbara M Lund, Alan G Kitchell and Clive S Impey



In Memoriam Gwylim Elis Jones

Gwylim Elis Jones worked in agricultural bacteriology for the whole of his career at a time when that discipline was rapidly evolving. After graduating in botany from the University of Wales, Bangor, he joined the Ministry of Agriculture laboratories at Hove, Sussex as an Assistant Advisory Bacteriologist of the National Milk Testing and Advisory Scheme. Whilst there, he was involved in researching the effect of refrigeration on the bacteriology of farm milk supplies, early work which contributed to the eventual adoption of the present-day system of collection from farms. After two years he transferred to the National

Agricultural Advisory Service (NAAS) at Aberystwyth and subsequently with NAAS to Wye College. The period at Wye was followed by a move to the Ministry of Agriculture, Fisheries and Food at Cambridge. Whilst at Cambridge he was greatly involved in the emergency measures to bring an end to the Foot and Mouth Disease outbreak which occurred in the 1960's. He then worked on plant diseases and pests, in particular the outbreak of Fireblight in fruit trees, carrot rot and the problem of carrot fly infestation. Retirement from the Brooklands Avenue site was in 1982.

Elis Jones built up an international reputation during his career, so much so that the Post Office learnt to deliver unerringly much mail from overseas addressed minimally to "Elis Jones, Cambridge".

Elis Jones joined The Society of Agricultural Bacteriologists in 1944, two years before its change of name to The Society for Applied Bacteriology. He was elected to committee in 1951, having been initially co-opted the previous year. In 1958 he became Honorary Treasurer and served in that office for a number of years between 1960 and 1970.

Education was a continuing important interest to Elis Jones, both at school level and higher education. He recognized the importance of scientific content to the education and training of laboratory technicians and in 1950 was sufficiently concerned about the absence of bacteriology from the syllabus of the new City and Guilds Laboratory Technicians Certificate course to bring it to the attention of committee so that influence could be brought to bear. Following retirement he devoted much time and physical work to supporting local schools and their fund-raising efforts.

Other interests included gardening and sport. While working with NAAS at Aberystwyth he had captained the cricket team and later in life he held the post of Secretary and other positions for a local golf club. Most important to him, however, was his family. Until the end of his life he retained an avid interest in science and particularly in his final years, genetics research and computing.

Elis Jones died on 20th April, 2004. He is survived by his wife, three children and four grandchildren.

David Post

In Memoriam: Professor David A A Mossel



ON 30th AUGUST 2004, professor David A A Mossel suddenly died, at the end of a day of fruitful discussions with colleagues and still occupied with many ideas for the coming period. At an advanced age but still energetic, he died on the job. It characterised his way of life and it probably would have been his own choice as well.

Mossel, born in Amsterdam in 1918, studied medicine at Leiden University and was trained in food microbiology by Sir Graham Wilson, Cambridge and Professor R. Buttiaux, Lille. He obtained his PhD degree in 1949 in Utrecht after defending his thesis on water binding and water determination in foods.

As a food microbiologist, his interest was primarily focused on transfer of microorganisms by food. He felt the significance of integrated quality control in the food chain with respect to contamination with and outgrowth of pathogenic microorganisms as the cause of a number of diseases and even death. This concept was developed by Wilson, but it was Mossel's merit to demonstrate its significance everywhere. He initiated many research projects and taught numerous courses all over the world, those at the Institut Pasteur (Paris, Lille) and in South America mentioned by name here. He served as an extraordinary professor at Leuven University (Belgium),

as an honorary professor at the San Marcos University at Lima (Peru), and as a visiting professor at many universities elsewhere. As he spoke five languages fluently, it was never a problem to make himself understandable. His personality was of great importance in fulfilling this task. Mossel was an eminent speaker who knew how to motivate his students.

In 1973, Mossel was appointed as a full professor at Utrecht University. He kept this position until his retirement in 1984. This did not mean that he terminated his activities. It is not possible to sum up all his activities as an emeritus professor. We restrict ourselves to mentioning his role in the *Eijkman Foundation* which aims to create postgraduate training and start research projects in the field of public health microbiology. The crown of this work was the module 'Public Health Microbiology of Food and Drinking Water' in the International Distance Learning, MSc Education for Public Health Science: Food and Drinking Water (University of Hertfordshire, Hatfield, UK). The international recognition for his work was reflected in several *doctorates honoris causa*, and in a number of honorary memberships. His work was strongly supported by the inconspicuous but ever-stimulating help of his wife.

National honours were heaped upon professor Mossel, the greatest of which were: Her Majesty The Queen of The Netherlands elevated professor Mossel to Knight of the Netherlands' Lion and the President of Utrecht University awarded professor Mossel the University's Silver Medal for academic merit.

Mossel propagated his message for more than fifty years. This period has now come to an end. Fortunately, his work will be continued using the opportunities of a new century.

F van Knapen, C Struijk and A Ruiter

DAVID MOSSEL DIED suddenly on 30th August 2004, after a normal busy day participating in the opening ceremonies of the new academic year at the University of Utrecht. David was one of our longest-standing members (latterly an Honorary Member), his application for membership, accompanied by a statement of his

qualifications of PhD and DSc, was approved by the Committee of the Society for Applied Bacteriology on 19th September 1949. The President was then Mr H J Bunker, Secretary Mr D A McKenzie and Treasurer Mr L J Meanwell.

Most people of David's age would have by now retired to a quiet life, tending the garden, but David lived his life and his passion for food safety to the full and right up to the last moment.

David was born of Jewish parents in Amsterdam in 1918. Later he converted to the Catholic Church. He was studying medicine at the University of Leiden when war broke out. He joined the resistance and was imprisoned by the occupying Nazi forces in 1942. Initially his imprisonment was relatively benign, as he was working for the Dutch electrical company, Philips, who managed to protect many detainees by asking for their assistance in their research and development effort. Later he was moved through Europe from one concentration camp to another, (Auschwitz, Mauthausen, Melk and Ebensee) but managed to survive as a result of his medical duties, which exempted him from some of the harshest treatment, enabling him to practice microbiological skills, particularly in the detection of TB among the prisoners, and of VD among the guards — the latter work earning him much-needed extra food. By May 1945 he was working in a former Austrian salt mine, now a construction plant for weapons, and was liberated by the British. He spent a few months working with the American army — no doubt honing up his English — before returning to the Netherlands, and learning that both his parents had died in a concentration camp in 1943. He was repatriated to a recovery centre in Valkenburg, where Princess Juliana visited the repatriates guided by Burgomaster P Hens. Here David met Hens and later Hen's daughter Helen, whom he married in 1947. In 1946 he became assistant in the food microbiology section at Utrecht University. In 1948 he was appointed Head of the Department of Food Microbiology at the Central Institute of Nutrition and Food Research ('TNO') Utrecht/Zeist. He obtained his PhD in 1949 from the University of Utrecht on water binding and water determination in foods.

From then on his career went from strength to strength. Probably as a result of seeing the misery caused by food shortages and unsafe food, and conscious how narrowly he escaped death in the war, he devoted his life to improving the safety and keeping-quality of food worldwide. His exceptional linguistic ability (he spoke English, French, German, Spanish and Italian, as well as his native Dutch), charm and skill as a teacher were huge assets in this task. In addition to his teaching, he published over 300 research papers, and about ten food microbiology textbooks. His overall theme can be summarised as '*prevention of foodborne microbial diseases and development of methods for validation and verification of the efficacy of intervention technologies.*'

Probably the two publications by which he would most like to be remembered are the review he wrote with Maurice Ingram (The physiology of the microbial spoilage of foods. *J. appl. Bact.*, **18**, 232-268, 1955), and his book, published by John Wiley in 1995 (*Essentials of the Microbiology of Foods, a textbook for advanced studies*). This book was the culmination of about 20 years' work, and summarises David's philosophy for achieving safe and wholesome food. Wiley never succeeded in persuading him to reduce the huge numbers of citations, so 202 of the 649 pages are references. The book listed many of his own papers, and during the nine years since the book was published he has written about 60 more.

Microbiologists whose views and work influenced David most include Sir Graham Wilson, author of *Topley and Wilson* and late director of the Central Public Health Laboratory, London, and Professor Maurice Ingram (whom he visited first in Cambridge soon after he started work at TNO), as well as Professors Buttiaux and Beerens (Institute Pasteur, Lille) and Professor Jacques Monod (Paris). His latest published paper (May 2004 *Int. J. Food Microbiol.*) is entitled '*Assessment of the microbial integrity, sensu G.S. Wilson, of piped and bottled drinking water in the condition as ingested.*'

He taught at the University of Louvain (as a full professor from 1968), and the University of Utrecht (full professor from 1973-1984, then Professor Emeritus). He was a founder member, in 1953, of the ICFMH (International Committee for Food Microbiology and Hygiene), whose journal, the International Journal of Food Microbiology, begun in 1984, is highly

successful. This organisation now holds international food microbiology symposia biennially — the latest, *Food Micro 2004*, was held in Slovenia, and David was due to attend. David was honorary president of the ICFMH for the last ten years until his death.

He was also involved in the founding of the International Commission for Microbiological Specifications for Foods (ICMSF) in 1962 and a member until he resigned in 1975. Hence he was involved in the first two of their seminal *Microorganisms in Foods* series: 1. Their Significance and Methods of Enumeration (1968) and 2. Sampling for Microbiological Analysis: Principles and Specific Applications (1974).



David Mossel lecturing at the ICFMH Workshop at the University of Stellenbosch, South Africa, 8 - 13th December 2003

He held visiting professorships in many countries including Peru, Spain, US, Philippines, Thailand and Australia, as well as being awarded honorary doctorates, and memberships of numerous national societies. Besides numerous food microbiology courses in Latin America (for which he had a special affection) Spain and other countries, he taught at the annual WHO Postgraduate Course in Food Microbiology in Lille, France, from 1957, and at the biennial WHO Advanced Food Microbiology course at Guildford, UK. His linguistic feats at these courses were legendary.

James Barnett lectured in English on yeasts at the Lille course one year, with David giving a simultaneous translation into French — except, according to James, it was not a mere translation, useful additions were made (and appreciated by James, who knew more French than he appeared to). My own experience after making a huge effort to give a ten minute paper in French at a meeting in Lille was less flattering — David, as chairman, complimented my French by comparing it with Winston Churchill's...at least he did not use Edward Heath as a comparison!

David was, of course, an intrepid traveller, and had many exciting tales to tell. In September 1970 he was flying to New York by Pan-Am when his jumbo-jet, and several other planes, were hijacked by the PFLP to the Middle East. He was chosen as spokesman and successfully persuaded the female guerrilla to allow the passengers off before blowing up the plane at Cairo. From then on, he avoided Pan-Am. Since his 'retirement', David has been concentrating on teaching his main message — integrated food quality control from conception to consumption. This has been via the *Eijkman Foundation* in collaboration with Corry Struijk, training postgraduate students and initiating research projects in public health food microbiology. In collaboration with the University of Hertfordshire, the *Eijkman Foundation* has recently set up a module on Public Health Microbiology of Food and Drinking Water, which is part of a distance learning MSc in Education for Public Health Science.

David's imposing presence (he was well over six feet tall) will be much missed, not only by his wife, Helen, his five children and eight grandchildren, but also by the whole international food microbiology community. There is no doubt that he will long be remembered with affection, and that his message and work will live on after him.

I am grateful for help in writing this from various people, including James Barnett, Wilhelm Holzapfel, Alan Kitchell, David Post, Terry Roberts, Niels Skovgaard, Corry Struijk, Mike Van Schothorst and especially Mrs Helen Mossel and her daughter, Margreet. Please visit <http://www.dpdanthony.name/mossel> if you would like to convey your condolences.

Janet Corry



MED • VET • NET

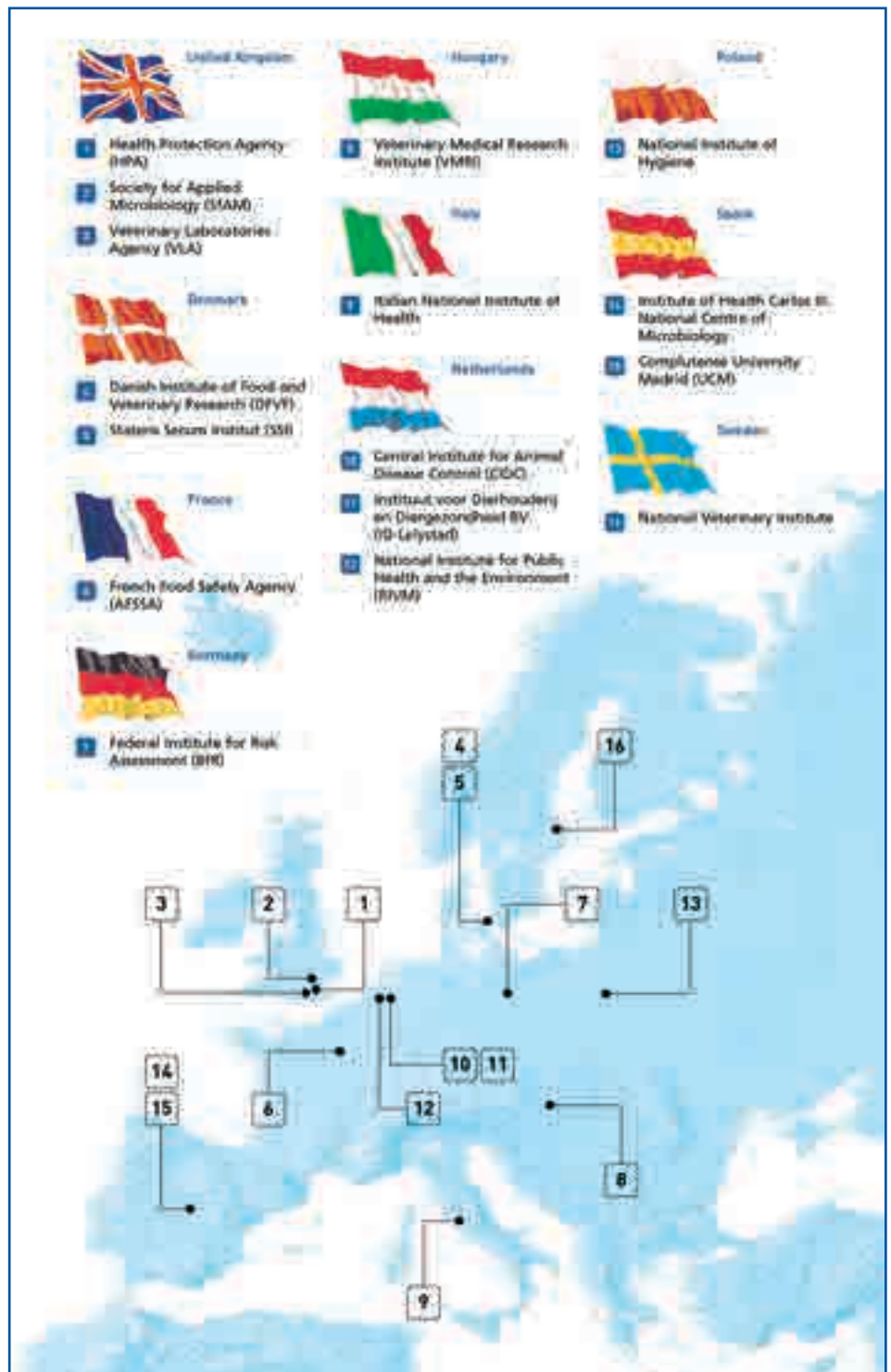
Teresa Belcher reports on this exciting new EU initiative

IF YOU CAST YOUR MIND back to the June 2004 issue of *Microbiologist*, Hon President, Peter Silley, outlined an exciting new initiative of the European Union — **MED-VET-NET**. This initiative was officially launched on 1 September 2004, and I am delighted to report that I have taken up the position of Communications Director for the network.

First, let me provide a brief overview of the network and zoonoses. The EU recently identified 23 zoonotic agents which must be monitored by member states in both human and animal populations. Traditionally, research on zoonotic agents in Europe has been highly fragmented, and so, in order to address this, the EU 6th Framework Program has provided financial support for five years to develop a Network of Excellence for the Integrated Research on the Prevention and Control of Zoonoses. Comprising of 16 European partners and over 300 scientists from multiple disciplines, **MED-VET-NET** will provide the appropriate environment for these scientists to share and enhance their knowledge and skills, develop collaborative projects and present joint research within and outside the network.

Why zoonoses research?

Diseases transmissible from animals to humans (zoonoses) are the cause of many of our serious public health problems. In fact, nearly two thirds of all known human pathogens are zoonotic. Zoonoses have always represented a risk to humans, but recent events such as Severe Acute Respiratory Syndrome (SARS), have demonstrated that these risks are increasing and have the potential for serious global consequences. These changing risks are due to increasing global travel, changes in livestock production and trade, growing contact between man and exotic animals, as well as shifts in human eating and food preparation habits. At the same time,



human populations are becoming more susceptible while the organisms themselves are mutating and becoming more virulent, and developing new transmission routes.

Food supply is an increasingly global industry and because many zoonotic agents are food-borne or have reservoirs in livestock, the microbiological safety of food is now recognised as a major public health concern for the European Union (EU). Integration activities and SfAM's involvement

MED-VET-NET is structured into three integration activities and four joint scientific activities (thematic areas).

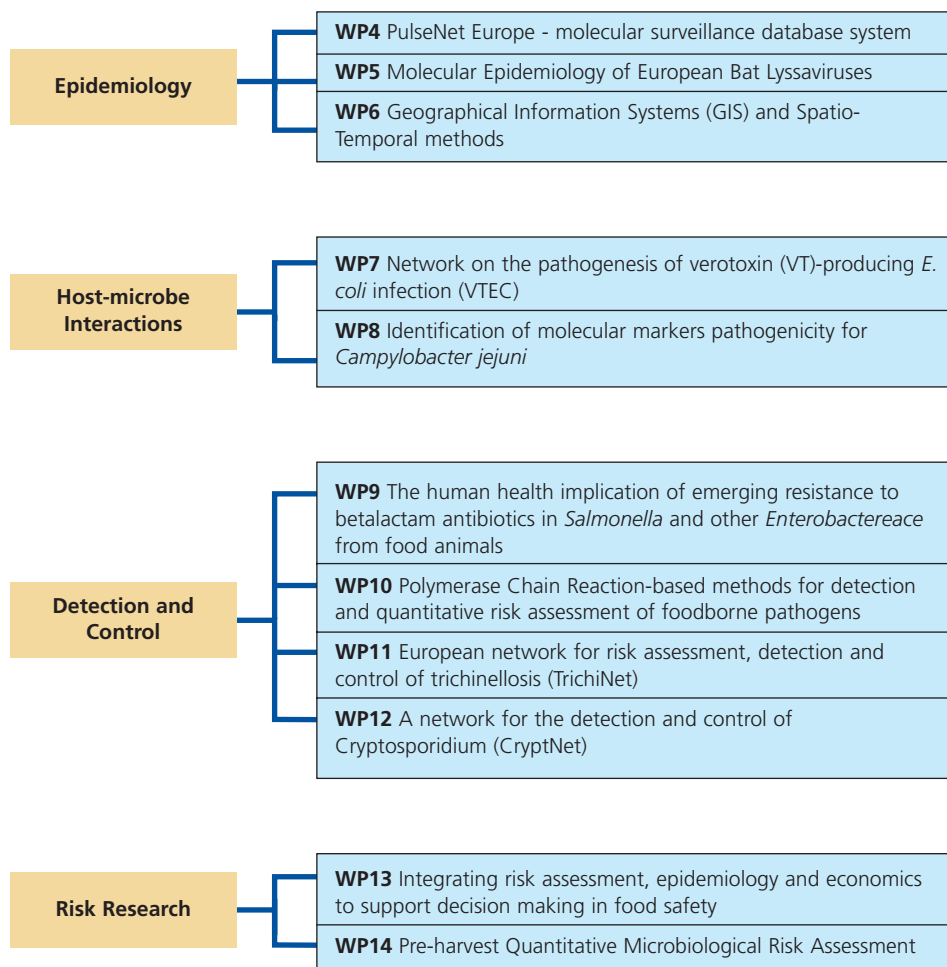
The Integration activities comprise of:

- **'Virtual Institute'** (Workpackage 1) – coordinated by the French Food Safety Agency (AFSSA), and is headed by Dr Andre Jestin. This workpackage aims to develop an effective management structure with administrative procedures, processes, reporting and review to enable the smooth running of the Network.

- **'Strategic Scientific Integration'** (Workpackage 2) – lead by Professor Diane Newell of the Veterinary Laboratory Agency (VLA). This area will provide the platform for the coordination and integration of scientific skills and expertise throughout the Network.

- **'Spreading Excellence'** (Workpackage 3) – is the responsibility of SfAM, under the guidance of Dr Peter Silley. This involves 'spreading the excellence of knowledge' both within the Network as well as externally. This includes the formation of a Communication Unit who will work on an internal website for scientists to share news, achievements, and reports; an external website with information for the public and other stakeholders; a monthly newsletter; fact sheets on zoonoses; and stories on the network's research. strategies for advising our various stakeholders (politicians, media journalists, consumers, general public) about **MED-VET-NET** will also be developed, and a training and development program for 'Scientific Communicators' within each Institute will be established.

Chart showing the Workpackages in each thematic area



Scientific Activities

The Scientific Activities comprise the experts appointed by each Partner institute. There are four thematic areas which will be active throughout the life of the project and comprise research topics which are timely, developing and contribute to the overall research capacity of the network. Workpackages are formed within these thematic areas and will run initially for 18 months (see above chart).

First meetings already underway

Following the launch of the network, a number of meetings have already been held. The first was the MVN First Thematic Meeting held from 8-10 September at the Royal Holloway College in Surrey, UK. Each institute sent four representatives to first provide an overview of the themes and the scientific work packages. Current work and strategic plans for each thematic area were discussed in the break-out sessions.

From 21-22 September, 2004, a "Kick-

off" Management Meeting took place in Utrecht, The Netherlands. This involved meetings of the Governing Board, the Advisory Panel and the Coordinating Forum of the Network. Professor Joost Ruitenbergh from the National Institute for Public Health and the Environment (RIVM) in The Netherlands, gave a Keynote Lecture on "Public health risks from emerging zoonotic diseases".

In future **MED-VET-NET** columns in *Microbiologist*, I hope to outline the scientific progress in each of the Workpackages, and also progress we make in communicating the achievements of the network.

Further Information

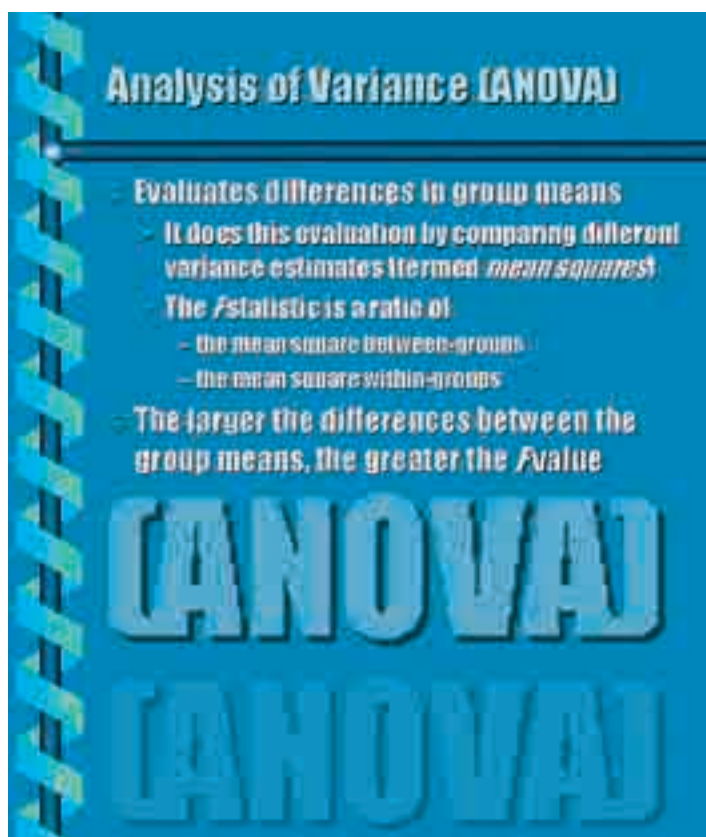
- For more information, visit our website at <http://www.medvetnet.org/> or contact me at the SfAM offices in Bedford on: **+44 (0)1234 271020**.

Teresa Belcher

Med-Vet-Net Communications Director

The use of Analysis of Variance (ANOVA) in applied microbiology

Richard Armstrong and Anthony Hilton discuss the uses of this important tool



STUDIES IN applied microbiology often involve comparing either several different treatments or the influence of two or more factors at the same time.

For example, an investigator may be interested in the degree of microbial contamination on coins collected from three different premises and the data analysis might involve a comparison of microbial numbers from the three different locations. In another example, one may wish to compare the pattern of transfer of bacteria from different dishcloths, either rinsed or not, onto a food preparation surface such as a cutting board (Hilton & Austin 2000). In this case, two factors may influence

microbial numbers, *viz.*, the type of dishcloth and rinsing treatment. An investigator may wish to establish whether both factors influenced microbial numbers independently or whether there was an interaction between them, *i.e.*, does rinsing have the same effect on the numbers of bacteria transferred when different dishcloths are used? The most appropriate method of statistical analysis of such experiments is analysis of variance (ANOVA) (Snedecor & Cochran 1980, Armstrong *et al.*, 2000, 2002).

Analysis of variance is the most effective method of analysing more complex data sets. It is, however, a method comprising many different variations, each of which apply in a particular

experimental context. Hence, it is possible to apply the wrong type of ANOVA and to draw erroneous conclusions from the results. This article is an introduction to ANOVA and describes first, how ANOVA came to be invented, the logic on which it is based, and the basic assumptions necessary for its correct use. Second, the application of the method to the analysis of three data sets drawn from experiments in applied microbiology is described.

The origin of ANOVA

An experiment was set up to measure the degree of bacterial contamination on 2p coins collected from three different premises, *viz.*, a butcher's shop, a sandwich shop, and a newsagent. A sample of four coins was collected at random from each premises. The number of

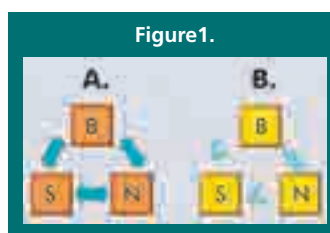
(Armstrong & Eperjesi, 2001). A significant value of Student's 't' indicates that the null hypothesis should be rejected and that there is a real difference in bacterial numbers between the two premises.

This method of analysis could be extended to three or more different premises. To compare all pairs of premises, three t-tests would be necessary (Fig 1). There is a problem, however, in making these tests because not all of the comparisons are independent. For example, if bacterial numbers from the butcher's shop were significantly greater than those from the sandwich shop but similar to those from the newsagent, it follows that numbers in the newsagent should be greater than those in the sandwich shop (Fig 1).

However, the latter comparison would not have been tested independently but follows inevitably from the first two comparisons. To overcome this problem, ANOVA was developed by Sir Ronald Fisher in the 1920s and provides a single statistical test of the null hypothesis that the means of the bacterial numbers on coins from the three premises are identical.

The logic of ANOVA

The data from the experiment described above are shown in Table 1. Each of the three premises is represented by measurements of bacterial contamination on four coins and therefore, the experiment is described as a one-way ANOVA with four replications in a randomized design. In an ANOVA, the total



bacterial colonies present on each coin was estimated by dilution plating. If only two types of premises were involved then the null hypothesis that there is no significant mean difference in the numbers of bacteria at the two locations could be tested using Student's 't' test (Snedecor & Cochran, 1980). The statistic 't' is the ratio of the difference between the means and a measurement of the variation between the counts on the individual coins pooled from both groups

variation between the observations (x_{ij}) is calculated and then partitioned into portions associated with differences between the three premises and the variation between the replicate coins within premises. The calculations involved are shown in Table 1. The sum of squares of the deviations of the x_{ij} from their mean (X^*) is used as a measure of total variation while the sum of squares of the three premises means from their overall mean is a measure of the treatment effect and is calculated from the column totals (T_i). Variation between the coins within each location (residual or error variation) is calculated as the sum of squares of the x_{ij} in each column from their column mean and then added together to give the error sum of squares or can be obtained by subtraction.

If there are no significant differences between the means of the three premises, the twelve observations are distributed about a common population mean ' μ '. If this is the case, then the variance (also called the mean square) calculated from the between premises sum of squares and the error sum of squares should be estimates of the same quantity. Testing the difference between these two mean squares is the basis of an ANOVA and the statistics are set out in an ANOVA table (Table 1). To compare the between premises and error mean squares, the sums of squares are divided by their appropriate degrees of freedom (DF). The DF of a quantity is the number of observations minus the number of parameters estimated from the data required to calculate that quantity. Hence, the total and between premises sum of squares each have eleven and two DF respectively, one less than the number of

observations or groups. This is because the mean of the ' x_{ij} ' values and the mean of the three group totals were calculated from the data to obtain the sums of squares. The error sum of squares has 9 DF because the column means are used in the calculation, i.e., there are three DF in each of the three columns making nine in total.

The between premises mean square is then divided by the error mean square to obtain the variance ratio. This statistic was named 'F' (hence, 'F-test') in honour of Fisher by G.W. Snedecor (Snedecor & Cochran, 1980). The value of 'F' indicates the number of times the between premises mean square exceeds that of the error mean square. The probability of obtaining a statistic of this magnitude by chance, i.e., from data with no significant differences between the group means, is obtained from the F distribution. If the value of 'F' is equal to or greater than the value at the 5% level of

probability, then the null hypothesis that the three premises means are identical is rejected. In this case, a value of $F = 4.89$ was obtained which has a P value of 0.037, i.e., there is less than a 5% of chance of obtaining an F ratio of this magnitude by chance. This result indicates a real difference between the bacterial counts from the three premises. Note that this analysis relates only to the three premises studied. It would not be possible to make a general statement about all premises of this type from these data. This would require a random sample of each premises to be obtained so that an estimate could be made of the variation in bacterial counts between similar premises.

Comparison of group means

The F-test of the group means is only the first stage of the data analysis. The next step involves a more detailed


examination of the differences between the means. In many circumstances, this involves making *post-hoc* tests between the group means. The subject of *post-hoc* tests a complex topic and a variety of methods are usually available for making such tests in statistical packages. The most commonly used tests and the assumptions necessary for their correct application have been discussed in detail previously (Armstrong *et al.*, 2000) and only two examples, *viz.*, Fisher's protected least significant difference (PLSD) and Scheffe's test, will be considered in this article. The results of the *post-hoc* tests applied to the present data are shown in Table 1. These tests vary in the degree of error control they provide and in particular their sensitivity to making a 'type one' error, i.e., rejecting a null hypothesis when it is true. By contrast, a type 2 error is accepting the null hypothesis when a real difference is present. Fisher's PLSD is the most 'liberal' of the methods available and the most likely to result in a Type 1 error. All possible comparisons of the group means are tested and the method uses the t-distribution to determine the critical value to be exceeded for any pair of means. This test indicates that bacterial counts were significantly higher on 2p coins from the butcher's compared with the sandwich shop and newsagents but that counts from the later two premises were similar. By contrast, Scheffe's test is one of the most conservative of the *post-hoc* tests giving maximum protection against making a type 1 error but increasing the probability of making a type 2 error. In this case, the test does not indicate any significant differences between the group means. Which test should be used in each circumstance depends on the objectives of the 

TABLE 1. The number of bacteria isolated from 2p coins collected from three types of premises

Replicates	Butcher's	Sandwich Shop	Newsagent
1	140	2	40
2	108	21	5
3	76	0	5
4	400	42	0
Mean	181	16	13
SE	74.16	9.80	9.24

Total sum of squares (SS) = $\sum(x_{ij} - X^*)^2 = 142238.917$, Between groups (premises) SS = $\sum(\sum T_i - H^*)^2 = 74065.167$, Error SS = Total SS - Between groups SS = 68173.75

ANOVA table

Source	DF	SS	MS	F	P
Between Premises	2	74065.167	37032.583	4.89	0.037
Error	9	68173.75	7574.861		

Post-hoc tests

Comparison	Mean diff.	Fishers PLSD	Scheffe's Test
Butcher's/Sandwich	164.75	P < 0.05	NS
Butcher's/Newsagent	168.5	P < 0.05	NS
Sandwich/Newsagent	3.75	NS	NS

Abbreviations: SE = Standard error of the mean, x_{ij} = Individual counts, X^* = overall mean of all 12 bacterial counts, T_i = Column total, H^* = mean of the three column totals, DF = degrees of freedom, MS = Mean square, F = variance ratio, P = probability, NS = not significant

experiment and on the relative consequences or costs of making a Type 1 or Type 2 error.

Assumptions of ANOVA

ANOVA makes certain assumptions about the nature of the experimental data that have to be at least approximately true before the method can be validly applied. An observed value x_{ij} can be considered to be the sum of three parts: 1) the overall mean of the observations (μ), 2) a treatment or class deviation, and 3) a random element drawn from a normally distributed population. The random element reflects the combined effects of natural variation between subjects and errors of measurement. ANOVA assumes first, that these errors are normally distributed with a zero mean and standard deviation 's', second, that although the means may vary from group to group, the variance is constant in all groups, and that effects of individual treatments are additive and not multiplicative. Failure of an assumption affects both the significance levels and the sensitivity of the F-tests. Experiments are usually too small to test whether these assumptions are likely to be true. In many biological and medical applications, in which a quantity is being measured, however, the assumptions hold well (Cochran & Cox, 1957; Ridgeman, 1975). In many applications in applied microbiology in which bacterial numbers are being estimated, the assumptions may not hold. There are two common problems when the data comprise numbers of microbes. First, small whole numbers with many zeros are unlikely to be normally distributed and second, a wide range of bacterial numbers may be present leading to heterogeneous variances. The

latter problem is evident in the example analysed in Table 1 where the standard errors for the three premises vary markedly. If there is doubt about the validity of the assumptions, significance levels and confidence limits must be considered to be approximate rather than exact. If there is only a single significant figure, the assumptions are more doubtful and if the data are small whole numbers, then the assumptions are unlikely to hold. If the assumptions do not hold, then a transformation of the x_{ij} into

effects of a number of different factors can be studied at the same time. Combining factors usually requires fewer replications than studying each factor individually in a separate experiment. In addition, the analysis reveals the possible synergistic or interactive effects between the factors and is often the most interesting information from a factorial experiment.

To illustrate the analysis, an investigator wished to study the influence of type of dishcloth (cloth or sponge) and prior rinsing of the

varied both with dishcloth and rinsing and whether the two factors had an independent influence on numbers of bacteria.

The resulting ANOVA (Table 2) is more complex than that of a one-way ANOVA because the between groups or treatments sums of squares is partitioned into three factorial effects, *viz.*, the main effects of dishcloth type and rinsing and the interaction between the two factors. In the present example, there is a main effect of dishcloth type ($F = 20.99$, $P < 0.01$) and of rinsing ($F = 28.92$, $P < 0.001$) suggesting significantly more bacteria were transferred from the cloth than the sponge and significantly fewer after rinsing both materials. In addition, there is significant interaction between the factors ($F = 20.97$, $P < 0.01$) suggesting that the effect of rinsing is not the same for the two types of dishcloth.

Examination of the treatment means suggests that the sponge transfers a smaller proportion of its bacterial load to the food preparation surface compared with the cloth. This effect is probably attributable to organisms being more exposed on the surface of the cloth and therefore more liable to be transferred compared with the more cavernous sponge (Hilton & Austin, 2000).

A more complex factorial experiment

An investigator wished to examine the pattern of survival of bacteria on £5 notes. The data comprise numbers of bacteria of two species, *viz.*, *E. coli* and *S. epidermidis*, inoculated on to the surface of £5 notes and subsequently measured at ten time intervals (Table 3). Survival of the two bacteria on cover-slips was examined as a control. The objectives of the experiment were first, to determine whether there was a

Table 2. Influence of type of dishcloth and rinsing treatment on the number of bacteria transferred to a food preparation surface

Replicates	Cloth		Sponge	
	Rinsed	Not rinsed	Rinsed	Not rinsed
1	1.0×10^5	7.8×10^7	3.9×10^5	8.0×10^5
2	2.3×10^4	5.0×10^7	9.0×10^3	4.0×10^5
3	3.9×10^5	4.1×10^7	8.5×10^4	2.0×10^5
Mean	1.7×10^5	5.6×10^7	1.6×10^5	4.7×10^5

ANOVA table

Source	DF	SS	MS	F	P
Material	1	2002.83	2002.83	20.99	$P < 0.01$
Rinsing	1	2760.42	2760.42	28.92	$P < 0.001$
Interaction	1	2001.33	2001.33	20.97	$P < 0.01$
Error	8	763.49	95.44		

Abbreviations: SE = Standard error of the mean, x_{ij} = Individual counts, X^* = overall mean of all 12 bacterial counts, T_i = Column total, H^* = mean of the three column totals, DF = degrees of freedom, MS = Mean square, F = variance ratio, P = probability, NS = not significant

another scale will often allow an ANOVA to be carried out. For example, in Table 1, a transformation of the data to a logarithmic scale is likely to equalize the variances for the three premises. For a more detailed discussion of the use of transformations see Snedecor & Cochran (1980) and Armstrong & Eperjesi (2001).

Factorial experiments

The ANOVA described above is an example of a single factor experiment, the variable involved being type of premises. In a factorial experiment, however, the

material on the number of bacteria transferred to a food preparation surface (Hilton & Austin, 2000). Each dishcloth was inoculated with 1 ml of a 10^8 cfu ml⁻¹ *E. coli* culture and after 10 min, the cloth was wiped over an appropriate area of cutting board. Additional pieces of cloth were inoculated but rinsed in sterile running water before wiping. The data comprise the number of bacterial colonies obtained on nutrient agar from two types of dishcloth, rinsed and unrinsed, and are shown in Table 2. The objectives of the experiment were to determine whether bacterial loading

difference in survival of bacteria on control surfaces as against £5 notes and second, to determine whether the two species exhibit different survivorship curves on the various surfaces.

This experiment is considerably more complex than the previous examples. First, three factors are involved, *viz.*, bacterial strain, type of surface, and time interval and this results in a 'three-factor' factorial. Second, the fact that repeated measurements of bacterial numbers are made on each surface leads to a 'repeated measures' design (Armstrong *et al.*, 2002). In the ANOVA, there are two major factors (bacteria and type of surface) with time constituting the repeated measures factor. A common mistake made by investigators is to analyse such an experiment as if it comprises three completely independent factors.

The ANOVA appropriate to these data is shown in Table 3. There are significant main effects of bacterial strain ($F = 140.81$, $P < 0.001$) and type of surface ($F = 111.48$, $P < 0.001$) which suggests first, greater numbers of *S. epidermidis* at the beginning of the experiment and second, greater numbers of bacteria on the £5 notes compared with cover-slips. There is an interaction, which is only just significant, between type of surface and bacteria ($F = 8.13$, $P < 0.05$) indicating that differences between surfaces varied with species. The main effect of time ($F = 5707.7$, $P < 0.001$) reflects the general decline in numbers over the period of the experiment. This decline, however, varies with type of surface ($F = 47.33$, $P < 0.001$), a more rapid and pronounced decline in numbers being observed on cover-slips compared with £5 notes and with species ($F = 168.7$, $P < 0.001$); a much

TABLE 3. The survival of two strains of bacteria on two different surfaces (EC = *Escherichia coli*; SE = *Staphylococcus epidermidis*). Each figure is the mean of two replicates

Time (hrs)	Notes		Control		Notes		Control	
	EC	EC	EC	EC	SE	SE	SE	SE
0	3800	3800	3800	3800	5400	5400	5400	5400
1	800	430	0	0	600	800	7	22
3	500	351	6	0	560	560	6	10
5.5	446	249	1	0	700	764	0	2
24	0	1	0	0	272	171	0	0
27	0	0	0	0	54	2	0	0
30	0	0	0	0	79	42	0	0
48	0	0	0	0	124	105	0	0
51	0	0	0	0	7	14	0	0
54.5	0	0	0	0	2	10	0	0

Analysis of variance					
Source	DF	SS	MS	F	P
Bacteria	1	909298	909298	140.81	P <0.001
Control/notes	1	719911	719911	111.48	P <0.001
Bacteria x control/notes	1	52480	52480	8.127	P <0.05
Main-plot error	4	25480	6457.6		
Time	9	146528276	16280919	5707.7	P <0.001
Time x	9	4332765	481418	168.7	P <0.001
Bacteria Time x	9	1215098	135011	47.33	P <0.001
Control/notes					
3-factor interaction	9	65389	7265	2.54	P <0.5
Sub-plot error	36	102687	2852		

Abbreviations: SE = Standard error of the mean, x_{ij} = Individual counts, X^* = overall mean of all 12 bacterial counts, T_i = Column total, H^* = mean of the three column totals, DF = degrees of freedom, MS = Mean square, F = variance ratio, P = probability, NS = not significant

more marked decline being observed in *E.coli* compared with *S. epidermidis*. There is also a significant three-factor interaction, but higher order interactions are usually too complex to interpret easily.

Conclusion

Experiments combining different groups or factors and which use ANOVA are a powerful method of investigation in applied microbiology. ANOVA enables not only the effect of individual factors to be estimated but also their interactions; information which cannot be obtained readily when factors are investigated separately. In addition, combining different

treatments or factors in a single experiment is more efficient and often reduces the number of replications required to estimate treatment effects accurately. Because of the treatment combinations used in a factorial experiment, the DF of the error term in the ANOVA is a more important indicator of the 'power' of the experiment than the number of replicates (Ridgman, 1975; Armstrong *et al.*, 2002). Finally, it is important to consider the design of the experiment because this determines the appropriate ANOVA. Some of the most common experimental designs used in the biosciences and their relevant ANOVAs are discussed in detail by Armstrong *et al.*, (2002). If there is doubt about the design or which ANOVA to use, the researcher should seek advice from a statistician with experience of research in applied microbiology. Once committed to an inappropriate experimental design there may be little that a statistician can do to help.

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Guessing the Future: a thing of the past? Predictive Food Microbiology and Risk Assessment

SfAM JANUARY MEETING ● NORWICH, UK 12-13 January 2005

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Overview

During the last few years there have been substantial advances in predictive microbiology and risk assessment. Use of predictive models facilitates the quantification of the growth of microorganisms in foods, and can be underpin risk assessments for the growth of pathogens in foods. Speakers at this challenging meeting will address important topics such as:

- Recent progress in predictive food microbiology and the ComBase Initiative
- Development of food spoilage models for use by industry
- Use of expert systems
- Risk perception and communication
- Risk characterisation and exposure assessment
- Practical approaches to risk assessment in the food and water industries

January Meeting 2005

Programme

Wednesday 12 January 2005

- 12.00–12.40 Overview: history, background and development of PM and RA (Liase with MZ)**
Leon Gorris, University of Wageningen, The Netherlands
- 12.40–14.00 Lunch and poster session**
- 14.00–14.40 Predictions of bacterial kinetics under dynamic conditions using the Combase database**
Dr J Baranyi and Dr Y Le Marc, Institute of Food Research
- 14.40–15.20 Expert Systems in Food Safety**
Carol Adair, Unilever Research
- 15.20–15.50 Tea**
- 15.50–16.30 Development of spoilage models for use by the food industry**
G Betts, Camden and Chorleywood Food Research Association, UK
- 16.30–17.10 Quantification of hurdles: predicting the combination of effects – is it possible?**
R Lambert, Nestle , UK
- 17.10–17.50 The link between Quantitative Microbiology and Quantitative Risk Assessment**
Marcel Zwietering, University of Wageningen, The Netherlands
- 18.00 Ordinary General Meeting**

Thursday 13 January 2005

- 09.00–09.40 Application of risk assessment in management of safety of retail foods**
A Kyriakides, J. Sainsbury plc, UK

- 09.40–10.20 Risk characterisation and exposure assessment**
Serve Notemans, TNO, Zeist, The Netherlands
- 10.20–10.50 Coffee**
- 10.50–11.30 Practical use of microbiological risk assessment by food companies**
P Voysey, Campden and Chorleywood Food Research Association, UK
- 11.30–12.10 Practical risk assessment: role of eggs in the transfer of *salmonella***
A Varnam and P Botey-Salo, London Metropolitan University, London, UK
- 12.10–12.50 Practical risk assessment: applications in the water industry**
Alan Godfree, United Waters plc, UK
- 12.10–14.00 Lunch**
- 14.00–14.40 Microbiological risk assessment and risk management: A Food Standards Agency Perspective**
P Cooke, Food Standards Agency , UK
- 14.40–15.20 Where next with Risk Assessment?**
P McClure and J Bassett, Unilever Research, UK
- 15.20 Close - please join us for tea**

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

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Spore forming bacteria — emerging and re-emerging issues

Old Ship Hotel, Brighton, UK ● 4th - 7th July 2005

The conference will consider recent advances in understanding in health, industrial and environmental issues associated with spore formers (following on from the 1994 meeting). It will review understanding of the taxonomy of spore formers and consider the physiological aspects, particularly those associated with spore structure and resistance. The health implications will be considered with respect to common infections caused by spore formers in both animals and man, the persistence of spores in food products, and also recent developments facilitating the use of spores as vaccine vehicles, probiotics and tumour targeting vectors. The environmental applications of spores will also be reviewed.

There will be sessions on:

- Spore formers the great survivors — a taxonomy and physiology update
- Spore formers in food microbiology
- Spore formers: human health issues
- Environmental applications of spore formers

There will also be an opportunity for oral and poster presentations. Further information will be published on the Society website and in the December issue of *Microbiologist*.



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Poems, Paintings and Penile Chancres

Tony Worthington talks about Syphilis:
a maddening disease with history

A Night in e

SYPHILIS, a potentially fatal sexually transmitted disease (STD) caused by the spirochaete *Treponema pallidum* (fig.1), once caused widespread epidemics and continues to remain a major cause of morbidity and mortality worldwide. Once called 'the great imitator' as the signs and symptoms of infection are indistinguishable from many other diseases, syphilis has been with mankind for hundreds, possibly thousands, of years and its origins remain an issue of some debate.

Throughout history, syphilis has been famously portrayed in art and literature and indeed, many renowned names have fallen victim to 'the pox'. Following the sharp decline in the number of cases of syphilis in 1958 after the widespread use of penicillin, levels have fluctuated periodically and have usually been correlated with behavioural changes and

educational campaigns. In 1998, advances in molecular research allowed for complete sequencing of the genome of *T. pallidum*, which will allow for a better understanding of the microorganism, potentially leading to improved diagnosis and treatment of infection. However, in contrast to these recent scientific advances, resurgence in unsafe sexual practices, has led to a recent increase in the number of new cases of syphilis and other STDs. And so the story continues...

The Origins of Syphilis

"A mysterious epidemic, hitherto unknown, which struck terror into all hearts by the rapidity of its spread, the ravages it made, and the apparent helplessness of the physicians to cure it." These were the words used by Sir William Osler (1849-1919), 'the father of modern medicine' and distinguished physician who ended his career as the Regius Chair in

Medicine at Oxford University, to describe the sudden appearance of syphilis in the early sixteenth century. Although the sixteenth century is widely documented in literature to be the era when syphilis caused havoc amongst the sexually privileged, the actual origins of the disease remain a subject of much debate. There are currently three theories related to the origins of syphilis: pre-Columbian, Columbian and evolutionary¹. The pre-Columbian school of thought believes that the origins of syphilis existed in the Old World before Columbus' discovery of America. Evidence for this theory resides as far back as 600 BC where the tertiary form of the disease appears to be noted in the early writings of Hippocrates in Classical Greece. Further evidence to support this has been found at the site of a fourteenth century Augustian friary in the port of Kingston upon Hull,

which provided palliative care and burial rites for "wretched souls". The bones of approximately two-thirds of the skeletons subsequently discovered at the friary were shown to have lesions highly characteristic of tertiary syphilis².

The Columbian school of thought deems that syphilis arrived from the New World; brought back by Christopher Columbus and his promiscuous crew during their first voyage. Evidence for this theory lies in the finding of bone relics with indications of syphilitic damage throughout the Americas and a lack of paleopathological documentation that syphilis was present in the Old World. Further circumstantial evidence is based upon the fact that several of Columbus' crew joined the army of the French King, Charles VIII, which conquered Naples in 1495; the region where syphilis (or as it was subsequently phrased *Morbus*

the arms of nus...



Figure 1. *Treponema pallidum* (Darkground Microscopy)

Gallicus - the French Disease) first reached epidemic proportions. It is also believed by supporters of this theory that the European spread of syphilis is due to the sexual activities of Charles's large corps of mercenary soldiers, who, upon demobilisation, returned to their homes throughout Europe².

A more recent approach is the evolutionary theory which states that a single microorganism responds to changes in environmental temperature and evolves to form distinct species and subspecies located in different geographical areas throughout the world. This may account for the distinct species of *Treponema* and sub species of *T. Pallidum*, being distributed worldwide. This theory is further supported by research which has demonstrated that experimental syphilis in rabbits can be modified by altering the environmental temperature. In addition, the

recent discovery of a syphilitic skeleton from between 1296 and 1445 in Rivenhall, near Chelmsford further casts doubt over the Columbian school of thought as this finding suggests that syphilis was already present in England before Columbus discovered the new world in 1492^{3,4}. *Bones are the memory chips of the body, surviving to tell the tale once everything else has disappeared...* And so the debate continues...

Syphilis in Art and Literature

Throughout history, syphilis has been frequently portrayed in Art and Literature by many famous names. However the word 'syphilis,' was first introduced into our language in 1530 in a poem by Girolamo Fracastoro, one of the greatest physicians and poets of his generation. Fracastoro could not resolve the origins of syphilis nor indeed identify the venereal

mode of transmission of the disease therefore he wrote a poem entitled '*Syphilis, sive Morbus Gallicus*' and devised a myth, giving the name syphilis to a fictional shepherd. The poem describes how *Syphilus* ('pig lover'), a pastoral shepherd is stricken with syphilis, albeit somewhat harshly given the circumstances, for having 'offended' Apollo:

*A shepherd once (distrust not ancient fame)
Possess these Downs, and Syphilus his Name;
Some destin'd Head t'attone the Crimes of all,
On Syphilus the dreadful Lot did fall.
Through what adventures this unknown Disease
So lately did astonisht Europe seize,
Through Asian coasts and Libyan Cities ran,
And from what Seeds the Malady began,
Our Song shall tell: to Naples first it came
From France, and justly*

took from France his Name...

Further historical references to syphilis can also be found in many plays, novels and paintings: in William Shakespeare's play *Measure for Measure* (1601-1606), Lucio refers to "thy hollow bones" — undoubtedly a reference to one of the side effects of mercury which was widely used to treat syphilis; in Voltaire's *Candide* (1759), Pangloss, Candide's mentor and teacher, participated in sexual jovialities with a maidservant and contracted syphilis which ravaged and deformed his body resulting in the loss of an ear and an eye; the Dutch artist Kees van Dongen (1877-1968) produced several illustrations for *Lassiette au Beurre*, an anarchist publication, which depicted the decline of a syphilis-riddled prostitute from poverty to death as a criticism of the social order at the end of the nineteenth century². ▣



Figure 2. Penile chancre in primary syphilis

Famous Syphilitics

“And of course Mozart died of syphilis...because every great man dies of syphilis!”

Faith Fitzgerald, internist and professor at University of California, Davis School of Medicine. Syphilis is no respecter of class, dignity or occupation and many ‘famous’ syphilitics have been documented throughout history. Although many of the diagnoses are made retrospectively from historical data and are therefore questionable, several syphilitics made reference to the disease in personal diaries. Famous documented syphilitics include; Christopher Columbus, King Charles VIII of France, Henry VIII and five wives, Pope Alexander VI, Ivan the Terrible, Voltaire, Wolfgang Amadeus Mozart, Ludwig van Beethoven; Abraham Lincoln, Charles Darwin, Edgar Allan Poe, Vincent van Gogh, Oscar Wilde, Adolf Hitler, and Al Capone to name just a few.

Syphilis: Clinical Manifestations and Treatment

Syphilis, if untreated is a chronic disease which is characterized by three main stages; primary syphilis, secondary syphilis and tertiary syphilis which are summarized in table 1¹.

Primary Syphilis

Approximately 30% of people who have unprotected sex with an infected partner will develop syphilis¹. Following contact with the microorganism, the treponemes disseminate throughout the body, however, multiplication occurs predominantly at the point of contact, with the formation of a primary lesion or chancre (figure 2) within 10 to 90 days post contact. The chancre is oedematous, infiltrated with inflammatory cells and serous fluid from the lesion contains numerous treponemes. The patient may also experience lymphadenopathy during primary syphilis. The chancre heals spontaneously within 2-6 weeks.

Secondary syphilis

Non specific symptoms of secondary syphilis including fever, headache, sore throat and arthralgias occur approximately 1-5 weeks after the chancre has healed. However, the most characteristic signs of secondary syphilis include a

red maculopapular rash anywhere on the body (figure 3) and moist, pale papules (condylomata lata) in the anogenital region, axillas and mouth, and contain high numbers of treponemes. These symptoms resolve spontaneously within 2-6 weeks, but may recur within 3-5 years post infection if the patient is not treated. In approximately 30% of patients, early syphilitic infection progresses spontaneously to cure without treatment.

In a further 30%, the untreated infection remains latent and the patient is asymptomatic. Duration of infection <1 year, which is asymptomatic, is defined as the early latent stage. As lesions are not usually present after the first year, the patient is considered non-infectious and this stage is defined as the late latent stage, however treponemes may still be passed from mother to fetus for up to 4 years post untreated infection (congenital syphilis). In the remaining



Figure 3 Macropapular rash in secondary syphilis

patients, syphilis progresses to the tertiary stage.

Tertiary syphilis

Tertiary syphilis is the most acute and destructive stage of the disease characterized by the destruction of tissue from a response to the presence of long-standing treponemal antigens with the development of granulomatous lesions (gummas) in skin, bone, viscera and the eye. The clinical-pathological manifestations are those of vasculitis and chronic inflammation. In cardiovascular syphilis, vasculitis involves the arteries leading to the formation of aortic aneurysms and aortic rupture. Degenerative changes in the central nervous system leading to neurosyphilis is a further complication of tertiary syphilis and may be due to direct invasion of the parenchyma by treponemes or by brain infarction due to vasculitis. Neurosyphilis may present in many forms depending upon the location of the lesions. Involvement of the dorsal columns of the spinal cord results in a loss of position sensation (tabes dorsalis) which may result in trauma to the knee and ankle joints (Charcot’s joint).

Generalised involvement of the brain leads to impaired motor function (paresis), loss of integrative functions and personality changes. This clinical manifestation is

TABLE 1. Stages of syphilis infection (adapted from Larson et al., 1998)

Stage	Incubation period (post infection)	Clinical Manifestations	Affected site
Primary	10-90 days	Chancre, lymphadenopathy	Skin mucous membranes
Secondary	6 weeks - 6 months	Multiple lesions, lymphadenopathy, fever, condylomata lata	Skin/mucous membranes
		Alopecia	Hair/eyebrows
		CNS involvement (asymptomatic/ symptomatic)	Meninges
Latent	Early: ≤ 1 year Late: > 1 year	Asymptomatic	
Tertiary	Months/years	Gummatous lesion	Tissue
		Aortic aneurysm Tabes dorsalis, dementia, Optical atrophy	Aorta Meningovascular
			Eye

referred to as 'general paralysis of the insane'. A further sign of neurosyphilis is the 'Argyll-Robertson pupil', which is characterized by failure of the pupil to respond to light but reacts to near and distant objects.

Treatment of Syphilis

As early as the late fifteenth century mercury was used for the treatment for syphilis and its use gave rise to the saying 'a night in the arms of Venus leads to lifetime on Mercury'. Mercury, along with its associated side-effects, remained the choice of treatment for syphilis for more than three centuries even though it lacked efficacy in the treatment of the tertiary stage.

The development of potassium iodide followed as an important advancement in the treatment of syphilis as it proved effective in treatment of late stages of the disease. However, in 1908, Paul Ehrlich isolated compound 606 (arsphenamine) in an attempt to cure sleeping sickness, and whilst the compound proved to be ineffective in the cure of the disease, it did destroy *T. pallidum*. In 1910, compound 606 was termed Salvarsan and was heralded as the *magic bullet* in treatment of syphilis. However, treatment with salvarsan was both expensive and painful with severe side effects within 6-8 hours following administration. Furthermore, patients thought to be cured of syphilis soon relapsed.

Physicians eventually discovered that treatment with salvarsan had to be supplemented by applications of mercury or bismuth ointments. In 1939, the most effective treatment of early syphilis consisted of alternating eight to twelve week courses of bismuth and salvarsan continuously for 60 weeks. Even then, this augmented treatment did not have ideal results⁵. In 1928,

the discovery of penicillin by Alexander Fleming was about to change the world of modern medicine and by 1945, penicillin became accepted as the treatment of choice for syphilis, although optimal doses were not finalized until 1960. Today, resistance to penicillin and its derivatives has not yet been reported amongst *T. pallidum* and these antibiotics remain the gold standard for the successful treatment of syphilis⁶.

Syphilis in the Twenty-First Century: Science versus Sexual Behaviour

Major advances in molecular research have allowed for the genomes of many microorganisms to be sequenced providing the information necessary to fully understand the aetiology of infection. In 1998, scientists sequenced the complete genome of *T. pallidum*⁷ generating a plethora of genetic information enabling scientists to enter the twenty-first century with the tools necessary to potentially eliminate a disease whose origins lie as far back as 600BC, if not before. The genetic map of *T. pallidum* identifies specific genes that are present or absent in the microorganism thus providing scientists with the crucial information necessary for the development of improved methods of treatment, diagnostic tests and also vaccines.

However, in sharp contrast to these recent scientific advancements, in 2001, for the first time in a decade, there was an increase in the numbers of cases of primary and secondary syphilis in bisexual and homosexual men in the USA^{8,9}. Reported figures in 2001 reached 6,103 which increased a further 12.4% by 2002¹⁰. The nationwide increase in rates of syphilis and other STDs has been

associated with a resurgence of unsafe sexual practices. Of further concern, is the fact that syphilis increases the risk of transmitting human immunodeficiency virus (HIV) by 3 to 5 fold.

Worryingly, the rise in the number of cases of syphilis is also being seen in the UK. Between 2001 and 2003 the overall diagnoses of infectious syphilis rose by 67% in males and 33% in females¹¹. In 2002, 1,163 new diagnoses of primary and secondary syphilis were made in Genitourinary medicine clinics in England, Wales and Northern Ireland.

The increase in syphilis in the UK is punctuated by a series of outbreaks occurring in high incidence areas including Manchester, London, Bristol, Newcastle Nottingham and Northern Ireland.

Conclusions

There is no doubt that syphilis has made its mark as one of the major infectious diseases in history, and whilst

it has been poetically captured in verse and colourfully portrayed in paintings, it remains the third most frequently sexually transmitted infectious disease worldwide today. Whilst recent advancements in science may potentially pave the way for reducing the global incidence of the disease, this must be concurrent with changes in sexual practice, particularly amongst high risk groups. Continued worldwide educational programmes promoting safer sexual behaviour are essential in helping to prevent the spread of STDs. Strategies need to include mass media campaigns, special awareness events (e.g. safer sex week), development of educational material, targeted community interventions and condom promotion.

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Verity Bevan investigates the second most common sexually transmitted bacterial infection

Gonorrhoea: An unlikely love affair

SINCE THE discovery of gonorrhoea in 1879 by Albert Neisser, this disease has become the second most common sexually transmitted bacterial infection.

Neisser learned and adopted the techniques of his peers to visualise bacteria using the newest microscopic methods. By staining smears with methylene blue and using oil-immersion techniques, he could observe bacteria using x1000 magnification. Originally, the organisms he saw were given the name micrococcus; however it was a colleague, Paul Ehrlich, who first coined the name gonococcus. Since then, the *Neisseria* group of organisms, so named after Neisser himself, has been added to and comprehensively characterised. The group also contains *Neisseria meningitidis*, well known for causing meningitis. Other members are largely commensals of the oropharynx and gastrointestinal tract. All the Neisseriaceae are Gram negative diplococci, producing oxidase positive colonies. The pathogenic *Neisseria* are distinguishable by detection of pre-formed enzymes, for which commercial tests are available. Both organisms are able to ferment glucose, but *N. meningitidis* is also able to ferment maltose. Gonorrhoea is responsible for infecting millions of people worldwide each year.

Trends in the prevalence of gonococcal infection are apparent. Rises in infection have been noted following wartime, as soldiers returning home passed infections onto their wives as a result of promiscuity whilst away from home. During the 1950s, the advent of penicillin saw a

decrease in infections; however, the rapid emergence of resistant strains in the 1960s and 1970s, saw the infection rate increase once again. The 1980s brought the new danger of HIV. A powerful campaign promoting the use of condoms and practicing 'safe sex' also drove down the rate of gonococcal infection, as well as other sexually transmitted diseases, (STIs). Nowadays, complacency is seeing infections on the rise once more. Treatments for HIV have been developed so intensively, patients are living longer and healthier lives. The classical 'it will never happen to me' attitude is proving a dangerous one, especially among the 18 to 25 year old age group where the increase is most noticeable. Other contributing factors are a decrease in the age at which individuals become sexually active, the number of sexual partners that they have and the advent of going abroad with the intention of having sexual relationships.



Figure 1.

The infection is only transmissible via direct contact with an infected sexual partner. It can affect males and females equally. Pathogenicity is influenced by the presence of pili, hair-like structures that help the organism attach to the

columnar epithelia of the urethra wall in males and the cervix of females. The vaginal wall is not able to be colonised as this is made up of squamous epithelia. An acute inflammatory response follows with the engulfment of the bacteria in to polymorphonuclear cells. It is thought that gonococcus is able to survive this hostile environment due to various mechanisms that protect it from the lysozymes and reactive oxygen species that are produced during inflammation. Migration of the organism in to the subepithelial space induces a purulent discharge, leading to the clinical signs of gonorrhoea infection. Despite an initial IgA response, the bacteria are able to switch the pili genes on and off, (phase variation), as well as rearranging the genes coding for pili and other surface markers. In this way it is capable of evading the immune system and eliciting the symptoms associated with gonorrhoea.

Symptoms are often very apparent. Approximately 90% of males experience a purulent discharge from the urethra. Females are more likely to be asymptomatic, with up to 50% presenting with no abnormal physiology. Other symptoms can include lower abdominal pain and dysuria. Pharyngeal infections are largely asymptomatic. Rectal infection may be accompanied by tenderness and a discharge, though this is relatively uncommon. It is advised that anyone suspecting that they are infected, should seek medical attention. The general practitioner is able to refer those most at risk to the Genito-urinary medicine, (GUM) clinic, where a comprehensive physical

examination may take place. A full sexual history will be taken to determine the level of risk for the individual. This will also indicate the primary sites that might be investigated. For example, homosexual males may require a rectal sample to be taken.

Samples are taken from the urethra in males as routine. Additional samples from the rectum and pharynx are taken in accordance with sexual history. Female samples are taken from the cervix, urethra and vagina. As with males, the rectum and pharynx may also be sampled. Typically all these specimens are screened for various other STIs to avoid repeating the procedure, which can be an uncomfortable experience.

Diagnosis of gonorrhoeal infection is subject to the isolation of the bacterium. Bacterial culture is both sensitive and cheap to perform. It has the added advantage that further tests can be carried out to determine antimicrobial susceptibility. In the GUM setting, the direct culture of genital specimens is the most effective method of isolation. Selective agars are now widely available for the culture of gonorrhoea. These often contain an antimicrobial cocktail to suppress the growth of normal genital flora, and prevent overgrowth with *Candida*. Common formulations such as Vancomycin, Colistin, Nistatin and Trimethoprim, (VCNT) or Lincomycin, Colistin, Amphotericin and Trimethoprim, (LCAT), are available from leading manufacturers. Confirmatory laboratory tests are performed on positive cultures. These include an oxidase test, a Beta-lactamase production test

and a Gonocheck, (EY Laboratories Inc.), to detect prolyliminopeptidase, a preformed enzyme produced almost exclusively to gonorrhoea (figure 1).

False positives can occur with organisms that are not Gram negative diplococci, therefore it is also important to check that the organism isolated has those characteristics. Together these elements constitute a positive identification of gonorrhoea. The culture may then have sensitivity testing performed using standard methods and according to laboratory protocol. Although the patient will already have been treated for the infection at the time of diagnosis, susceptibility testing remains necessary and has two main functions.

Firstly, it confirms that the infection was susceptible to the treatment given and that the infection would have been removed, secondly, to monitor the epidemiology of the organism, and any resistance trends that may appear.

Molecular methods are also available to detect gonorrhoea, although these have not yet been validated for genital specimens, however, urine samples can be used. Immediate diagnosis using Gram stained smears is also common practice in GUM clinics. This allows the patient to be counselled and treated on the day of assessment. Microscopic diagnosis is reliable for 90-95% of symptomatic males, versus 60-75% of asymptomatic patients. In females, the reliability is much lower, approximately 40%. This low figure is largely due to the presence of normal genital flora obscuring the presence of gonorrhoea in the smear. Male slides characteristically contain numerous pus cells, with obvious intracellular bacteria present. Gonococcus has a distinctive morphology to the trained eye, appearing as a

Gram negative 'bean-shaped' diplococcus. The use of neutral red over the more commonly used carbol fuchsin as a counter stain also enhances the appearance of the organisms (figure 2). Gram staining rectal and pharyngeal samples is not recommended as these sites contain commensal *Neisseria* that can be mistaken for gonorrhoea.

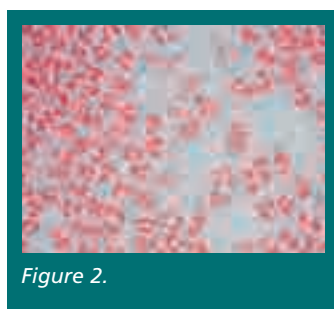


Figure 2.

All patients diagnosed with gonorrhoea are seen by a health adviser, who will explain the nature of the infection and the consequences it has on themselves and their partner. It is also stressed that compliance with the therapeutic regimen prescribed to them is crucial to a successful cure. The patient must also provide the names of previous sexual partners for follow up purposes. In symptomatic patients, all partners during the preceding fortnight are informed; however, if asymptomatic, all partners during the previous three months should be notified. The patient is also advised to abstain from further sexual encounters until after a follow up assessment.

Gonorrhoea infection is not always without complications. In males, transluminal spread of the organism can lead to infection of the epididymus and prostate. In females, pelvic inflammatory disease, endometriosis or salpingitis can lead to fertility problems. Disseminated infections such as arthritis, arthralgia,

tenosynovitis and skin lesions can also result from prolonged untreated gonorrhoea infection. These are extremely uncommon, occurring in less than 1% of cases. Gonorrhoea is also able to infect neonates on delivery. This is an infection of the eyes known as Ophthalmia neonatorum and is often apparent within the first two days of birth. Eye swabs can be taken to confirm diagnosis.

Treatment regimens are prescribed in accordance with national guidelines and will vary according to an individual's circumstance, for example, pregnant women, breast-feeding mothers and Beta-lactam sensitive patients must be suitably prescribed. Uncomplicated cases are treated using Ceftriaxone or Spectinomycin in the case of pregnant women. Previous regimens have used Penicillin as an effective treatment; however the emergence of resistant strains has led to this being withdrawn as a suitable treatment.

In recent years, resistance to Tetracycline and Ciprofloxacin has also been noted. If sensitivities are known it is possible to treat using Ciprofloxacin, Ofloxacin or Ampicillin. Ciprofloxacin and other Quinolones, as well as Tetracycline, should not be prescribed to pregnant women. Likewise, Ampicillin is unsuitable for patients known to be allergic to Beta-lactam antibiotics. Single dose therapies are highly recommended to increase patient compliance, and reduce the likelihood of re-infection.

Follow up appointments are arranged at the time of diagnosis, usually 2-3 weeks after original appointment. The purpose of this is to establish whether the infection has cleared, and to ensure the patient has not suffered any additional symptoms or side-effects following treatment. If

symptoms are apparent, retesting will be required. Asymptomatic patients largely do not require retesting as the efficacy of treatments usually deems this unnecessary. A second positive culture is usually attributed to reinfection or lack of patient compliance rather than drug failure.

On a national scale, the Gonococcal Resistance to Antimicrobials Surveillance Programme, (GRASP) also monitors the emergence of resistance. This study takes place during July and August each year. Isolates from a number of clinics and hospitals across England and Wales are sent to one of two reference laboratories. These laboratories perform susceptibility testing using agar dilution techniques. The data is then compiled and used to amend guidelines for the management of gonorrhoea as appropriate. Approximately half of all confirmed cases of gonorrhoea are diagnosed in the London area. Around a quarter are women, a quarter are homosexual males and half heterosexual males. Less than 10% of these can be attributed to unprotected sex whilst abroad; however, this proportion is increasing. An overall increase in infection of 7% was recorded between 2000 and 2001.

Gonorrhoea is likely to remain a prominent feature of future sexual health forums. The emergence of resistant strains is worrying, and should continue to be monitored. The need for new treatment regimens may be required in the near future. Until then the role of GUM clinics in the re-education of sexual behaviour is crucial for the control of these infections.

Verity Bevan

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REDUCE WHAT YOU PRODUCE

Microbes get to work on degradable plastic

You may have noticed that many supermarket shopping bags are now made from tapioca. No, not tapioca pudding — tapioca root starch, derived from the cassava plant. These bags biodegrade in 28 days when composted, which is a lot quicker than your average shopping bag. This is surely good news: bags made from bio-derived polymers reduce the use of oil and discarded bags will degrade in the environment. Microbiologists will give silent thanks to microbial organisms everywhere; an army of volunteer microbes, the original recyclers, have been enlisted to recycle shopping bags the way nature intended. Few would argue against the advantages of degradable plastics but they are not ideal for all applications.



There seems no alternative to this material especially for single use items such as loops and tips. But what about the disposal of these plastic items? The red-topped sweet jar, used as a bench-top dry-discard container has become ubiquitous. These containers are manufactured from an oil-derived polymer (PET) but anyone who has seen one come out of an autoclave will know that recycling it is not an option. But is recycling the

direction we should be moving in? Not according to Andrew Simmons of RECOUP who suggests:

“Our target should be to increase resource efficiency and consider whole life issues: it’s wrong to use recycling alone as the measure of environmental performance”.

So how does the sweet jar measure up in this regard? Due to their bulk, such jars are difficult and expensive to store and transport. What’s more their tower-like shape does not lend itself to the efficient utilisation of space when in use. Even short items cannot lie flat and tend to criss-cross and obstruct each other, which leads to more waste.

So how can the sweet jar be improved? What is needed is a purpose-designed bench top container that is not made from an oil-derived polymer. The answer could well be **Microb-in™**. Developed by **Adrianic**, based in Sheffield, **Microb-in™** is a new type of bench-top waste disposal container. The idea is an innovative adaptation of the familiar carton. Like all cartons it is manufactured from paper board which is energy efficient to manufacture. What’s more, **Microb-in™** stacks inside itself making it easy to store. It’s light and efficient to transport, using less fuel and creating fewer carbon dioxide emissions.

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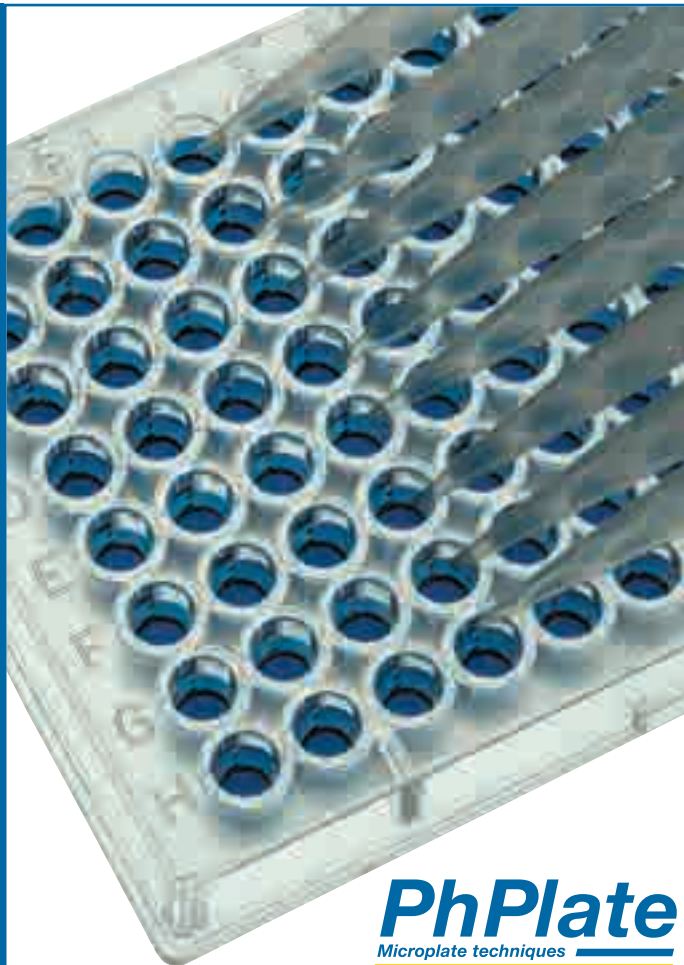
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Biodiversity conservation in Costa Rica: the INBio experience.

Peter Green reports on INBio's pioneering work

COSTA RICA IS LOCATED IN Central America between Panama and Nicaragua. The country has a stable and democratic political system, no army and roughly 4% of the species believed to exist in the world.

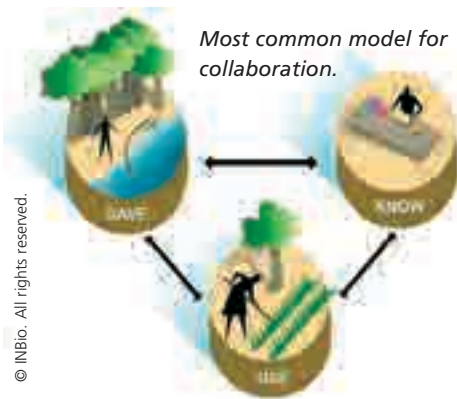
Mind-boggling? When focusing on the number of species per square kilometer, Costa Rica could be considered one of the densest countries in the planet. Just to give you an idea, it is estimated that more than 500,000 species inhabit this country. With this wealth of natural resources and a little vision, an institution within the country whose mission would be centred on biodiversity conservation seems like a logical step. This institution is known as the National Biodiversity Institute (INBio) which was created in 1989 under the Costa Rican law of associations as a nonprofit civil organization. It came about as part of a paradigm shift in the country's response to the issue of biodiversity conservation. Although initially Costa Rica had understood conservation to mean protection of resources, this concept gave way to a view of conservation as an opportunity to incorporate biodiversity into socioeconomic development.

INBio's mission is to *'promote greater awareness of the value of biodiversity as a way to achieve biodiversity conservation and improve the quality of human life.'* Its activities are carried out in close collaboration with the government and other public and private institutions, both domestic and international.

The Institute's ultimate goal is for knowledge to be used by society, and not merely to reach society. In short, INBio is a civil society organization that develops knowledge on the country's biodiversity and contributes this knowledge to various processes so that society will conserve its biodiversity and use it sustainably.

The main accomplishments of INBio can be summarized as follows:

1. INBio developed a conceptual framework for the protection, knowledge and use of biodiversity, which has now become a national policy contained in the National Biodiversity Strategy. The best way to conserve the country's biodiversity is by using it to improve the quality of life for Costa Rica.



2. INBio has become a national organization of international scope. The donor community, the international scientific community and the 106 official delegations of countries interested in learning more about INBio are an example of its international impact as a reference institution for sustainable management of biodiversity.

3. INBio developed an innovative system for compiling inventories of biodiversity. Because experts are in short supply all over the world, it coordinated 'taxonomic work teams' of paratonomists, technicians, curators and national and international experts.

4. It has made innovative use of modern information technologies with systems to support the collection, administration, generation and dissemination of information. For example, its own *Atta!* information system has received worldwide recognition as a pioneering initiative. It includes: bar codes to identify each specimen in the collection, geo-referencing for each of the more than three million specimens, multimedia information and an Internet site where the general public enjoys flexible, free access to the databases. This information system won INBio the *'Tech Museum 2003: Technology Benefiting Humanity'* award from the Tech Museum of Innovation located in San Jose, California.

5. INBio has developed various processes for sharing knowledge and information on biodiversity with diverse sectors of society for the primary purpose of supporting the formation of values and activities for sustainable use of biodiversity. For example, Editorial INBio has published 94 products, mostly for

educational purposes and to support ecotourism (now the country's primary source of income), and created INBioparque, a theme park whose purpose is environmental education, that received over 150,000 visitors in its first three years of operation.

6. A Bioprospecting Endeavor (systematic search for new uses of biodiversity), using modern scientific and technological approaches to seek out new products derived from Costa Rican wild organisms of interest to the chemical, pharmaceutical, agricultural and biotechnology industries. Research is guided by such criteria as guaranteeing improvement of the country's scientific and technological capacity and sharing equitably any benefits that may potentially derive from these products, considering protected wild areas as one of the beneficiaries. Collaborations are typically carried out via research agreements.

This Unit has been responsible for conducting research to find sustainable uses for genetic and biochemical elements of Costa Rican biodiversity. It has structured agreements for scientific cooperation with renowned universities, research centers and companies, both nationally and internationally. At the national level, one of our most outstanding programs has been *'Support to Develop Biodiversity Use by Small Enterprises'* (INBio/IDB/MIF Program) which began in 1999 under the terms of an agreement with the Inter-American Development Bank (IDB) and through non-reimbursable technical cooperation from the Multilateral Investment Fund (MIF). In addition to the research agreements, Bioprospecting offers services, consultancies and training in different areas. INBio has positioned itself as a leading institution in its field and seeks to continue to advance biodiversity research through partnerships as a Center of Excellence for the Management of Science and Technology for Conservation and Sustainable Development.

Peter Green
NCIMB, Aberdeen, UK

Further Information

■ For more information on Atta, please visit: <http://atta.inbio.ac.cr/>

Peter Green reports on the first award of the SfAM International Development Fund

Culture Collection Training Course: INBio, Costa Rica

PRESERVATION AND UPKEEP of biological resources, in the case of collections of organisms which serve as the bedrock of reference material necessary for research, is by no means a simple matter. Costa Rica's National Biodiversity Institute (INBio) recognized a pressing need to fill a knowledge gap to adequately preserve and maintain the microbial collections scattered throughout the different research organizations within Costa Rica and to bring together a group of their representatives to discuss this situation.

This prompted INBio, a non-profit, public interest, NGO, to seek training for national scientists in this area. Through one of our collaborators, Dr. Marcel Jaspars at the University of Aberdeen, we began our contact with the National Collections of Industrial, Food and Marine Bacteria (NCIMB) in the United Kingdom. With the help of the Curator at NCIMB (Dr Peter Green) we submitted an application to the new *SfAM International Development Fund* (IDA). The funds were allocated to a three day training course to be held at INBio and with the participation of three experts from UK: Dr. Peter Green, Curator, NCIMB, Aberdeen, UK; Dr. Paul Kirk, Senior Mycologist CABI Bioscience, Egham, UK and Professor Michael Goodfellow, University of Newcastle upon Tyne, UK. We worked on this application and on November 2003, we received the good news that it had been accepted. Hence, the preparations for the "Culture Collection Training Course" in April 2004 started.

The main aim of the course was to bring together national researchers and for the experts to lay out the basics of preservation, maintenance and handling of microbial collections. Nineteen researchers from eight organizations were invited to participate at no cost since their expenses were covered by the SfAM IDA grant. Their educational background ranged from B.Sc. to Ph.D in areas such as: Chemistry, Microbiology, Biotechnology, Biology, and Agronomy. The youngest participant was 21 and the oldest in their forties. The list of participating researchers and their affiliations are shown in the panel on the right (also see picture at right)

Specifically, the grant money provided was used to cover the costs related with the experts travel and accommodation, the meals, equipment and materials (including a certificate of participation) needed for the workshop as well as for providing a set of books to each of the participating organizations and two 2-year SfAM memberships for two outstanding young researchers.

In summary, 16 presentations were given by the experts and participants in the form of a roundtable. On the third day, the participants were given the opportunity to have one-on-one sessions with the experts with whom they could tackle their specific and current concerns regarding management of the collections. The lunches and informal dinner also provided a space for networking and exchange of scientific and business views.

In addition to each of the participants receiving the training course materials, three of the most salient points from the discussion fora, under the guidance of the UK experts, are summarized below:

1. The eight organizations share common problems, to name a few: limited or non-existent funding to maintain the collections, limited personnel and none dedicated to this task, problems with existing infrastructure (no space to house the collections, equipment failure) and lack of information on how to solve problems on how best to preserve the specimens and avoid contamination.

2. Participants acknowledge that pooling of resources and generation of common guidelines for culture collections make good sense. Hence, participants have begun discussions on the possibility of structuring a Costa Rican Federation of Culture Collections and its implications. Topics such as: how should the federation be constituted? Which are the priority

organisms that should be a part of the collection? Physically, where would these collections be housed? How would information be archived? What could each of the institutions contribute? Which would be the funding mechanisms for the federation? Who should spearhead the process and become the leader in the implementation of a federation?

3. The University of Costa Rica, particularly its Faculty of Microbiology, was mentioned as a likely candidate for spearheading this initiative. Results from an initial meeting at the University to discuss the idea of a federation are pending.

INBio is very satisfied with its work as a facilitator for this initiative. The Institute recognizes the value of the results of the course and is confident that these initial meetings will be the stepping stones for further discussion and a tangible outcome in the form of some formal culture collection structure and network within Costa Rica.

INBio and NCIMB would like to thank SfAM for their support in making the training course a reality.

Peter Green
NCIMB, Aberdeen, UK

LIST OF PARTICIPANTS AND AFFILIATIONS

Course tutors:

Dr. Peter Green, Curator, NCIMB, Aberdeen, UK, Dr. Paul Kirk, Senior Mycologist CABI Bioscience, Egham, UK; Profesor Michael Goodfellow, University of Newcastle upon Tyne, UK

National Researchers:

Ana Sittenfeld, CIBCM-UCR; Catalina Murillo, INBio; Caterina Guzmán, UNA Cindy Araya Castillo, ICP; Eduardo Hidalgo, CATIE; Erick Hernández, ITCR; Giselle Tamayo, INBio; Iván Rodríguez, INBio; Jorge Blanco, INBio; José Matías, INBio; Loengrin Umaña, INBio; Lorena Uribe, CIBCM-UCR; María Isabel Ríos, INBio; Marielos Mora, CIBCM-UCR; Miguel Obregón, INA; Myriam Hernández, INBio; Nefertiti Campos, INBio; Norman Rojas, Faculty of Microbiology-UCR; Silvia Soto, INBio.

Further Information

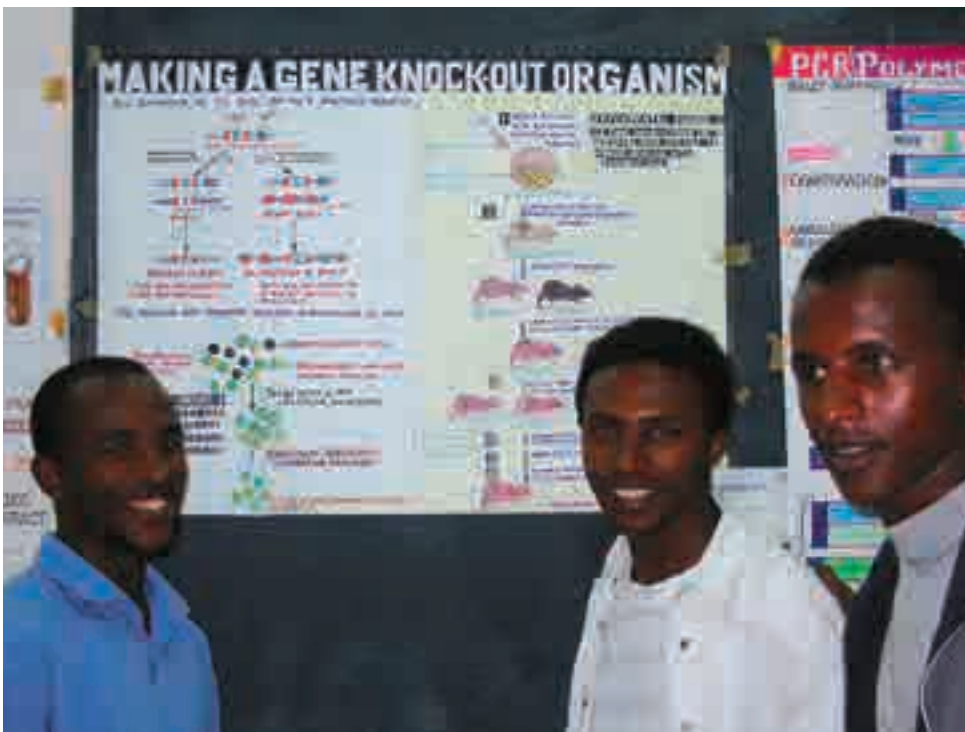
- www.inbio.ac.cr
- www.ncimb.co.uk
- **SfAM Endangered Culture Collection Fund:** www.sfam.org.uk/prizes.php



The Course members

Education in Ethiopia

In the sixth in a series of articles, **Dr Jenny Search** reports on her continuing two-year voluntary service overseas placement at Debu University in Ethiopia



I HAVE SPENT THE summer break trying to prepare courses for the next academic year.

There will be new courses in microbiology and parasitology so I am collating some materials and preparing teaching aids that can be used after I have left. Last semester I taught a course about research methods and design in which the students had an assignment to prepare a poster presentation. Some of them are so good they can be used in other courses as teaching aids.

For microbiology, I printed out some colour pictures of the results of biochemical

tests to use in labs as we don't have all the chemicals necessary for the tests. I couldn't find a laminating service in Awassa so my colleague Mintesinot and I spent one afternoon sealing the pictures between two sheets of overhead transparency film with a candle, to try and protect them from the ravages of 60-odd students in a practical class!

In the next academic year we will have final year students for the first time so we are trying to prepare some small research projects for them. This is not easy as only one of the members of staff is actively involved in research

at the moment, so it's not just a question of tagging a student onto an already established project.

I came across one idea for a project whilst browsing through the microbiology books in the University library. I read about hot chilli sauce having antibacterial activity on raw oysters and thought I could apply the idea in a local context. One of the major ingredients of Ethiopian food is a mixture of spices called 'berbere'. This is a red powder made of crushed red chillies and other spices and used in almost every Ethiopian dish. I searched the internet for information and found out the chemical that gives chillies

their hotness, capsaicin, is reported to have an antibacterial effect. For example, Dorantes *et al.*, reported that capsicum extracts from various hot peppers inhibited the growth of *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Bacillus cereus*.

I tried out a preliminary experiment to determine if berbere had an antibacterial effect and to find out if it was worthwhile investigating further as a student project. After a failed first attempt I surfed the Internet further and quickly discovered that capsaicin is not soluble in water. So I tried again and

Far left: This photo shows three third year biology students and a poster about gene cloning that they made for the "research methods and design" course last semester

Left: This is me in the lab trying out an experiment to see if 'berbere' (the red powder in the paper cone) has any antibacterial effects

below: This shows me melting some overhead transparencies with a candle to protect the photo of some biochemical tests. I will use these in a microbiology practical class as a teaching aid, because we don't have all the chemicals necessary to carry out the tests

Below right: Clean and dirty 5 birr notes



observed that a 5% solution of berbere in glycerol or cooking oil inhibited the growth of *Staphylococcus aureus*. I also found that dried alcohol extracts from berbere and chilli peppers had an inhibitory effect. A favourite delicacy here is a dish called kitfo which is raw minced meat so my plan is for a student to look into the effects of berbere on bacterial growth in the meat. Kitfo is often served with other spice concoctions which can also be investigated. Interestingly, one of my colleagues told me that Ethiopians often feel some discomfort in their stomachs if they eat a meal that does not contain berbere!

Some other ideas Mintesinot and I have been discussing include: looking at antibacterial activities of preparations of traditional medicines, seeing if the paper currency here (which is often extremely dirty as you can see from the pictures of a new and used note above!) harbours any pathogenic bacteria and investigating some local beliefs such as squeezing lime juice on food stops you from getting food poisoning. The research may not be very sophisticated, we have to try and come up with projects that can be done here and now and that may have some relevance to the people of Ethiopia.

Further Information

■ Dorantes, L, *et al.*, *Antimicrobial activity of capsicum extracts against some pathogenic bacteria*. Proceedings of the 16th International Pepper Conference, Tampico, Tamaulipas, Mexico. 2002

■ www.neal-jenny.info

■ www.vso.org.uk.

■ The Faculty of Natural Sciences at Debu University: <http://home.no/dufns>

Jenny Search

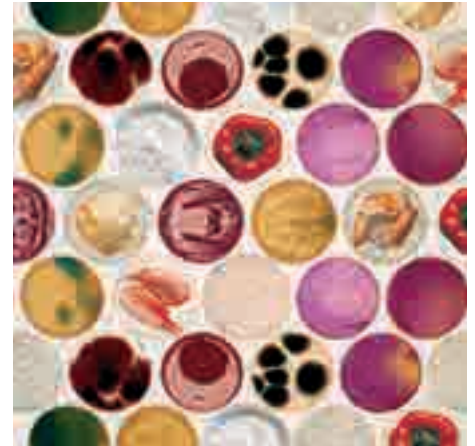
Debu University, Ethiopia



Sfam Summer Conference Report

DAIRY & FOOD MICROBIOLOGY challenges and opportunities

sfam SUMMER CONFERENCE ● CORK, IRELAND 12-15 July 2004



DAIRY SCIENTISTS and food microbiologists came together to share ideas on the impact of microbiology towards animal sciences and human health at the SfAM Summer conference on Dairy and Food Microbiology. The conference, subtitled, 'challenges and opportunities', drew a panel of 21 invited speakers from Ireland, the UK, the USA, the Netherlands and Belgium.

This year's Summer Conference was perhaps the most ambitious and innovative event devoted entirely to agri-food education ever to be held in Ireland. The Society took the leading role, working in partnership with The *Alimentary Pharmabiotic Centre*, *BioSystematica*, *Don Whitley Scientific* and *Safe Food*. The event brought together a wide range of microbiological and educational organisations, each of which sponsored individual trade shows. Over 100 people attended at least part of the event, the maximum number present at any one time being 120. Core organisers of the event were Peter Silley (Hon. President of the Society), Margaret Patterson (Hon. Vice President of the Society), Arthur Gilmour (Organising Committee), Martin Adams (Hon. Meetings Secretary) and Joanne Mulligan.

There were many stimulating special lectures by leading figures in the animal sciences, food biotechnology, human health and novel processes, such as Dr D O'Rourke, Dr A Fox and Dr P Kiely. The conference began on Monday 12th July 2004 with a welcoming reception for delegates in the Devere Hall of University College Cork (UCC). There were excellent poster sessions and trade exhibitions that were not only highly informative, but also attractive and stimulating, and commonly surrounded by a buzz of intense interest. There were entertaining evening activities and dinners, the most interesting of which took place on Tuesday evening when delegates toured the Old Middleton Distillery. This was followed by a lively Irish Night, a traditional cultural event, organised by committee members and the UCC, which had delegates mesmerised by the traditional and progressive alcohol industry and local cultures.

The Plenary Sessions: Tuesday 13 July 2004

The morning session was given over to current problems and the future obstacles of *Animal Health and Zoonoses*. The session began with thought provoking discussions upon current diseases in cows, tuberculosis, brucellosis, Crohn's

and Scrapie diseases. Mastitis, induced by bacteria in the dairy cow, is said to be one of the most disruptive and expensive of such diseases. The morning's session reviewed the treatment of the present situation discussing control of international boundaries. The use of antibiotics for treatment and non-antibiotic dry cow products raised the question of the assessment in their usage. The talk on tuberculosis highlighted some significant developments in tuberculosis studies; control and eradication strategies, and effective diagnosis and assessment of animal vaccination. This was followed by a plenary session on bovine brucellosis, which was believed to have devastated British troops based in the Mediterranean during WW2, as a result of the consumption of non-pasteurised milk.

One of the most interesting talks discussed the association of *Mycobacterium paratuberculosis* and Crohn's disease. Higher detection rates of *M. paratuberculosis* in gut samples from Crohn's patients, remission caused by anti *M. paratuberculosis* antibiotic therapy and substantial improvement in disease condition, have been recognised as evidence supporting a link between *M. paratuberculosis* and Crohn's disease.

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Another interesting talk followed discussing animal health including the fatal disease Scrapie — for which there is as yet no reliable diagnostic test for live animals. Genetic breeding and the culling of genetically susceptible animals were proposed as possible alternatives. The talk emphasised the attempt to define genetic rules of susceptibility and their application to other species, such as deer.

The plenary sessions in the afternoon discussed Food Biotechnology, focusing on food grade enzymes, probiotics, the production of food ingredients and the use of GM microorganisms in agri-food sectors. There was an overview on enzymes derived from plants, animals and



microbial sources, focusing on the commercial production of microbial enzymes on an industrial scale. Details of the three main microbial sources; yeast, bacteria and fungi, were described. The discussion on probiotics highlighted the importance of genome studies. Such information provides the fundamentals of the origin, metabolic capability and functionality of probiotics.

Perhaps the most interesting talk during this session, was the use of bacteriocins from bacteria to control other undesirable bacteria in food. Nisin, a recognised bacteriocin, is produced by *Lactococcus lactis*, and is used as an alternative to chemical inhibitors in the food industry. This presentation illustrated this concept with examples of controlling food pathogens in various fermented foods, the use of lactic acid bacteria, the extension of shelf-life in heat



treated products and the possibility of developing functional foods with bacteriocin as an ingredient. This was followed by a presentation on the usage of GM organisms in the agri-food sector, particularly to produce crops with beneficial traits, such as improved nutritional qualities. However, risks remain, and the impact on the biodiversity of culturable and non-culturable microbial populations remains questionable. It appears that greater understanding of microbial-plant interactions at the molecular level is needed to biocontrol strains and facilitate risk assessment in the agri-foods sector.

The morning session on Wednesday consisted of presentations on *Human Health*, focusing on the various diseases caused by *Campylobacter jejuni*, *Salmonella*, *Escherichia coli*, and viruses in food-borne illness. The first session started with a talk on *C. jejuni*, which causes the most gastrointestinal infections in the UK. The enigmatic nature of these infections is due to the fact that the source of infection remains unknown in many cases. The discussion attempted to shed light on these problems, giving hope that new techniques such as phenotyping methods and nucleic acid sequence-based typing methods may more accurately define the causes of this sporadic enigma. This was followed by a presentation on combating *Salmonella*, which causes food-borne infections on an international scale. Two networks involving both laboratory-based microbiologists and epidemiologists have been recognised to help combat this infectious agent across international boundaries: Enter-Net and Pulse-Net, based in Europe and the USA respectively, working together to facilitate intervention strategies and collaborative action.

E. coli is known to be pathogenic and produces cytotoxins. However, a verotoxigenic *E. coli* strain differs in its zoonotic potential and may be carried by healthy adult domestic and wild ruminants, and has been isolated from foodstuffs of animal origin. The next presentation highlighted the various aspects of bacteriology, virulence and typing of verotoxigenic *E. coli* in humans, animals and foodstuffs, using results from veterinary medicine, human medicine and food microbiology studies.

The talk on viruses in food-borne illness reviewed the main agents of potential food-borne virus infection, their stability and potential routes for

2004 Summer Conference Report

infection. More interestingly, the potential for viruses as a threat to food safety, was also debated.

The afternoon sessions were allocated for presentations by students and non-students. The presentations covered such diverse topics as '*Lactobacillus acidophilus* and *Lactobacillus casei*,' '*Applications of bacteriocin-containing fermented wort to eliminate beer spoilage bacteria*,' '*Effects of surfactants on polycyclic aromatic hydrocarbon degradation*' and '*Inhibition of Nisin activity in meat*.' The presentations from non-students covered general microbiological topics such as '*The usage of mathematical modelling to measure single-cell lag times*,' '*The epidemiological*



comparisons of Listeria monocytogenes in foodstuffs,' '*Genomic approaches to understanding the adaptive response of C. jejuni*,' '*the survival of Salmonella spp. on eggshells*,' '*The selection of anti-Salmonella lactic acid bacteria from porcine*' and '*The comparative genomics of L. lactis ssp. cremoris and ssp. lactis*.'

The last day of the conference was allocated to plenary sessions on Novel Processes and Products, focusing on food processing and commercialisation. This session discussed the advances in thermal processing to develop novel food packaging and processing methods, as well as the challenges posed by microbial resistance to pressure treatment in foods. It was agreed that the reliability of these processes will benefit many innovative new process technologies, and speculations were aired on the future applications of pressure-treated foods. Effective microbial inactivation was also

discussed, highlighting the sensitivity of different microbial groups and the kinetics and mechanisms of inactivation. Commercialisation was hotly debated using probiotics as a model, and emphasizing the development of probiotic platform technologies for human and animal healthcare products, as well as research and development advances in gastroenterology, immunology and microbiology. Undoubtedly, clinical evidence to support such claims is important.

The 2004 Summer Conference has been an important milestone in the field of Food Safety and Microbiology.



Liong Min Tze School of Molecular Sciences, Werribee Campus, Victoria University, Melbourne, Australia.

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

An investigation on the source of Campylobacterosis. John Graham reports on his work project



DURING JULY AND AUGUST 2004 I was given the opportunity to work in the Food Microbiology branch of the Food Science Department, Queens University of Belfast. I gladly accepted, as this work experience scheme would be a great way of learning and applying the necessary technical skills to allow me to compete in the job market.

The project I was assigned, aimed to discover whether there was a link between outbreaks of *Campylobacter* in humans and food poisoning from poultry. In order to do this I would compare strains isolated from poultry with strains isolated from clinical outbreaks of *Campylobacter* from humans.

Campylobacter, in particular *C. coli* and *C. jejuni* is one of the most common causes of foodborne illness in Britain. *Campylobacter* takes the form of abdominal cramps, profuse diarrhoea and sometimes vomiting and fever. As most patients usually recover without treatment after several days, many cases are not reported. Outbreaks are rare so it is therefore a less well known bacterium than *Salmonella*.

The samples I analysed consisted of 100 Spanish strains; this was because staff in the laboratory had compiled a database to show *Campylobacter* variation between countries and my samples were therefore to be compared with results from previous work. Of the 100 strains, 50 were isolated from poultry and 50 from human clinical cases. All

cultures arrived on swabs, however due to their sensitive microaerophilic nature some samples could not be revived on blood agar.

The main aim of my investigation was to highlight links (if any) between chicken and human *Campylobacter* using AFLP (Amplified Fragment Length Polymorphism), a genetic fingerprinting assay. Bionumerics software was used to analyse the AFLP profiles. This software clusters isolates into groups according to their genetic banding pattern. A value of 90% profile homology was applied to indicate an identical sample.

Other methods were also used to verify speciation, including a multiplex PCR-based assay, which could determine the genus and species of the bacterium isolated and could also detect bacteria of similar genus i.e. *Arcobacter*. Another identification test I used, was the phenotypic identification kit "MAST ID". This consisted of three tests: indoxyl acetate; urease and hippurate hydrolysis to identify *Campylobacter* isolates. These tests were used to confirm the Spanish speciation and that of my AFLP results.

After analysis of the samples I found that AFLP divided the *Campylobacter* strains into two distinct clusters, one consisting of only *C. coli* and the other *C. jejuni*. Both clusters contained sub clusters, however the *C. coli* isolates showed a genetic homology >70% whilst *C. jejuni* isolates were more loosely clustered showing homology >49%. This result may be due to the fact that there were only 15 *C. coli* isolates as opposed to 67 *C. jejuni* isolates.

No similarity between chicken and human isolates was observed. A similarity was apparent between *C. coli* strains with the three human strains segregating completely from twelve food isolates at 70%. This showed that no human infection was apparent from *C. coli* in chickens, however to verify such a result, a larger number of *C. coli* isolates should be collected and tested.

C. jejuni showed a similar result in the majority of cases, with food isolates segregating from poultry isolates, however four out of the five *C. jejuni* sub-clusters showed some level

of similarity between food and poultry strains. Two clusters showed one human and one poultry isolate, grouping at 93% and 86% similarity respectively. This indicates that one particular clinical *C. jejuni* isolate was identical to one isolated from a chicken, whilst the other pair of isolates also show very high similarity. All other isolates indication of similarity between food and poultry types clustered <81%.

These results highlight that contaminated poultry is a potential cause of campylobacteriosis in humans. Despite only one pair in 82 *Campylobacter* isolates being identical (i.e. having >90% similarity), this is a reasonably good result as *Campylobacter* have a non-clonal nature, wide genetic diversity, and they may change their genetic structure to survive in a different host.

Overall I was pleased with the outcome of my investigation and it generated ideas for further analysis, such as whether the strains analysed are pathogenic, non-pathogenic or carriers for virulence factors. This would give us further insight into the cause of campylobacteriosis.

I would like to thank my supervisors Lynn Moran and Robert Madden and all other staff involved at the Food Science Division for their friendliness and help over my ten-week project. I am grateful to SfAM for the opportunity to work in the field of microbiological research, as it gave me an invaluable first-hand insight into the work and procedures carried out in a research lab; all of which I found both enjoyable and challenging.

John Graham

Development of an in vitro model of anaerobic biofilm growth of *Pseudomonas aeruginosa*

BEFORE ENTERING THE final year of my Biomedical Sciences degree at the University of the West of England, Bristol, I was eager to gain laboratory experience during the summer vacation. The SfAM 'Students into Work' Grant gave me the opportunity to work in a Microbiology research laboratory for seven weeks on a subject area that was completely new to me. The aim of the project was to study



biofilm growth of *Pseudomonas aeruginosa* and look at the effect of growing this microorganism under low-oxygen conditions.

Anaerobic growth of *P. aeruginosa* in biofilms is of interest because it has recently been found that *P. aeruginosa* grows in this way in the lungs of cystic fibrosis (CF) patients. Cystic fibrosis is a genetically inherited disease, characterized by faulty ion transport in the epithelial membranes of affected tissues. It can result from a variety of mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The main clinical problem for CF patients is the deterioration of pulmonary function. Faulty CFTR function leads to abnormal fluid secretion and in CF airways mucus secretions are abnormally thick. This mucus becomes a breeding ground for opportunistic pathogens such as *P. aeruginosa*, *Staphylococcus aureus* and *Haemophilus influenzae* amongst others, as well as some true pathogens (e.g. *Mycobacterium* spp.). However, with the progression of CF airway disease, *P. aeruginosa* predominates and it is believed to grow in the form of a biofilm.

Bacteria growing as biofilms are much more difficult to control or eradicate with antimicrobials than their free-floating counterparts. Once *P. aeruginosa* establishes itself in the CF airway, it seems impossible to eradicate. Approximately 90% of CF deaths are due to *P. aeruginosa*-related infections.

P. aeruginosa is able to grow anaerobically by denitrification (which requires nitrate as an alternative electron acceptor) and by fermentation of arginine. As many antimicrobials require the presence of oxygen for their activity (e.g. the aminoglycosides, such as

tobramycin and gentamicin) and other agents have reduced activity when there is no oxygen present, then it is important to look into new ways of treating *P. aeruginosa* infections.

The first objective of this project was to become familiar with the Sorbarod *in vitro* biofilm culture system, using it to grow biofilms of *P. aeruginosa* under aerobic conditions. *P. aeruginosa* PAO1 was grown in an iron-limited, chemically defined growth medium (CDM) which contains a nitrate source (KNO₃) necessary for anaerobic respiration. All biofilms were continuously perfused with aerated CDM and samples of eluted medium and cells (eluate) were taken regularly for viable counting. Steady-state biofilms could be established after 24 hours of culture and usually eluate cell numbers were followed over the course of three or four days. The overall biofilm population was also determined by the resuspension of biofilm cells and viable counting, at the end of each experimental run.

The second objective was to modify the system in order to enable it to be run under low oxygen or fully anaerobic conditions. The units housing the biofilm apparatus were modified and a gas mixture of N₂ and CO₂ was introduced into the system whilst perfusion with non-aerated CDM continued. As before, viable counts were followed over the course of several days and compared to the data acquired from running the system aerobically. The system was also easily modified to allow perfusion of an antimicrobial agent through the biofilms and some preliminary work was carried out to compare drug efficacy under aerobic and reduced-oxygen conditions.

Working in a laboratory at UWE Bristol has given me valuable practical experience in Microbiology, to add to the experience I had already gained working on an Immunology project in a laboratory in Malawi. The week-to-week running of the project required a large amount of forward planning and preparation and I gained much confidence from the experience. I sincerely thank my project supervisor, Dr Shona Nelson, and the entire Microbiology staff for their support, especially Colin MacKenzie for his help with setting up the modified Sorbarod units. Finally, many thanks to SfAM for their sponsorship.

Mukanthu Nyirenda

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.

The President's Fund ▼

The Application of 'Ecotoxicogenomics' in Ecotoxicology

ECOTOXICOLOGY encompasses the fields of ecology and toxicology and is concerned with protecting all environmental areas from adverse effects by new and existing synthetic chemicals. Ecotoxicology attempts to estimate where these chemicals go when they reach an environment (their fate), and what ecological impacts they have when they get there (their effect). Genomic array technology is a new, powerful tool that is being used in the field of ecotoxicology, which may assist in our understanding of the fate and effect of toxicants on different environments.

The term genomics encompasses the sequencing and organisation of whole genomes, however, it is also used to collectively describe new '-omics' technologies (transcriptomics, proteomics & metabolomics). In an organism, the measurement of mRNA levels provides insight into the genetic information that is being transcribed, this is known as 'transcriptomics' or functional genomics. The study of protein expression ('proteomics') yields information about the transcribed sequences end up as functional units within the cell. However, 'metabolomics' or 'metabonomics' is the measurement of metabolites, which provide information about the way functional proteins act to produce energy and process materials (Phelps *et al.*, 2002).

In a human context, gene expression profiling has previously been used to assess the response of toxicants that affect humans (Kramer *et al.*, 2004) and this technology was termed 'toxicogenomics' (Iannaccone, 2001). As the name suggests, this technology encompasses genomics together with mammalian toxicology. From this, Snape *et al.*, (2004) recently proposed the term 'Ecotoxicogenomics' which describes the incorporation of new genomic technologies (transcriptomics, proteomics and metabolomics) into environmental studies. The authors go on to define ecotoxicogenomics as 'the study of gene and protein expression in non-target organisms that are important in responses to environmental toxicant exposures.'

The power of transcriptomics is the ability to study the simultaneous expression of thousands of genes, in an attempt to understand the phenotype of an organism. The methodology involves the use of microarrays which take the form of glass slides, plastic, silicon, nylon or nitrocellulose supports of differing size and shape, onto which thousands of discrete spots of nucleic acid are spotted (in the form of cDNA clones, PCR products or oligonucleotides). Each spot corresponds to a specific gene. A typical ecotoxicology transcriptomics experiment may involve:

- 1) Growth of cultures under various conditions or collection of environmentally perturbed samples, plus untreated controls.
- 2) Harvesting of cells/biomass and extraction of total ribonucleic acid (RNA) from each sample.
- 3) Generation of radioactively- or fluorescently-labelled copy deoxyribonucleic acid (cDNA) from RNA samples
- 4) Hybridisation (binding) of labelled cDNA samples to microarrays
- 5) Imaging and quantitation of microarray expression patterns, using imaging equipment and software.
- 6) Analysis of array expression patterns, using expression software e.g. GeneSpring™.

In ecotoxicogenomic studies, the RNA is obtained from toxicant-exposed tissues, biomass, effluents or cells. By comparing toxicant challenged genomic profiles with controls, we can attempt to identify unique toxicant induced signatures.

Replicate experiments are essential in all scientific areas in order to perform thorough statistical analysis. However, reproducibility is an often overlooked issue within microarray data, but it is vital to validate gene expression profiles and to support, with confidence, any biological functions in response to stressors that the gene array results may indicate. Continued improvements in statistical analysis and bioinformatics software are needed to facilitate the enormous data mining challenge that array results present.

Data quality assurance of microarray experiments is made difficult by the amount of data measurements produced and by the large number of potential sources of variation that may be introduced throughout the process. Potential sources include steps in sample collection and preparation (RNA extractions, labelling and amplification), elements of the hybridisation process (hybridisation solution, temperature, time and extent of cross-hybridisation), elements of the commercial array printing process (purity of probe solution, print head status, robot movement, differences between batches), elements of scanning (length of exposure) and elements of spot identification and quantification (Hess *et al.*, 2001). Therefore, standardisation is imperative if microarray experiments are to be compared. A well planned experimental design and conformation to MIAME guidelines will help reduce the amount of variation introduced into array experiments, whilst also ensuring that the experiments are comparable and reproducible. The Minimum Information About a Microarray Experiment (MIAME) documents are a set of guidelines compiled by Brazma *et al.*, 2001 that aim to outline the minimum information required to interpret unambiguously and potentially reproduce and verify an array based gene expression profiling experiment. A specific set of guidelines for use during toxicogenomic studies are also available. The MIAME/Tox document is based on the original MIAME document produced by the microarray gene expression database (MGED) Society, but it has been adapted to suit toxicogenomics data capture and exchange. MIAME/Tox aims to define the core of information that is common to most toxicogenomic experiments.

After standardisation, experimentation and data-mining, a general limitation of expression profiling is that it is often difficult to relate changes in gene expression to whole organism responses that have biological significance. However, continued use of gene expression profiling by biologists together with data analysis support from bioinformaticians and statisticians is aiming to rectify this. This issue must be addressed prior to the wider integration of ecotoxicogenomics into ecotoxicology. Finally, I would like to thank SfAM for awarding me a President's Fund Grant which gave me the opportunity to further my work in Microbiology.

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Angela Brown

Fluorescent *in situ* hybridisation detection of bacteria using molecular beacons

MOLECULAR BEACONS ARE A relatively modern type of nucleic acid probe that have the ability to detect specific nucleic acids in homogenous solutions (Tyagi & Kramer, 1996).

They consist of an oligonucleotide flanked by a fluorescent moiety and a quenching moiety; both attached via linker molecules. The oligonucleotide contains the specific sequence located centrally with self-complementary regions at either end, thus the molecule forms a stem-loop structure with the fluorophore and quencher being held in close proximity. If the fluorophore is excited by light of the appropriate wavelength, whilst in the closed state, the quencher will absorb the energy emitted by the

fluorophore and dissipate it as heat. In the presence of complementary target, the loop region of the beacon will bind to the target sequence. The beacon-target hybrid is longer and more stable than the complementary stem, thus the beacon will undergo a spontaneous conformational reorganisation that will force the stem apart spatially separating the fluorophore and quencher, and fluorescence will be restored. Molecular beacon fluorescence will also increase when the beacon stem is denatured, such as when exposed to high temperatures or pH.

Fluorescent *in situ* hybridisation (FISH) is a culture-independent method of identifying and enumerating specific micro-organisms (Amann *et al.*, 1990). It is a key technique in microbial ecology, and relies on a fluorescently-labelled nucleotide probe hybridising to its specific target site within the ribosomal RNA (rRNA) molecule. Samples are analysed by fluorescence microscopy and/or flow cytometry, with flow cytometry being the tool of choice for a high rate of sample throughput where each cell is individually analysed. However, the sensitivity of FISH using rRNA-targeted oligonucleotide probes has always been a problem. Amongst several issues that compromise the technique, the number of ribosomes per individual cell is one of the more important factors. Cellular rRNA content has been proved to be directly correlated to the growth rate of microbial cells, indeed 10^4 to 10^5 copies of 5s, 16s and 23s rRNA are estimated to be present in a single *E. coli* cell from an exponential phase culture. Using standard rRNA FISH with exponential phase cultures, target cells and non-target cells can easily be discriminated. However, sensitivity problems start to arise when small, slowly growing or stationary phase cells are analysed due to low cellular rRNA content. Low fluorescence signals cause target cells to be mistaken for non-target cells. This is a very significant problem when it comes to analysing the relative abundance of specific microbes in the mixed populations of oligotrophic environmental samples. Much effort has been directed into increasing the sensitivity of FISH over recent years allowing the analysis of more types of environmental sample. Recently, DNA and PNA molecular beacons were successfully used to replace mono-labelled linear probes for FISH with lab cultures of bacteria (Xi *et al.*, 2003). In this work, a

DNA molecular beacon was designed targeting numerous *Pseudomonas* species, the specific sequence of the beacon being a slight modification of a previously published *Pseudomonas* probe (Braunhowland *et al.*, 1993). A direct comparison of the fluorescent intensities from exponential-phase target (*Pseudomonas putida*) and non-target (*E. coli*) cells hybridised with the molecular beacon and linear probe was made. Hybridisation conditions for both types of probe were optimised regarding hybridisation solution composition, temperature and probe concentration. The fluorescence intensities from *P. putida* cells hybridised with the beacon and linear probe were similar. However, *E. coli* cells that hybridised with the beacon gave approximately half the fluorescence of *E. coli* cells hybridised with the linear probe, thus giving the beacon approximately double the signal-to-noise ratio of the linear probe. The removal of unbound probe by washing decreased the background fluorescence for cells hybridised with the linear probe, to a level similar to that seen when hybridised with the molecular beacon. However, washing cells for flow cytometric analysis generally involves a centrifugation step which leads to cell loss and the adherence of cells to each other, or non-target cells or debris. The decrease in background fluorescence from the molecular beacon is thought to be a result of the beacon being "dark" when in a non-hybridised state, the linear probe however is of equal fluorescence whether hybridised or unhybridised.

A variety of environmental samples (soil, river water and activated sludge) were spiked with *P. putida* cells, FISH with molecular beacons and linear probes were then used to detect the added cells. Hybridisations with beacons gave a more distinct separation of target cells from non-target cells and debris present in the sample. When hybridised with non-spiked environmental samples of activated sludge and river water, the molecular beacon enabled detection of more cells in every sample analysed than that detected with the linear probe. Fluorescent CFU counts were made on environmental samples using *Pseudomonas* selective agar (Oxoid) to estimate the number of *Pseudomonas* present. Cells detected by FISH reflected the number of cells detected by culture-based methods.

These results suggest that the increase in signal-to-noise ratio of the beacon

enables more target cells to be detected in environmental samples. The use of molecular beacons in FISH represents another advancement in present technologies, however it should be noted that the most appropriate type of FISH protocol to use will depend on many factors such as target cell characteristics and analysis method.

I would like to thank SFAM for awarding me a grant to help present this research at the recent ISME10 meeting in Cancun, Mexico.

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Jeremy Lenaerts

Cereal Fermentation: an asset to developing African countries

THE MAJOR CEREALS, maize (*Zea mays*), millet (*Pennisetum typhoideum*), rice (*Oryza sativum*) sorghum (*Sorghum vulgare*) and wheat (*Triticum aestivum*) are widely cultivated in many African countries contributing up to 70% of world production. Cereals are responsible for as much as 77% of total calorific consumption and contribute significantly to the dietary protein intake of people in African countries. However,

they are highly perishable in the fresh state due to the warm tropical climate, which facilitates microbial deterioration. The facilities for storage and preservation of are grossly inadequate for the huge amount produced annually. Much is sun dried and fermented to extend the shelf life.

A great majority of traditional cereal-based foods consumed in Africa are prepared by natural or spontaneous fermentation. Fermented foods can be described as palatable and wholesome foods prepared from fresh or heat-treated raw materials. They have desirable attributes such as pleasant flavours, taste, and aroma with improved processing qualities. The majority are used as infant food and dietary staples for adults whilst some are used as beverages.

Fermentation is one of the oldest methods of food processing in African countries. The type of product desired dictates the pretreatment used. The dried grains are sorted, washed, steeped, milled and sieved before fermentation for cereal gruels. Dried products like bread only require milling and sieving, whilst malting prior to milling and sieving, are required for beverages. Most cereal-based products are heat-treated before consumption.

Fermented products can be categorised based on the raw materials used. This includes maize-based foods such as "Ogi" for the Yoruba speaking people in Nigeria and "kenkey" for the Ghanaians and Millet-based foods like Kunuzaki — a beverage common in northern part of Nigeria. Others are rice-based like Busa.

The metabolic activities of the microorganisms employed contribute immensely to the characteristic organoleptic properties of the fermented food. Most bacterial fermentations are carried out by lactic acid bacteria (LAB), such as *Lactococcus*, *Enterococcus*, *Carnobacterium*, *Sporolactobacillus*, *Leuconostoc*, *Lactobacillus*, *Streptococcus* and *Bacillus*. Yeasts like *Saccharomyces* bring about alcoholic fermentation while some common mold species such as *Aspergillus*, *Penicillium*, *Fusarium* and *Cladosporium* may be involved in certain products.

LAB consist of gram positive rods or cocci, which are anaerobic, microaerophilic or aerotolerant catalase-negative organisms. They produce lactic acid from degradation of sugars. They can be homofermentative or heterofermentative. Many of them are used in the commercial production

of many fermented foods. Fermentation of cereals involving LAB is a common household practice in developing African countries. However, the people may not be aware of the various benefits to their general wellbeing.

Modern means of preservation such as freezing and refrigerating are beyond the reach of many. The LAB converts the sugars into organic acids such as lactic and acetic acids, which lower the pH to a level that inhibits undesirable spoilage and pathogenic organisms. This action is facilitated by lowering of oxidation reduction potential and competition for essential nutrients. Ogi, a fermented corn product can be kept for 28 days by decanting and replacing the supernatant water. The production of other inhibitory compounds such as alcohols, hydrogen peroxide which reduces oxidation potential, diacetyl that inactivates enzymes like transketolase and is effective against gram negative bacteria and fungi, bacteriocins which inhibit essential metabolic activities of a range of bacteria — all these contribute to this antagonistic effect.

These factors invariably confer safety to the consumer as their bacteriocidal effects have been proven on many pathogenic strains of *Listeria monocytogenes*, *pathogenic E coli*, *Yersinia enterocolitica*, *Salmonella spp*, *Streptococcus*, *Staphylococcus spp* and *Shigella spp* (Motarjemi, 2002, Opere *et al.*, 2003). Bacteriocin producing strains are sometimes used as protective cultures to improve the microbial safety of foods. Nisin produced by *Lactococcus lactis* has practical application in food processing.

The use of starter cultures should be encouraged as the nature of flora involved in natural fermentation is complex and varies from crop to crop. It has been found that food poisoning organisms and coliforms are present with the LAB during this type of fermentation. Pure culture inoculation improves processing technique, predictability and consistence to ensure maintenance of product quality and confers safety to consumers.

The activities of microorganisms increase the rate of degradation of the raw materials making it possible for more nutrients to be utilised. The alpha and beta amylase of LAB converts the starch in the cereals to sugars for fermentation.

The fermentation process has been shown to lower the levels of proteinase inhibitors in cereal gruels thereby increasing the proteolytic activity of some

fermenting bacteria. This provides simple proteins, peptides and amino acids especially essential amino acids such as lysine, leucine, isoleucine, methionine and even tryptophan thus improving the protein quality of the food.

The fermenting organisms with probiotic properties aid digestion in humans through their activities on prebiotics present in the gut thereby increasing the quantity of digestible carbohydrates.

The organic acids produced by LAB in carbohydrate based foods like cereals is responsible for the sourness and tanginess relished in Ogi — a fermented corn product. Diacetyl and acetoin contribute to the desired organoleptic properties of fermented cereals. The high alcohol content of beer, pito and burukutu is valuable in traditional medicine and this is the drink of choice at important festive or traditional gatherings.

The involvement and presence of LAB in fermented foods has been found to have many beneficial roles in human health. This is a great asset to developing countries where adequate medicare is lacking in many areas. The antimicrobial activities of *Lactobacillus spp* has encouraged the use of fermented products as a simple method of treatment and prevention of gastro intestinal infections which are very common in infants (Olukoya *et al.*, 1994). In Nigeria, there is high incidence of diarrhoea and this is ranked as the second major cause of morbidity and mortality in children in the country (Alabi *et al.*, 1998). The problem is due to low levels of personal hygiene and public sanitation as well as low socio-economic status of most families, even in urban areas. Therefore, the use of fermented cereals as weaning foods offers great potential if improved food hygiene is practised.

The role of probiotics in human nutrition cannot be disassociated from the increasing awareness of the many benefits of fermented foods.

Many studies have shown that fermenting LAB has probiotic properties and some are being used on commercial bases. Their beneficial effects include provision of intestinal microbial balance, detoxification of carcinogens, assisting in lactose digestion in lactose intolerant individuals and the enhancement of immune responses. Other merits include bioaccessibility of lipids, reduction in allergenicity of foodstuffs and most importantly, control of many intestinal

disorders of microbial origin.

Current research interest in the field of biotechnology needs this valuable asset to help mankind. Resources for adequate scientific research work are lacking in many parts of developing African countries and as such concerted efforts should be made to exploit this golden gift to the human race.

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Simbo Aboaba

In-use Indicators of the Performance of Bactericidal Formulations

THE PUBLIC'S ATTENTION IS being increasingly drawn to incidences of microbial infections that are aggravated by cross contamination. These range from food-related cases concerning *Listeria monocytogenes*, *Campylobacter jejuni*, *Salomonella enteritidis* and *Escherichia coli* 0157:H7; HIV infections and the problems of antibiotic resistant bacteria in hospitals.

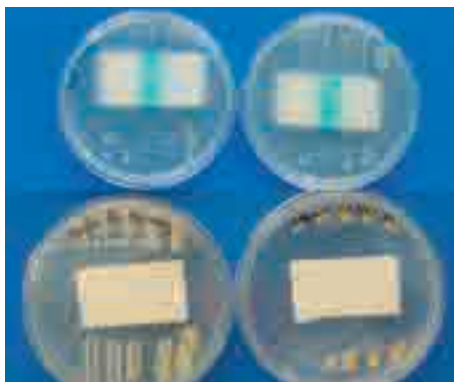
As microbiologists in particular, we applaud the calls for compliance with the basic standards of hygiene and cleanliness in catering establishments, health-care centres and in the home. But

the ten-fold increase in food poisoning over the last ten years in the UK (PHLS figures) seems to show no sign of reversing. Assurance for safe food is needed. NASA and the Pillsbury Company gave us the concept of Hazard Analysis and Critical Control Point (HACCP) in 1971 which was designed as an alternative to end-product sampling and testing. Since then HACCP has undergone a number of refinements under the auspices of bodies such as the National Advisory Committee on Microbiological Criteria for Foods and the International Commission on Microbial Specification for Foods. This went on until 1993 when Guidelines for application of HACCP were finally adopted internationally and accepted as a food safety system that could minimise or eliminate non-tariff trade barriers.

However results of research undertaken in the UK by the UK Food Standards Agency indicated that although HACCP system may be in place, a lack of knowledge and access to expert advice limits the system's effectiveness. Food manufacturers, food retailers and caterers may be aware of "hazards" and "critical control points" in their processes, but verification of effective operation of control was not happening. Effective removal of microbial load at critical points hinges on rigorous adoption of liquid sanitisers and the use of sanitiser-impregnated wipes. These wipes should meet the relevant European Standards or, for the USA, the Environment Protection Agency Registration requirements. The question is: at what point during their use will the wipes fail to meet these requirements?

This is where our project comes in! I work on an EU funded project with the acronym REAIPIC (Reliable and Emphatic Activity Indicator Products for Cross Infection Control) a title; I am reliably informed, thought up over a pint in the Blue Pig Working Men's Club, Hebden Bridge, West Yorkshire.

A major aim of the project is to produce two types of impregnated wipe capable of delivering biocidal performance to at least the requirements of the European Biocides Directive 98/8 and which incorporate reliable indicators of their performance. The first approach derives from existing technology invented back in the late 1970's; a nonwoven wipe impregnated with a robust biocidal formulation bound to which was a 'security stripe'.



We have extended the concept of a biocide-bonded indicator in such a manner that a clear message is revealed when the wipe's performance falls below the requirements of the European Biocides Directive. In essence we have developed a wipe with a stripe, which, as the stripe fades in use, reveals a visible indication that the operative should stop using the wipe. In this first approach we have relied on a composite biocide of biguanide, quaternary ammonium compound and E.D.T.A, a biocide with a broad spectrum of activity. This biocide is impregnated into the nonwoven substrate, pre-printed with a simple logo (during our earlier trials this was simply 'NO'). Then the indicator stripe is overlaid concealing the logo, bound directly to the biocide and not the wipe. Thus as the wipe is used and the biocide is deposited on the cleaned surface, the stripe fades revealing the logo.

The development of our second type of impregnated wipe has been far more challenging and has resulted in a novel system for the production of peracetic acid and hydrogen peroxide at temperatures below 4°C. An organic manganese catalyst has been utilised in formulations to generate the active biocide of peracetic acid and hydrogen peroxide. The properties of these formulations possess high levels of bactericidal, virucidal and sporicidal activity in a short contact time (< 5 minutes) at temperatures below 4°C, the effect and residue chemistry of which are environmentally and toxicologically benign. Peracetic acid has been recognised as a disinfectant for more than 50 years (Greenspan & MacKeller, 1951), as a liquid it is unstable and has a pungent odour. By 'impregnating' our formulation in to a nonwoven matrix we have overcome these inherent problems, providing both component separation and stability prior to activation. We aim to

utilise the addition of a dye which undergoes a colour change, related to a shift in pH, as our performance indicator in this particular wipe system.

By incorporating a clear and emphatic indicator of performance both in biocide formulations and within the biocide impregnated wipes, we may have addressed some of the shortcomings of the HACCP system. Clearly we still have some way to go to prove our applications in the market place, but a relatively simple technology could lead to significant reductions in microbiological infections and cross contamination.

I am grateful for the financial support from the Society for Applied Microbiology President's Fund which has enabled me to attend the 104th ASM meeting in New Orleans USA.

Christopher Hodgson

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Cytokines and Chemokines in Infectious Diseases

Various Authors: Edited by Malak Kotb and Thierry Calandra.

Humana Press 2003. ISBN 0-89603-908-0. Price \$145.00

(Source: Humana Press).

Reviewed by Adam K. A. Wright

The discovery of the cytokine Interferon in 1957 through the combined efforts of Alik Isaacs and Jean Lindenmann heralded a new and complex field. The plethora of cytokines and chemokines being characterised continues to grow and each time sheds an important light on the orchestration of the immune response.

Since the beginnings of immunology were stirred up from the 'golden era' of microbiology, the development of cytokine biology has ensured that their paths will remain tied together. It is now impossible to study the pathogenesis of infectious diseases without a good knowledge of the human (Immune) response to infection and colonisation and this includes cytokine responses.

As with many biological processes, separating material into sections to make it more palatable for digestion is a difficult process. Many textbooks dealing with micro-organisms, for example, have either opted for a taxonomical approach or one that perhaps takes a medical viewpoint, dividing the information into organ systems.

Malak Kotb and Thierry Calandra (Editors) have taken the former approach, writing a book that represents the interface of immunology and microbiology. An excellent introduction by Charles Dinarello sets the scene, filling a gap in this complex field. He briefly discusses the very cytokines that are relevant to infectious disease then the advantages and disadvantages of using knockout mouse models, before moving on to polymicrobial sepsis as an experimental model. Sepsis becomes a recurrent theme throughout the book and perhaps for good reason. Sepsis and its resultant syndrome still demonstrate mortality rates (30-50%) that have not changed much in the preceding decades, despite the best supportive care available.

The pressure to reduce this rate has never been greater in light of an increasing number of hospital admissions with sepsis. In contrast, advancements made in anti-cytokine therapy (e.g. in rheumatoid arthritis), have allowed the development of an additional dimension towards treatment.

The unappealing appearance of the front cover, and bland diagrams should not put people off. Inside there is a large volume of work that has methodically covered an extensive range of experimental, and sometimes conflicting, interesting data.

The editors approach to this book has been to focus on the infectious disease itself then discuss the major cytokines and chemokines involved. Particularly in chapters dealing with bacteria, the tendency has been to list some of the major cytokines or chemokines as subheadings and briefly discuss some of the experimental work in certain models of infection. Whilst this may make it difficult to understand overall concepts, those who wish to see the role of a particular cytokine can do so with ease, through looking up the relevant sub-heading.

The first section deals with host susceptibility to infection. At first glance a description of Leptin, which is a class I cytokine, appears an odd choice to begin this section on susceptibility. One of the major factors however relies on a weakened immune response, which can and is commonly brought about by starvation and in mouse signalled by low leptin levels. Thus an 'environmental' approach is complemented well by the second chapter which looks at cytokine gene polymorphisms.

From this point on, a taxonomic approach is evident encompassing first Gram-negative and positive bacteria, mycobacteria, miscellaneous bacteria (focusing on Lyme arthritis and pneumonia), fungal (*Aspergillus fumigatus*; *Candida albicans*), parasitic (Leishmaniasis; *Toxoplasma gondii*) and finally viral infections (HIV; Hepatitis). There are generally just a few chapters in each of the aforementioned sections and because of this only certain infections or aspects of an infection are discussed. Better (more memorable) figures of the mechanisms illustrated throughout this book can be found elsewhere, as here they are represented with little imagination, being black and white and with no use of illustration. This is perhaps

then not the place to look if you wish to compare and contrast a particular cytokine response between two infectious diseases. It is also not a book that undergraduates (and some postgraduates) will want to pick up to unravel the complex association of infection and the cytokine response. However if one's work is associated with a particular chapter topic or sepsis, then its comprehensive nature provides an excellent starting point.

The final chapters detail cytokines as therapeutic agents (IFN- γ ; IL-2; G-CSF and GM-CSF) and anti-cytokine based therapies in septic shock, streptococcal toxic shock syndrome and necrotizing fasciitis. These latter two chapters indicate that although there have been many setbacks, particularly with using cytokines as therapeutic agents, there remains areas that benefits from anti-cytokine intervention and thus hope.

Functional Genomics-A practical Approach

Edited by Stephen Hunt & Frederick Livesey. Price: £ 45.00.

ISBN: 0-19-963774-1. 12 October 2000, reprinted 2001, 2002.

Oxford University Press.

Reviewed by Efstathios Giotis

There have been many books already published, covering theoretical as well as technical aspects of functional genomics. The title of this book is not misleading. As part of the 'Practical Approach' series of textbooks it is designed to provide the reader with purely practical information on the key methods most widely used in functional genomics and in particular, in the area of gene and protein expression profiling.

The book is quite short and gets straight to the point with chapters which may be read independently from each other. In the brief, well-prepared introductory chapter the editors review the current status in functional genomics, describing the scope of the text and discussing future developments in gene expression profiling. The subsequent chapters commence with the aims of the chapter and contain a brief description of the theoretical background underlying the method, a step-by-step outline of the technique presented, followed by

technical considerations. The topics covered include large-scale approaches such as cDNA microarrays, serial analysis of gene expression (SAGE), differential display and suppression subtraction hybridisation as well as proteomics methods such as the two-dimensional gel electrophoresis of proteins, mass spectroscopy and others. There are over 100 protocols, which could easily be photocopied for everyday use, from authors who are either developers of a particular method or expert users.

It is necessary to emphasise that the editors selected an informative approach, providing useful information concerning cost, technical requirements, limitations and even the ease of use of the technical approaches described, in order to help the new researcher to apply functional genomics methods in their area of interest. Troubleshooting sections and a description of the common problems encountered are also helpful as well they enable the reader to make a quick comparison of the different technologies. It is also interesting that the authors quite often question the usefulness and the efficiency of certain methods. The data covered is of high scientific quality, clearly explained and well referenced. A considerable amount of data is presented in the form of useful and practical tables and schemes in black and white colours. The text is well designed and includes a supplier's list. The subject index at the end of the book is a very convenient tool for the reader who requires a fast research on a topic of interest.

The aim of this textbook was to gather a respectable number of current functional genomics methodologies in one volume with detailed, practical descriptions. However, it could perhaps have included some more theoretical background to assist those readers who are perhaps less familiar with some of the methodologies described in several chapters. Given that the book was edited back in 2000 and considering the speed of scientific advances, additional relevant chapters would be a valuable addition to future editions of the text.

Overall, this edition, unlike many other similar publications, is reader friendly and offers a good starting point for someone who is interested in the field from a practical point of view. That is why this book is an indispensable laboratory manual for researchers and postgraduate students engaged in functional genomics research or related disciplines.



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:

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The website is the best source of detailed information on the Society and its 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.

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- Online access to the Society's three journals OR hard copies of the journals.
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- Up to three members of company staff attending Society meetings at members' rate. (This means a 50% discount on non member registration rate).

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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.

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.

Special interests

The Society has six very active Interest Groups:

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