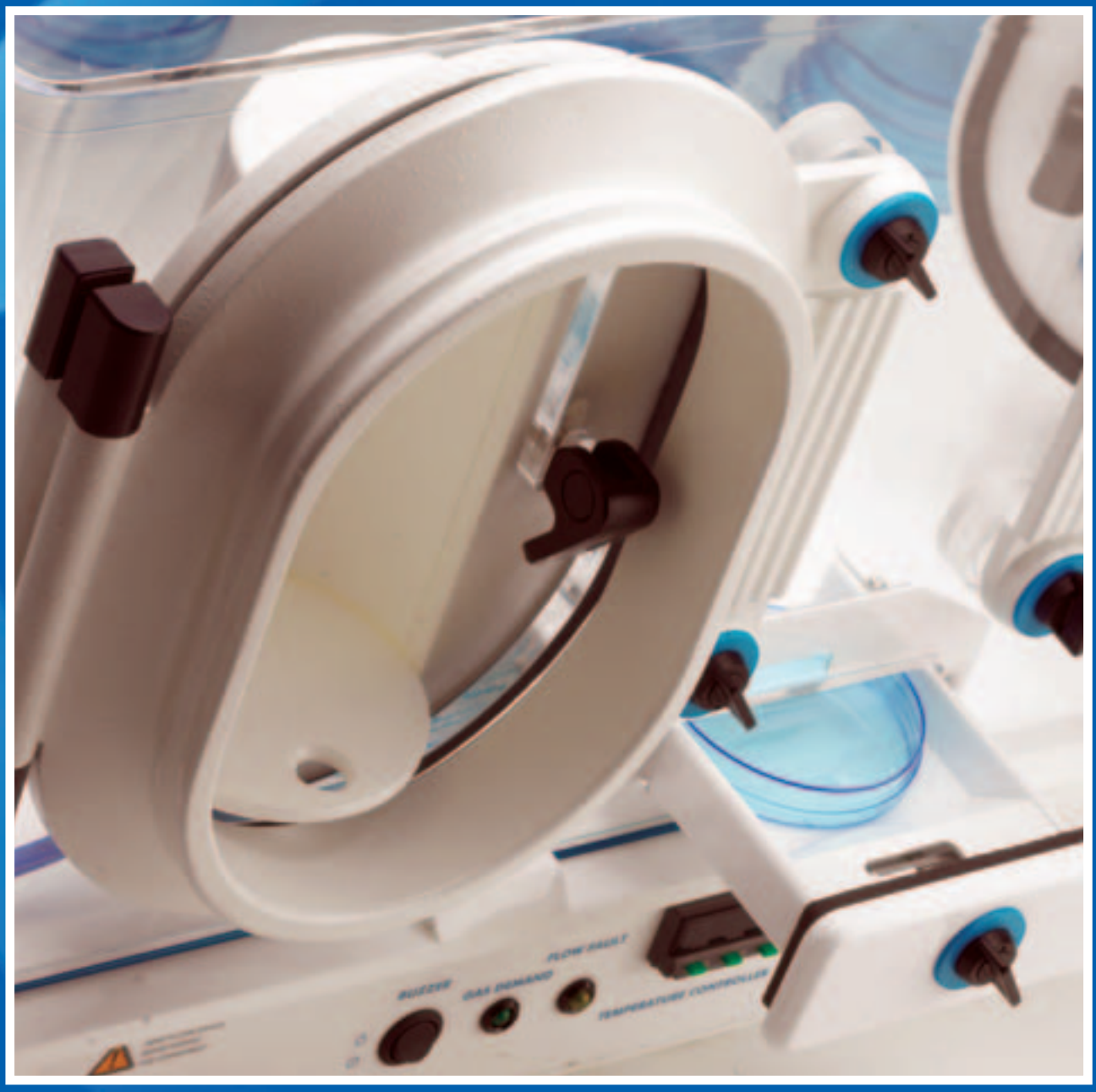
Microbiologist
The magazine of the Society for Applied Microbiology ■ June 2007 ■ Vol 8 No 2 ISSN 1479-2699

Biofuels

Stuart Shales and Stephen Smith explore the complex issues of Biofuels from a microbiologist's perspective

excellence in microbiology

don whitley scientific



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Microbiologist the magazine of the Society for Applied Microbiology

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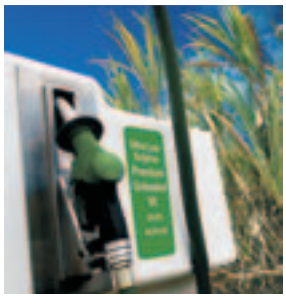
editorial

Lucy Harper talks about the environment and biofuels



With the environment so high on the political agenda everyone is aware of environmental issues such as climate change, carbon emissions and recycling. I'm sure we've all considered our carbon footprint in terms of the amount and modes of transport we take, how much waste we produce and the amount of energy we use. Factors involving transport which affect our carbon footprint, include whether or not we travel by public transport and if not, the number of cars we own and the frequency and mileage we travel.

Only this morning, I watched a discussion on the BBC Breakfast news regarding the widening of the first UK motorway, the M1. A large-scale road-widening scheme is underway to widen the M1 from three to four lanes on some of its busier stretches. The aim of this scheme is to ease traffic congestion, however, some argue that this will only temporarily ease the problem. Eventually the second law of thermodynamics may be mimicked, and more cars will fill the roads resulting in similar levels of congestion, but along four lanes of motorway instead of three. Ploughing money into an improved public transport system is thought by some to be a more favourable approach to encourage people away from their cars. In theory this would help not only the congestion problem, but also the environmental impact of travel. However, if travelling by public transport is impossible with the current infrastructure, how can we try to limit the environmental impact of our travel?

One of the possibilities is the introduction of biofuels, that is: "Fuel produced from renewable resources, especially plant biomass, vegetable oils, and treated municipal and industrial wastes. Biofuels are considered neutral with respect to the emission of carbon dioxide because the carbon dioxide given off by burning them is balanced by the carbon dioxide absorbed by the plants that are grown to produce them. The use of biofuels as an additive to petroleum-based fuels can also result in cleaner burning with less emission of carbon monoxide and particulates" (American Heritage Science Dictionary).

The use of biofuels as a replacement for fossil fuels, is a complex issue fraught with economical and political concerns such as, do we have the capacity to produce the crops required for the commercial production of biofuels? Should our land be put to such use or should farmers concentrate on food crop production? Should biofuels be used for transport purposes or are they better utilised in the production of combined heat and power (CHP) (http://news.bbc.co.uk/1/hi/sci/tech/6636467.stm)? If you hold particular views on this sometimes contentious subject and you'd like to share them with us here at SfAM then please do send them to me, the Editor at lucy@sfam.org.uk.

The microbiological perspective of biofuel production forms the theme for this issue of the *Microbiologist*. Our first feature article concentrates on the microbiologists' view of biodiesel production (page 26). The production of bioethanol through the fermentation of yeast forms the basis of the second feature article (page 30). While researching this editorial I found out that Lotus have designed a version of their 'Exige' car with an engine that runs on E85 bioethanol — 85% bioethanol, 15% petrol (http://www.reuk/Lotus-Biofuel-Supercar.htm).

This is only a test vehicle at present, but results look promising. Not only is this the most powerful road version of the Exige, but using bioethanol results in as much as 70% less net emissions of carbon dioxide. There are some drawbacks however, including finding a petrol station which sells bioethanol fuel.

contribute

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

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

Lucy Harper

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

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

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benefits

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

- Substantially reduced rates for attendance at Society meetings and conferences
- Access to the members areas of the Society website
- Many generous grants and awards
- FREE access to three acclaimed journals

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

GRANTS & AWARDS: Many grants, awards and prizes are available to members including the W H Pierce Memorial Prize and Prizes for Student Oral Presentations and Posters at the Summer Conference. In addition to these substantial awards, the Society has funds to assist members in their careers as microbiologists. These include The President's Fund, Conference Studentships, Sponsored 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 PDF application forms available to download from the members area.

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

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, Environmental Microbiology* and *Microbial Biotechnology.* 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.

MEETINGS: We hold two annual meetings. The January Meeting is a one-day meeting with parallel sessions on topical subjects. 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.

WEBSITE: The website is the best source of detailed information on the Society and it's many activities. It has fully interactive membership areas where you can book your place at Society meetings, find exclusive *Sf*AM documentation and much more.

membership options

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

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

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

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

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

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

■ **Retirement membership** is available to Full Members once they have retired from their employment and have completed at least 20 years membership of the Society. Retired members are entitled to all the benefits of Full Membership except access to *Journal of Applied Microbiology*, *Letters in Applied Microbiology* and *Environmental Microbiology.*

JOIN US!

You can apply for membership on, or offline. To apply offline, please contact the Membership Co-ordinator, Julie Wright on **01234 326846**, or email **julie@sfam.org.uk.** Alternatively, write to her at:

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

www.sfam.org.uk

president's column

Yes — it really could be you!

Margaret Patterson reviews the many grants and awards available to Society members. The web site (www.sfam.org.uk/grants.php) has full details and application forms.

The summer season is almost here and we are all being tempted by a variety of conferences and meetings planned for the rest of the year, yet it seems to be increasingly difficult to attend these events.

This is partly due to the problem of getting time away from the lab or office, and partly the problem of finding sufficient funding to meet the costs of travel and accommodation as well as registration fees. It therefore seems timely for me to remind all Full, Student and Honorary members of the Society that they are eligible to apply for a wide range of grants and awards. This includes the President's Fund which is specifically to assist members with funding to attend meetings and conferences. Indeed, this is an important benefit of membership of S*f*AM, but unfortunately not many of our members take advantage of this superb award. In fact, the levels of funding set aside for the grants and awards has been increased in 2007 so there is more reason than ever to consider applying for an award or grant.

Here is a summary of what we currently offer.

The President's Fund

This is available to all Full, Student and Honorary members who require funding to assist them in attending any relevant conference (not just those associated with S*f*AM). The maximum value of the award is £1,000. You have to be a member for least one subscription year and be a fully paid up member at the time of the application. Successful applicants can re-apply for another President Fund every two years. There is no closing date for this award.

Student's into Work scheme

The Society offers Full Members the opportunity to give undergraduate students (who are studying microbiology as part of their degree programme), or those who have recently graduated, the chance to gain work experience for up to 10 weeks. This award is also available to those who want some 'refresher' experience before they return to work, for example after a career break. The grant gives £160/week to the worker and up to £50 to the employer for consumables (see page 45).

Sponsored Lecture Grant

This is available to Members who are also members of other groups, clubs or societies who would like to invite a guest speaker to talk on any aspect of applied microbiology but cannot afford to. The grant covers up to £500 for speaker's travel and subsistence costs.

Conference Studentship Grant

This is aimed at student members who would like to attend a Society meeting but cannot afford the fees or travelling expenses. The award covers registration, accommodation, meals and modest travel costs. Preference is given to students who are presenting a paper or poster at the meeting.

Regional meetings Grant

This is a joint initiative between S*f*AM and SGM to promote microbiology in all geographical areas where we have members. Grants of up to £2,000 are available to support one-day microbiology meetings. Both Societies will promote the meeting on their websites and in their members magazines.

Endangered culture collection

This is available to anyone who has been a full member for at least three years, or to another individual if the application is supported by an existing S*f*AM member of at least three years. The aim is for the Society to assist UK experts to visit endangered culture collections either *in situ* or in the country of origin. It is also to provide short-term relief and pay for relocation of cultures to a willing recipient when collections cannot be maintained *in situ*. The total value of the award is £5,000.

Overseas Development Award

This is available to anyone who has been a full member for at least three years, or to another individual whose application is supported by an existing S*f*AM member of at least three years. If it is for a non-member, they must reside in a developing country or in

Eastern Europe and require assistance to visit a laboratory for training. The value of the award is up to £5,000.

Laboratory fellowship

This Fellowship was launched in 2006 and is available to Full members who work in private, academic or government laboratories. Grants of up to £1,000 per week (to cover travel, accommodation and consumable costs in the host laboratory) for up to four weeks are available to support staff wanting to visit another laboratory to be trained in a new technique or learn new skills.

New lecturer Grant — a new award for 2007

This grant is available for newly appointed lecturers or researchers in their first academic post in Higher Education, to pump-prime their microbiological research. Applicants should currently be Full Members of SfAM and have been a Full member for at least two years prior to their application. Funding of up to £10,000 is available for a successful applicant.

Applicants can apply for this grant within three years of the start date of their first appointment. Applicants need not necessarily be in a 'Microbiology' lecturer post but their research field and proposed project should be in the field of Applied Microbiology. This grant is not available to Research Fellows or Post Doctoral Researchers. The closing date for the award will be in September each year and the first awards will be made in the autumn of 2007 (for more details see page 10).

I have received some feedback which suggests that many people think that the awards are very competitive and the chance of being successful is low. However, I would again urge all members to submit an application for funding if they feel they could benefit. If you are a Full or Student member, you have an equal right to be considered for funding and the increased levels of funding we have allocated to awards means that there is a good chance that reasonable applications, which meet the criteria, will be successful. The application forms are not difficult to complete and I would encourage you all to consider making an application. If you do, it really could be you who is successful in getting an award!

Dr Margaret Patterson
President of the Society

ceo's column

Philip Wheat reports on the latest developments within the Society

I am writing this column after the one day Spring meeting the Society held at Manchester Metropolitan University. I have now had a chance to reflect on the day and by all measurements the meeting can be described as very successful (see full report page 21). We are already turning our attention to planning next years Spring event. The intention will be to move the meeting around venues in different areas of the United Kingdom. The most likely venue for next years' meeting will be Aston University in Birmingham.

Whilst on the topic of SfAM meetings, the preparations for our 2008 Winter meeting (Wednesday 9 January 2008, Royal Society, London) are nearly complete. Similar to recent Winter meetings, once again there will be concurrent sessions on offer. These will be *'Quality Control and Accreditation in Applied Microbiology and Research'* and *'Microbiology of Alcoholic Beverages.'* I am sure the meeting will be very popular so look out for full details in forthcoming issues of *Microbiologist* and on the website. Early booking for this meeting is recommended as places are limited.

Details of the SfAM 2007 Summer Conference can be found on page 23 of this issue. Once again this meeting is proving to be popular and a number of delegates have already reserved their place. The topic of the meeting, *Microbiology of Fresh Produce*, could not be more relevant with the recent US FDA report warning of a serious foodborne *E.coli* O157:H7 outbreak from fresh Spinach. As well as a full scientific programme there is an excellent series of social events on offer, including a comprehensive Trade Exhibition, dinner at the Millenium Stadium (an optional tour of the stadium prior to the dinner is also available) and drinks and canapés at the imposing Museum of Wales. There will also be a more informal quiz night at the conference hotel (Park Plaza, Cardiff). Once again places for this meeting are limited so early booking is highly recommended.

We have previously reported that the Society is seeking to become Incorporated but remain a registered charity. I can report that this is going ahead, but progress has been slower than anticipated. Several drafts of the proposed Articles and Memorandum of Association produced by the Society's solicitor have been reviewed by myself and the Trustees, and the latest drafts have just been returned with further comments. I am now hopeful that by this years' Annual General Meeting (4 July 2007) I will be in a position to report that these governing documents have been lodged with both Companies House and the Charity Commission so that the process of incorporation can be finalised.

As I have previously reported, the Society will be attending a number of events over the summer months. If you cannot make it to the SfAM 2007 Summer Conference I hope to meet as many members as possible at these meetings.

Philp Wheat
Chief Executive Officer

membership matters

Come and see us in **2007!**

Once again in 2007 S*f*AM will exhibit at several international conferences. We will be attending the *American Society for Microbiology* meeting in Toronto in May. This will be followed with two exhibitions in July (IAFP, Orlando, 8 - 10 July and *International Food Technology*, Chicago, 29 -31 July). In addition to attending these three meetings we will also attend and exhibit at the *14th International Workshop on Campylobacter, Helicobacter and Related Organisms* to be held in Rotterdam, The Netherlands, 2-5 September 2007 and the *13th European Congress on Biotechnology*, Barcelona, Spain, 16-19 September 2007.

History Book

Have you requested your copy of the History of the Society book? We still have a few remaining copies for any members who wish to get hold of one. Write to: communications @sfam.org.uk or phone us on **+44(0)1234 326661** and we will send a copy to you. Postage & packaging is free of charge so contact us soon to be in with a chance of getting hold of a copy.

Grants and **Awards**

Grants and awards — for all full ordinary and full student members!

Want to attend one of our meetings, visit a laboratory overseas, arrange for a student to gain some work experience in your laboratory, organise a one-day meeting in your region? Can't find the money to support this worthwhile venture? Then apply for a S*f*AM grant. We have an extensive range of grants and awards available to all full ordinary and full student members for these and additional activities relating to the greater understanding of applied microbiology. For more information please visit: www.sfam.org/grants.php

new **lecturer grant**

Are you an early career scientist who's just begun their first permanent appointment in a Higher Education Institute? Do you need some funds to pump-prime your microbiological research? If you have been a member of S*f*AM for at least two years and you have not already received a major research grant from other sources, then you can apply for the **New Lecturer Research Grant.**

You can apply for this grant within three years of the start date of your first appointment. You need not necessarily be in a 'Microbiology' lecturer post but your research field and proposed project should be in the field of Applied Microbiology. This grant is not available to Research Fellows or Post Doctoral Researchers. The closing date for applications is 30th September each year.

Terms and Conditions

1. Only applications from academics in their first academic appointment in a Higher Education Institute will be considered for this award. Applicants should apply within three years of the start date of their appointment.

2. Applicants must have been a Full Member of S*f*AM for at least two years to be eligible to apply.

3. S*f*AM will not fund the full economic cost of research.

4. S*f*AM should be acknowledged in any publications that arise from the research. In addition, a feature report about their S*f*AM funded work (500-1000 words) must be provided for *Microbiologist* magazine.

5. The recipient must present a poster or oral presentation of their work at a S*f*AM conference.

6. There will be an allocation for travel to the conference.

7. In any given financial year there are a restricted number of these grants available. The closing date for applications is 30th September in each year. Applicants must apply at least eight weeks before the proposed start of any project.

8. A successful applicant cannot re-apply to the fund.

9. A successful candidate will receive funds payable to their institution only. Normally payments are not made to an individual recipient.

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.

Germany

J. Kuever; P. Natskoulis

Hungary

T. Toth

India

D. Garg; R. K. Gaur; D. Kaushik; S. Mutnuri

Ireland

D. Bougoure; P. Bourke; J. Gutierrez; K. Jordan; B. Kelly

Jordan

S. Saleh

Netherlands

K.A. Van Heij-de-Boer

Nigeria

C.I. Ayolabi; R.E. Uzeh

Sudan

A.O.Y. Hashim

Taiwan

C-H. Yang

Thailand

S. Lumyong

United Kingdom

J.R. Bailey; T.R. Blower; E. Button; J. Caddick; S.J. Casey; H. Cini; I.T. Cocking; A. Cody; B.A. Daramola; A-M. Dean; V. Dixon; S. El-Sabbahy; T.J. Evans; T. Gristwood; A. John; C.A. Jones; B. Kwakye-Awuah; A.S. Lamb; B.D. Lane; O. Larkin; L. Lasecka; A.A. Leahy-Gilmartin; C. Lord; J. Nahl; V.S. Pokkuluri; S. Poulter; K. Rajakumar; R.L. Robins; K. Safadi; D.J. Scanlan; R. Shahzad; L-L Shillam; S.N. Smith; E. L. Sproston; S. Taylor; R. Villa; S. Wong; K. Woodward; C. Wright

USA

A. Asceric; D. Bruen; T. Gallagher; V. Gomez-Alvarez

CORPORATE

Southern Group Laboratories Ltd

Not a member?

For full information on the many benefits of S*f*AM membership, turn to page 6

society *for* applied sfam microbiology

Photo Competition

Have you taken an outstanding photograph of your beloved bugs? Do you know someone who has and you'd like to see their work in print? Perhaps you've taken a photograph while attending a S*f*AM conference which you think is worthy of reproduction?

Due to popular demand, S*f*AM are running the photography competition again this year. We are looking for twelve eye-catching images to use for our 2008 calendar which we will be giving to all out members as a Christmas gift.

To enter this competition, please send your photographs to the Editor in the form of JPEG files which must be a minimum size of 7 x 7cm at 300dpi (800 x 800 pixels). Alternatively, you can send the original photographs in hard copy to the Society Office and we will return them to you after they have been scanned.

Photograhs will appear in one of two categories:

1. Scientific — e.g., a colourful image using bacteria
2. Non-scientific but with a S*f*AM theme e.g., taken at a S*f*AM event

The closing date for entries for this competition is 28 September 2007

mailbox

From: Dr Gavin Clark
Subject: Friendly society

Please thank the Society for the copy of its history; the book arrived without a scratch, and makes very interesting reading. I was in Edinburgh as a student when Tam Gibson was head, followed by Anna Stirling; two very different people, but both with a passion for their discipline which was passed on to those around them — and on to me if I am to believe the students' comments when I retired.

I wrote to Mrs Stirling (she always said she had put more into the Mrs than the PhD, and prefered to be so addressed) until she died, latterly via her daughter because Mrs Stirling became totally infirm.

I came to the College at Kings Buildings at a time when Ron Board was there, and his reputation for life was still spoken of in hushed tones!

I saw Ron in Toronto a few years ago; which was a real pleasure.

What I look back on were the close friendships of the department, achieved without living in each others pockets. It was this same enjoyment of shared professionalism and a open friendliness that is spoken of in the *History of the Society* as something that set it apart from other societies. It is absolutely true. In all my years attending meetings around the world, I remember the meetings of our Society as uniquely 'cosy' without a hint of inbreeding.

From: Dr Ron Bishop
Subject: Happy history

Many thanks for sending me the copy of Max's history of the society. It's beautifully produced and I look forward to being reminded of pleasant times when I used to be a microbiologist (never thought my picture would be in it, though!). It was very interesting to see a photo of your new offices — quite a difference from the old Blore Tower! I hope you're all finding it convenient and comfortable.

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

If you feel you could be our next winner for 2007, 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.

World Rabies Day 2007

World Rabies Day is an initiative organized by international health professionals to increase global awareness as to the ongoing and unnecessary tragedy of human rabies. Our goal is to engage 55,000 people across the world to take action on this day, one person participating for every victim of rabies that died needlessly during the year. As a member of the international community of public health, we urge you to join forces with us to make a difference in rabies prevention and bring an end to the suffering of rabies. **Website: www.worldrabiesday.org**

Lost Members

We have a few members on our database who have moved on without telling us! This means that we cannot maintain their membership as we would like.

If you know of the whereabouts of any of these members, then please could you either inform Julie Wright, the Membership Co-ordinator (julie@sfam.org.uk) or give the member a 'gentle nudge' for us. We have listed the members' names below together with their last known location. Thank you for your help.

Miss Kirsty V Fletcher, Wirral, UK
Miss Catherine M Loc-Carrillo, Leicester, UK
Dr J F S Dempster Ireland
Dr M A Halablab, King's College, London, UK
Dr R T Mitchell, Health Protection Agency, London, UK
Miss Elizabeth J McMinn, London School of Hygiene & Tropical Medicine, UK
Mr W D Gemmell, Glasgow, Scotland
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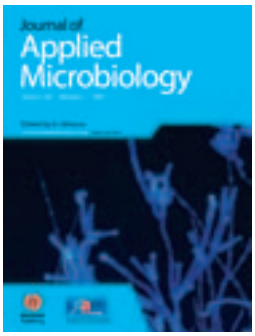

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4) Probiotics and their fermented food products are beneficial for health. S. Parvez, K.A. Malik, S. Ah Kang, H.-Y. Kim. Vol. **100**, No. 6, June 2006.

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1) Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. A. Nostro, M.P. Germano, V. D'Angelo, A. Marino, M.A. Cannatelli. Vol. **30**, No. 5, May 2000.

2) Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris* pathovars. S. Satish, K. A. Raveesha, G. R. Janardhana. Vol. **28**, No. 2, February 1999.

3) Antibacterial activity of selected plant essential oils against *Escherichia Coli* O157:H7. S.A. Burt, R.D. Reinders. Vol. **36**, No. 3, March 2003.

4) A rapid and efficient assay for extracting DNA from fungi. D.W. Griffin, C.A. Kellogg, K.K. Peak, E.A. Shinn. Vol. **34**, No. 3, March 2002.

5) Screening and mutagenesis of a novel *Bacillus pumilus* strain producing alkaline protease for dehairing. H.Y. Wang, D.M. Liu, Y. Liu, C.F. Cheng, Q.Y. Ma, Q. Huang, Y.Z. Zhang. Vol. **44**, No. 1, January 2007.

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3) Environmental predators as models for bacterial pathogenesis. Hubert Hilbi, Stefan S. Weber, Curdin Ragaz, Yves Nyfeler, Simon Urwyler. Vol. **9**, No. 3, March 2007.

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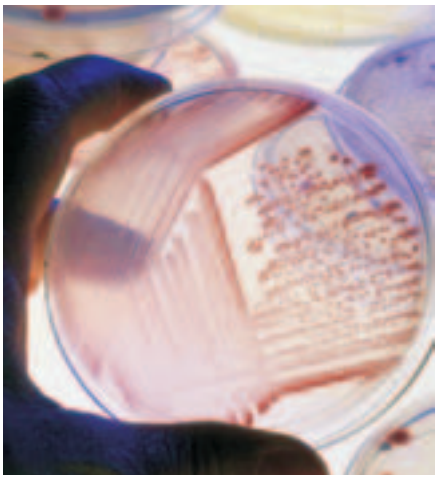
Current Issues in Intestinal Microbiology

Editor in Chief G. W. Tannock (at time of this publication, current Senior Editor I.R. Rowland)
Horizon Press. ISSN 1466-531X
Price On-line open access
Reviewed by Judith Evans



This is a peer reviewed journal, published twice yearly, both electronically and in print, consisting of review papers covering subjects relating to human and animal intestinal microbiology, particularly ecology of the intestine, pro and prebiotics. The microflora of the gut, and their interactions with their specific host are currently receiving a great deal of interest from commercial companies manufacturing foods and digestive supplements that can be bought by the general public. The gut flora are generally not well understood, only a tiny percentage of the microbes present are thought to have been cultured and the cryptic nature of most mean this is an area of research needing further study and Current Issues in Intestinal Microbiology is a forum for publishing such research.

The three papers in this edition cover very different subjects: bacterial biofilms in the human gastrointestinal tract, virulence genes of *Vibrio (V.) cholera* and microencapsulation of probiotic bacteria.

The first paper begins with a brief introduction to bacterial biofilms and the types and location of bacteria known to inhabit the gut. The authors move on to discus the role of these organisms in healthy and diseased states and whether the presence or absence of specific organisms is diagnostic of specific diseases. The authors acknowledge study of biofilms is difficult and suggest areas such as tissue culture, continuous culture and novel fermentation experiments that may be important for facilitating further study.

The second concentrates on *V. cholerae*, an organism well suited to existing in marine and estuarine environments where biofilm formation may help survival of the organism.

The lifecycle within the host is governed by virulence genes. This paper is a summary of what is known about regulation of those virulence genes by *V. cholerae* biotypes classical and El Tor within the host. It offers a clear description of the components involved in the cascade regulation of genes required for colonisation of the human small intestine.

Probiotics are reportedly beneficial to the consumer in a number of ways, and their survival during food production, storage and passage through the stomach and small intestine is a major concern for manufacturers. This last paper is a fascinating review of the problems associated with delivery of stable, viable probiotic bacteria in large quantities for industrial food production. Different methods of physically protecting the cells from the various environments they are exposed to are described and illustrated, along with the types of foods differently prepared probiotics are added to. There is, quite surprisingly, a list of suppliers of various probiotics. The conclusion of the piece is flagging the future direction of the industry.

Gut health is currently high on the agenda of anyone with a television. There is a large market waiting to be tapped, and food manufacturers compete to entice the general public to buy products that will "do them good". This journal comes as a bit of a relief from such vague claims, with clearly written articles, illustrated with figures and photographs as appropriate. A visit to the online archive at www.horizonpress.com/ciim/online.html to get more up to date information is highly recommended.

Color Atlas of Medical Bacteriology

Luis M. de la Maza; Marie T. Pezzlo; Janet T. Shigei; and Ellena M. Peterson. ASM Press, Washington D.C., 2004. ISBN: 1-55581-206-6 pp 316, Price: **$139.95 (£92.00)**

Reviewed by Sally Cutler

This colour atlas provides not only an impressive collection of over 650 colour illustrations, but a good supportive summary on each of the groups of organisms addressed. Consequently, it is a valuable resource for those in diagnostic laboratories or involved in teaching diagnostic bacteriology. The book is organised into 38 chapters covering a good range of bacterial species likely to be encountered in a diagnostic setting. It clearly describes the features of each group, including differential tests to accurately identify these organisms. Of particular value is the concise background information provided on commonly used diagnostic tests. This is nicely summarised in the final chapter which focuses on these tests as a separate entity.

The authors state in their preface that they did not attempt to provide in-depth information, however, links to further reading would further increase the value of this book to students wishing to pursue their studies. The authors must be complemented on the exceedingly high quality of the illustrations included. This could however be further improved by the inclusion of arrows in some instances where a specific features are being discussed. This was particularly notable in figs. 34-3; 34-5; 34-8 and 37-2. The inclusion of a zone size indicator is also essential for figure 24-6, where *Brucella melitensis* should be resistant to both test dyes in this inhibition test, however, a zone is clearly visible around one of the dye impregnated discs. The authors state that a zone size of at least 5mm is required for susceptibility, however no measure is provided.

The only illustration that was of dubious quality was a dark-field micrograph of *Treponema pallidum*, where the authors do comment on the poor quality of this image. Those images depicting antimicrobial susceptibility assays tended to be over-inoculated without the desired semi-confluent growth. The zone of inhibition around the kanamycin disk for *Veillonella* (fig. 28-17) defeated my powers of optical resolution! The apparent change in colour of CIN agar between plate A and B of figure 13-7 was a surprise, and may well be a photographic artefact. The continual reference to the superiority of carbol fuchsin over neutral red as a counterstain in Gram films, though valid, was repeated in most chapters until it became almost tiresome.

The authors provide useful cautionary notes of value to those undertaking the diagnostic tests described, for example, the possibility of some MRSA strains of *Staphylococcus aureus* to be negative with latex based clumping factor/protein assays and the fact that some coagulase negative *Staphylococci* can conversely be positive in this assay. Similarly, potential morphological confusion of *Listeria monocytogenes* with *Enterococcus* spp. or *Streptococcus pneumoniae* in direct clinical films and biochemical inconsistencies following incubation at different temperatures for *Yersinia* spp. (chapter 13), provide helpful warnings.

The general coverage of bacterial genera is comprehensive, even including organisms such as *Tropheryma whipplei*, the causative organism of Whipple's disease. Taxonomic nomenclature ranged from impressively up-to-date, to sadly out-dated depending on the chapters. Chapter 12 on *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia*, *Pantoea* and *Raoultella*, gave current nomenclature, but also included former names enabling cross-referencing with other texts, however, in contrast, the chapter on Leptospires failed to embrace the nomenclature agreed by the IUMS in 2002 whereby the *Genus species* serovar each appear, thus giving names such as *Leptospira interrogans* serovar Ballum or *L. interrogans* serovar Canicola. The use of summary tables is also somewhat inconsistent between chapters.

Several errors and omissions flaw this otherwise excellent book. The reported association of *Brucella* spp. with insects and ticks, though not inconceivable given their phylogenetic relationship with other arthropod-borne microbes, has not been reported in the literature to my knowledge. Though it is generally accepted that cultivation is the "gold standard" for brucellosis, in their chapter the authors state the need for serology as "culture alone is not reliable", giving a somewhat confusing message to the reader. Phage susceptibility is a useful adjunct for differentiation between *Brucella* species, but is not mentioned by the authors.

When discussing the natural reservoirs for *Leptospira*, the authors have omitted to mention livestock, however they later mention dairy farmers as an occupational at risk group. When discussing *Borrelia* spp. the authors state that *Borrelia valaisiana* "appears to be a human pathogen" in contrary to the view generally accepted in scientific literature. While, the scientific terminology for the human clothing louse changes from *Pediculus humanus humanus* on page 267 to the more recently used *Pediculus humanus* by page 273.

Disappointingly, the authors fail to recognise the genus status of *Anaplasma*, instead including this phylogenetically distinct clade under the *Ehrlichia* in chapter 37. The authors initially use species names in this chapter, but then revert to the former terminology of "HME" and "HGE." Furthermore, they describe *Coxiella burnetii* as forming spores. Although the small condensed variants have been likened to spores, they are not conventional bacterial spores and this terminology could potentially confuse the reader.

Whilst recognising that evolution of diagnostic approaches may soon resign the tests they describe to the history books, the authors state that "in the meantime let us enjoy the beauty of the bacterial world", an aim that they have fulfilled admirably. In answer to the authors' question of whether this "final product is worth the wait" I think we can reply in no uncertain terms, yes!

Despite these minor criticisms, the book should be highly recommended to those involved with teaching or diagnostic bacteriology.

our policy on the media

We will:

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

Science Media Centre

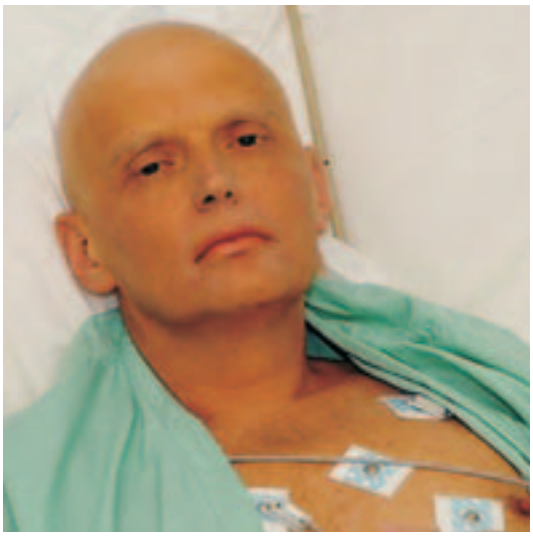
Fiona Fox discusses the success of the SMC five years on

The Science Media Centre has just celebrated its fifth birthday and many have remarked on the success of this unique venture — now used by every single major national news outlet in the UK. Set up in April 2002, the Centre was the scientific community's response to a strong sense that scientists had failed to get their voices heard in the media furores over controversies like BSE, GM crops and MMR.

The Centre continues to promote our philosophy that the most effective way of improving the quality of media coverage of science is for more scientists to engage more often with the big stories of the day. In the early days this was a nice theory, now it is a proven fact; one of the joys of my job is travelling the country demonstrating to scientists how we have been able to improve the quality of reporting by making it easier for journalists to get the best science and the best scientists into their stories.

On all the big breaking news stories last year; like the TGN1412 clinical trials disaster at Northwick Park, or the Russian dissident, Alexandr Litvinenko, poisoned by polonium-210, the contamination of Cadbury's chocolate with *salmonella*, or the outbreak of bird flu in the Bernard Matthews turkey farm — the Science Media Centre was offering the best scientists with the best information as those stories were breaking and developing. All of these had the potential to become huge scare stories, exaggerated and sensationalised in ways which could have real impact on people's lives. But we can now show how having an abundance of great scientists with real expertise on these issues helped create a more balanced and accurate media debate.

The Science Media Centre is not a traditional press office charged with generating positive media coverage about specific areas of science or for one institution. We are openly media driven and if the media wants to talk about MRSA, bird flu, GM crops or climate change, we



media**watch**

microbiology in the news

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

are happy to help them do so. That said, the Centre has developed a unique series of 'background briefings' for national news media to help more scientists seize the media agenda and attempt to inform the framework of coverage on controversial topics. Amazingly, the Centre now averages one press briefing a week and it's not at all unusual for us to conduct two or three. We have executed several of these with our friends at S*f*AM, including one on the benefits or myths of pro-biotics. Despite the fact we call them 'background briefings' last year 52 out of 55 briefings generated positive coverage in the national news.

Our success at running media briefings and the expertise we've gained handling controversial subjects, has in the past year seen

more and more scientists and science press officers approaching us to run their media briefings. This is a really exciting part of the job and last year we either organised or hosted the media launches of key reports like the Prof Gordon Duff's Expert Scientific Group findings into what went wrong at Northwick Park, the Weatherall report into current and future use of primates in medical research commissioned by the Wellcome Trust, MRC, Royal Society and Academy of Medical Sciences and the controversial Nuffield Council on Bioethics report on the care of premature babies.

We also keep in close contact with the scientists on our database to check that they are happy with the reporting of major stories. Many of the media briefings we run are based directly on scientists' worries that inaccurate reporting of key facts is distorting public reactions. For example, in the middle of the bird flu crisis last year we were contacted by several scientists who had seen journalists reporting that chicken may be unsafe to eat — so in a matter of days we had arranged a briefing with leading microbiologists and food safety experts to correct that

impression and give the reporters an in-depth background knowledge of the conditions under which the virus can be contracted by humans. Again, with five years experience behind us, we can show that taking the risk of talking to national news journalists about these alarming stories almost always pays off and we are the proud owners of some beautiful press cuttings with balanced headlines calming fears and killing scare stories.

Struggling to meet the needs of the ever-expanding 24 hour media machine when a major science story breaks is the biggest single challenge for the Science Media Centre; basically we need more great microbiologists to join our database. We do not throw our experts to the hungry media wolves without support and we always call scientists first to make sure they are confident to speak to journalists on a breaking story. We also offer media training and a general Introduction to the News Media course for the media virgins. So if you are someone who has shouted at the TV or radio in the past few years because of inaccurate or sensational reporting of your subject area then please do get in touch with Claire Bithell (email: cbithell@ri.ac.uk) and we can talk you through the way it works.

While the mass media may not be scientists' preferred way of communicating science to the public, surveys continue to prove that it is the most significant influence on public attitudes to science. When people are terrified that they might get *Salmonella* from eating chocolate, MRSA from a routine visit to hospital or bird flu from eating chicken — they deserve to hear the facts from the best experts available and that could well be you!

Fiona Fox
Director, Science Media Centre

Med-Vet-Net introduce two new research workpackages

Workpackage (WP) 32 - Validation of public health surveillance

Public health surveillance

National food and public health authorities request information on the incidence and cause of zoonotic bacterial infections. Currently, collection of data on human infections, such as the food-borne pathogens *Campylobacter* and *Salmonella*, is not sensitive enough to give an accurate picture of the magnitude of the problem. Most countries do not have systems that determine the exact number of human infections, but use passive surveillance that relies on physicians and microbiology laboratories reporting infections. This data cannot be compared between European countries as each uses different methods, and physicians have different practices for the management of patients with suspected food-borne infections. More accurate surveillance of these infections would allow better control methods to be implemented.

Workpackage 32, *'Public health surveillance for foodborne infections: Design of epidemiological studies and applying sero-epidemiology to validate the surveillance pyramid'* was devised to look at this problem. In June 2006 an open invitation was issued to all EU member states and associated countries to participate in the project. Interested parties were invited to a workshop, where the selection criteria for serum banks and a tentative study design were agreed. Efforts to identify additional serum banks were made through a presentation at the EnterNet meeting in 2006, through literature research and personal contacts. The workpackage officially started on 1 September 2006 with participation of five EU countries and the US. Laboratory work has started and negotiations about access to existing serum banks in five other European countries are ongoing.

Approach

The project will use a cost-effective technique — sero-epidemiology — which has previously been used to study infections in animals, to detect human infections. By testing for bacterial infections in human sera, such as blood already stored in medical facilities, researchers aim to fill the missing gaps in health surveillance data. Concentrations of specific antibodies will be measured in sera from existing population-representative serum banks, and based on these results, incidence estimates will be generated with the help of a stochastic back calculation model, which accounts for inter-individual variations in antibody response.

"This project brings together experts in serology, epidemiology, mathematical modelling and community-based studies allowing us to integrate information from different approaches of studying human infection" says workpackage leader Dr Kåre Mølbak, Director of the Department of Epidemiology, Statens Serum Institut, Copenhagen: *"By making full use of existing European studies and new data we will be able to calculate the ratios between infected cases, cases with symptoms in the community and lab reports."*

Research Plan

A detailed research plan was developed during the kick-off meeting, which was held on 20 — 21 September 2006 in Prague, Czech Republic. In preparation for this meeting, the project team explored the availability of population-based serum collections and embarked on validation of the mix-ELISA for S. Enteritidis/S. Typhimurium.

Once they have agreed testing methods for sera, researchers will plan and agree protocols for community-based studies of these bacteria. The group plan to conduct a pilot study based on sera stored in a number of different countries. Information on the level of antibodies to food-borne pathogens will be translated to measures of disease frequency, which again will be compared with the officially reported figures. This will pave the way for the creation of novel, more accurate Europe-wide surveillance systems for gastrointestinal illnesses.

Major elements of the research plan include:

1. Preliminary development of resources and tools for sero-epidemiology.

1.1 An inventory of serum-banks

1.2. Analyses of an existing collection of patient-sera (follow-up study of 300 patients), to determine antibody decay profiles after *Salmonella* infection using a mix-ELISA rather than ELISA with serotype-specific LPS catching antigen.

1.3. Draft consensus protocols for the epidemiological, mathematical and serological methods will be developed by the team. After final acceptance, we will invite different European countries, where adequate sera are available to participate in the pilot study.

2. Laboratory analysis of sera from selected countries

2.1 Selection of sera collections.

2.2 Completion of serological tests for antibodies directed against S. Enteritidis/ S. Typhimurium in selected sera.

2.3 Completion of serological tests for antibodies directed against *Campylobacter*

med-vet-net

Med-Vet-Net is a European Network of Excellence that aims to improve research on the prevention and control of zoonoses by integrating veterinary, medical and food science research. Comprising 16 European partners and over 300 scientists, **Med-Vet-Net** will enable these scientists to share and enhance their knowledge and skills, and develop collaborative research projects. **Med-Vet-Net** officially commenced on 1 September 2004, and is funded to the value of €14.4 million for five years.

jejuni/coli in selected sera.

3. Community-based epidemiological studies

3.1. A protocol for community-based studies on infectious gastroenteritis will be developed, with different options of a costly prospective cohort study (using stool and/or serology) or a less expensive retrospective survey. Advantages and disadvantages of both approaches will be clarified. Particular consideration will be paid to how to integrate information from various approaches to determine the surveillance pyramid, including community-based studies, sero-epidemiology and risk assessment.

3.2. An attempt will be made to obtain sera from the population cohort of the planned second study on community incidence of infectious intestinal disease in the UK (IID-2). Enrollment to this study began in spring 2007 and the study subjects will be prospectively observed for episodes of acute intestinal infections for one year. Serum samples taken at the end of the observation period in 2008 (i.e. during the second half of workpackage 32) would provide a rare opportunity to compare serological findings with prospectively observed incidence of symptomatic infections with *Salmonella* and *Campylobacter*. However, implementation of this activity will depend on approval by the IID-2 steering committee and availability of additional funding.

Workpackage (WP) 33 - Early host responses to *Salmonella* and *Campylobacter*

Advances in host — pathogen research have mainly focused on studies on pathogens while studies of host-responses have lagged behind. Detailed insight into specific pathogen properties has however, not led to optimal control strategies for infection of humans and farm animals. Zoonotic infections with *Salmonella* and *Campylobacter* still remain an important human health problem. A better insight into host — pathogen interaction with a focus on host-responses may enable the development of optimal control strategies.

Workpackage 33, *'Early host responses to Salmonella and Campylobacter'*, began on 1 March 2007 and aims to contribute to our understanding of mechanisms for infection by focusing on the early host-response to *Salmonella* and *Campylobacter*. The project participants propose to analyse early host responses in the ileum of mice using micro-arrays, and to identify pathways induced upon infection. Currently, the hypothesis is that such early responses determine if exposure to these enteric pathogens will lead to bacterial clearance or replication, and subsequent colonization or disease.

Salmonella and *Campylobacter* are successful pathogens as exemplified by the burden of disease they cause in humans. Farm animals can be colonized with these pathogens and, to date, attempts to eliminate *Salmonella* and *Campylobacter* from the food chain have been only partially successful. Detailed insight into pathogen factors and highly sophisticated diagnostics and typing of these bacteria has shown that, although this knowledge has contributed to detection and epidemiology, it is not sufficient for optimal infection control. A better insight into host-factors involved in the elimination of these pathogens will allow a more complete understanding of the interaction between *Salmonella* and *Campylobacter* in the host. Insight into these mechanisms will eventually:

- improve the relevance of diagnostics to disease status;
- contribute to our understanding of epidemiological observations;
- contribute to the development of vaccines and therapeutics;
- provide input into risk-assessment research;
- enable a better understanding of individual susceptibility to infection.

The overall aim of the proposed project is to identify host-factors that are regulated in the intestine in response to infection with *Salmonella* and *Campylobacter*. This will allow a better understanding of the interaction of these pathogens with the host immune system and will not only contribute to the host-pathogen interaction field but will also be valuable to risk-research, epidemiology and detection and control.

The specific aims of the project are to:

1. Develop standardized infection protocols.

2. Characterize the early host-responses to *Salmonella* infection. These will be analysed using three different methods.

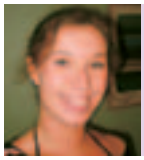
3. Preparation of a first report.

4. Comparison of responses to wild-type *Salmonella* and attenuated *Salmonella* mutants.

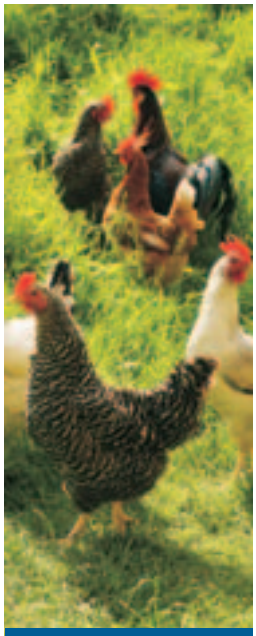
5. Characterize the early host-responses to *Campylobacter* infection.

6. Organize a workshop - when all experimental data are collected a workshop will be organized in which all results are presented.

7. Recommendations on the use of this approach to gain insight into host — pathogen interaction and possible contribution to risk research and epidemiology.



Teresa Belcher and **Jennie Drew**



information

For more information about **Met-Vet-Net**, visit: http://www.medvetnet.org/ or contact Teresa Belcher on: +44 (0)1234 271020

BIOSCIENCES FEDERATION

bio **focus**

Richard Dyer laments the loss of practical skills within the biosciences

The Biosciences Federation is a single authority representing the UK's biological expertise, providing independent opinion to inform public policy and promoting the advancement of the biosciences.

For further information visit:
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The Biosciences Federation is seriously concerned about the loss of practical skills across the full range of the biosciences. That is, from ecology to *in vivo* pharmacology and from taxonomy to biochemistry. The biosciences are practical subjects, and yet in our schools and universities the amount of practical experience that students acquire continues to diminish. This decline is likely to continue because we have lost and are losing teachers with practical skills.

For my A levels we went out into the fields and threw metre squares "randomly" on patches of grass and then proceeded to count the number of certain plants and insects within the square. Many of you will have had a similar experience at school or university and will probably remember, as I do, the enjoyment of these outings — and not just for getting your square around someone's neck! But this is now a rare educational activity. And the loss of training in field work is important because, for example, the subtle change in the distribution of lichens is an indicator of climate change. We have lost many lichenologists, and many of those who remain are close to retirement. To embark on a project in the field in this area now requires more than the usual attention to the competence of your supervisor: you could find yourself working on wrongly identified lichens.

The same is true for scientists with *in vivo* skills. Once again, I have fond memories of tracing dogfish cranial nerves — well, perhaps not so fond because I was not addicted to formaldehyde! But it was an introduction to animal work and developed a real awareness of how nerves pass through tissue and bone. The work brought a three dimensional understanding of line drawings and excited interests that I suspect would not have been ignited without this experience. Some will argue that a prosected dogfish can provide nearly all these educational elements — it is a debate that those involved in medical education know well. Nonetheless, some practice on cadavers seems preferable to the alternative for veterinarians, doctors and those using animals for research. Today, the pharmaceutical industry has great difficulty in recruiting in this area because few are qualified for the work.

Of course, not all bioscientists need to throw metre squares and cut up dogfish in order to make a research or teaching career in one of our disciplines. However they are likely to need to make up reagents correctly and this is not a skill that one can anticipate today in all graduate students. The point is, the decline in practical skills threatens the strength of the biosciences.

How has the present situation arisen? There is no single answer to this question, but the expansion of university bioscience courses is an important component of the answer. With doubling, trebling and quadrupling of student numbers in the biosciences, it has often proved too difficult to find and pay for the space and staff to enable practical work of a high standard to continue. Indeed, as you will know, many courses are structured to minimise the need for practical training. It is possible today to do an Honours degree in Pharmacology and, if you are predicted to obtain a lower second class degree, your Honours project will be in the library. Graduates lacking practical skills will not usually attempt to find the time for more practical work when teaching in secondary schools.

What can be done to reverse this deteriorating situation? Clearly, motivation and money are needed. Motivation comes from need and leads to money. The ecological and *in vivo* examples given above were chosen because they are in areas where the need is real and so is the possibility of extra resource. We do not think that we can usefully argue for an all-embracing single step solution to this problem, but we do think that we can target areas and work with others to achieve change. Indeed, we are quietly achieving significant success. The loss of practical skills is now part of the national agenda and resolution of particular needs is being discussed in a positive way with Government.

Richard Dyer
Chief Executive
Biosciences Federation

Spring Meeting 2007 Report

Broadening Microbiology Horizons

Manchester Metropolitan University, 11th April 2007

This meeting was the first in a series of Spring meetings which the Society will organise annually. The intention of the *Sf*AM Spring meeting is to provide topics which appeal to personnel working in Clinical Microbiology, in particular Biomedical Scientists.

The meeting was awarded seven credits under the Institute of Biomedical Sciences continuous professional development scheme. It attracted over 70 delegates and five trade exhibitors to the modern state-of-the-art facilities of the John Dalton building at Manchester Metropolitan University.

The morning session was opened by Professor **Andrew Fox** (Health Protection Agency, Manchester) whose intriguing title was *'Lumping and Splitting — latest developments in typing methods.'* He highlighted the plethora of typing techniques that were available. These include phenotyping techniques and genotyping methods. He commented that there have been major advances in typing methods with the introduction of genotyping methods, in particular with the advent of nucleic acid amplification techniques. He proceeded to present a very comprehensive insight to developments in this field.

The second presentation was given by Professor **David Denning**, Professor of Medicine and Medical Mycology at Wythenshawe Hospital, Manchester. He highlighted that there were three fundamental problems with invasive fungal infections. Infections with fungi are under-detected, slow to diagnose and often go completely undetected.

He then went on to explain why molecular diagnostics were becoming increasingly important in prompt diagnosis of invasive fungal infections. To demonstrate the importance of more rapid diagnosis he highlighted that patients suffering with these conditions, if diagnosed within 10 days had a mortality rate of 40%, compared to 90% if the diagnosis was made after 11 days or more.

The penultimate talk of the morning was given by Professor **Geoff Hanlon** from the University of Brighton. He described very eloquently the use of Bacteriophages in the treatment of patients with a whole array of infections. He commented that much work was being undertaken in this field, particularly in countries previously affiliated to the USSR. As well as describing the potential for use of Bacteriophages in treating infections, he went on to discuss some recent applications in treating food to ensure the elimination of pathogens e.g., *Listeria* species from cheeses.

The final presentation was given by Dr **Jean-Yves Maillard** of the University of

thanks

Thanks are due to all the speakers who freely gave of their time. Feedback questionnaires indicate that delegates found all the presentations to be of a high quality and very informative. Thanks are also due to the trade exhibitors (**Bioconnections**; **Cosmos**; **Don Whitley Scientific**; **LabM** and **Prolab**) who contributed to the meeting. This initial meeting proved to be a great success and will certainly become an annual event for *Sf*AM. Planning is already underway for next years meeting.

Cardiff. He presented an extremely informative update and review on microbiocides. He highlighted the usefulness of these agents in the control of hospital acquired infections. He described experimental work showing that synergistic *in vitro* effects could be demonstrated for some of these compounds when combined with certain agents.

Philip Wheat

information

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

You can also find details of this years' Summer Conference on page 23 of this issue of *Microbiologist*

The afternoon session began with an excellent presentation by Professor **Peter Gilbert**, University of Manchester on dental microbiology during which he comprehensively described polymicrobial communities. He explained that only 60% of the numerous different microbial flora had been successfully cultured and that poor oral hygiene could result in a number of conditions other than carious lesions (tooth decay) including gingivitis, peritonitis and eventually tooth loss. Halitosis was the result of a biofilm on the tongue with pathogens such as *Porphyromonas gingivalis*. The photographs, if nothing else, made you want to clean your teeth there and then! The graphic illustrations almost brought their own scratch and sniff ability and one could almost imagine the malodour that would be emitted from the mouths demonstrated during the lecture. Peter finished his talk with some fascinating new theories and some evidence on the role of periodontal pathogens in cardiovascular conditions such as stroke and endocarditis. Dr **Valerie Edwards-Jones** continued on the theme of scary pictures and chronic medical conditions, talking about the role of silver in controlling wound infections. She outlined the causes of chronic wounds and why wound healing mechanisms are compromised by bacteria infecting the wound. She described the formation of wound biofilms and the difficulties in treating these.

The role of silver as an antimicrobial agent since antiquity through to current day was described and the varying effects achieved with different silver salts. Nanocrystalline silver seemed to be more effective at achieving bactericidal levels which could be sustained over time. Possible problems with future resistance were discussed, especially since there was a rapid increase in the number of silver dressings available since the first one introduced in the late 1990's. Some of the dressings donate silver to the wound bed, others work by releasing silver into the dressing. Many had different levels of available silver.

Dr **Tony Fooks** gave an update on rabies outlining the effect of this deadly zoonotic virus on public health, veterinary and economic standpoints. Rabies still poses an enormous public health threat despite the availability of some biologicals for its control. New bat variants have been identified.

In Europe, classic rabies had decreased due to successful oral wildlife vaccination programmes but this was not the case for less rich Eastern Europe and throughout parts of Africa, Asia and in specific regions of the Americas. He described his work on the development of an oral vaccine for immunization programmes on dogs in the Far East. The vaccine had proven to have a high efficacy and further trials were being undertaken. Molecular epidemiology had helped improve the understanding of the epidemiology of rabies but further understanding on pathogenic mechanisms were needed to understand inter species transmission.

Finally, Dr **Andrew Sails** finished the afternoon session with a fascinating talk on the future of diagnosis of infectious disease and the concept of near-patient testing. He outlined how technological development had allowed practitioners to get nearer to the instant diagnosis of the presence or absence of a pathogen that could radically change their working practices.

Present technologies, such as immuno-chromotography and lateral flow devices, as well as other commercial products, were discussed. PCR systems were available to allow diagnosis of MRSA within the working day and agents of bioterrorism could be detected away from the laboratory. One of the major factors currently was cost, but the technologies were being miniaturized and costs were coming down. He described how the patient could purchase a Chlamydia test from Boots the Chemist, as well as tests for HIV and hepatitis. Other point of care testing may be available from the chemists in the near future as technology develops further!

Valerie Edwards-Jones

Summer conference 2007

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

Microbiology of Fresh Produce

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

Including sessions on:

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

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students session

"Getting the most from your PhD"

This brief session for postgraduates aims to provide opportunities for networking and discussion around the theme of maximising the opportunities that a PhD programme can offer.

Kate Exley will provide short inputs to structure the session on key themes including, recognising, developing and evidencing employability skills, networking and teaching.

Monday 2nd July

Chair: Margaret Patterson

14.00 onwards - Arrive and Register

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

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

20.00 **Evening at leisure**

21.30 **Quiz Night**

Tuesday 3rd July

Chair: Keith Jones

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

Session 1. Organisms and the plant

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

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

10.45-11.15 **Coffee/posters**

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

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

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

13.00-14.00 Lunch

Session 2.

Chair: Martin Adams

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

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

Public health aspects

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

15.45-16.15 **Tea/posters**

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

16.50-18.00 **Student Session Getting the most from your PhD**
Kate Exley, University of Leeds, UK
SEE PANEL ON PAGE 23

17.30-19.30 **Trade Show**

Wednesday 4th July

Session 3

Chair: Tim Brocklehurst

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

09.35-10.10 **Burkholderia cepacia and other opportunistic pathogens**
Eshwar Mahenthiralingam, Cardiff University, UK

10.10-10.45 **Risk Assessments for fresh fruit and vegetables**
John Bassett, Unilever, Bedford, UK

10.45-11.15 Coffee/posters

Intervention strategies for control

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

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

12.25-13.30 **Lunch**

Session 4.

Chair: Karen Stanley

13.30-14.30 **Offered papers**

14.30-15.00 **Tea/Posters**

Chair: Andrew Hall

15.00-16.00 **Student presentations**

16.00-16.30 **W H Pierce Prize**

16.30-17.00 **AGM**

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

Thursday 5th July

Session 5

Chair: Geoff Hanlon

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

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

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

10.45-11.15 **Coffee/posters**

The industrial perspective

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

11.50-12.25 **Issues with organic produce**
Carlo Leifert, Nafferton Ecological Farming Group, University of Newcastle, UK

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

13.00-14.00 **Lunch & Close**

For the latest information please visit the website at: www.sfam.org.uk/summer_conference.php

BOOKING FORM and INVOICE

SfAM SUMMER CONFERENCE 2 - 5 JULY 2007

Microbiology of Fresh Produce

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

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	Full Members	Student, Honorary, Associate & Retired Members	Student Non - Members	Non - Members
Full Conference Rate: inclusive of registration fee, coffee breaks, lunches, Society dinner and accommodation in the Park Plaza hotel for the entire conference	£500.00 ☐	£250.00 ☐	£500.00 ☐	£700.00 ☐
No Accommodation Rate:	£195.00 ☐	£100.00 ☐	£195.00 ☐	£350.00 ☐
Day Rate: 08.30-17.00 hrs per day, or part thereof, inc. of reg. fee, coffee lunch and tea	£100.00 ☐	£50.00 ☐	£100.00 ☐	£150.00 ☐

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☐ **I wish to attend the Society Dinner on Wednesday evening (This is included in the Full Conference Rate and the No Accommodation Rate but costs £50.00 for delegates who elect to pay the Day Rate).** Please note that numbers are limited:	£
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TOTAL AMOUNT REMITTED:	£

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Biodiesel: a microbiologist's perspective

Stuart Shales discusses the complex issues surrounding the use of biofuels from a microbiologist's perspective

The subject of biofuels is very topical and features heavily in the news media. This coverage has increased considerably since the publication of the 'Stern Report' earlier in 2007 (Stern, 2007). In his review Sir Nicholas Stern discusses the economic impact of climate change and proposes strategies for investment to mitigate the effects of carbon dioxide (CO_2) accumulation in the atmosphere and resultant climate change.

In 2005 in the UK, 38.1 million tonnes of fuel was used for motor transport, both freight and domestic (source: DTI). Of this, 18.7 million tonnes was petrol and 19.4 million tonnes was diesel (DERV). During the period 2004 to 2005, diesel consumption exceeded petrol for the first time in the UK, much of this being due to the high sales of new diesel cars for the domestic sector. This high consumption of crude oil derived fuels for motor transport together with that for rail, marine, aviation and power generation has a major impact on CO_2 emissions in the UK. One of the drivers for the introduction of renewable, CO_2 neutral fuels is the need to mitigate greenhouse gas emissions. There are also other drivers, including the security of fuel supply. It is predicted that workable oil reserves will peak in the next two or three decades at a time when demand for oil is growing dramatically, as nations such as China and India become more wealthy and car ownership increases. For the UK there is another related issue: until recently as a result of North Sea Oil production the UK, there was a net export of crude oil, however the North Sea reserves are dwindling and the UK is now a net importer of crude oil.

The UK Government has introduced a Road Transport Fuels Obligation which seeks a 5% substitution of motor fuels with renewable sources by 2010. On top of this, the EU has a target of 10% substitution by 2020. So what are the options for renewable fuels? Currently the principal biofuels are bioethanol and biodiesel — first generation biofuels — as substitutes for petrol and diesel respectively. One of the main aspects of recent press coverage concentrates on concerns relating to the competition of agricultural land use for food production versus biofuel production. This gives rise to the question "is there sufficient land for both?" The answer to this question is "no", particularly if all motor fuels are to be derived from renewable sources. To this end, second generation biofuels produced from plant biomass will become increasingly important.

The Microbiological Perspective

What role does microbiology play in biofuel production? For bioethanol the answer is immediately apparent as ethanol is produced by fermentation of sugars by yeast and some bacteria. The production of alcohol by yeast such as *Saccharomyces cerevisiae* has been used for several thousands of years in the formation of alcoholic beverages and now this technology is being applied to biofuel production. The Brazilian alcohol programme has been in operation since the early 1970s and now nations such as the United States are developing extensive alcohol production facilities (see page 30 for more details). In addition to bioethanol as a petrol substitute, there is growing interest in the production of biobutanol which has an energy density virtually the same as that of petrol, whereas that of ethanol is considerably less. Thus fuelling a car with butanol will provide more miles per gallon than ethanol. Biobutanol is produced anaerobially by some *Clostridium* species and has been undertaken commercially in the past.

This article will concentrate on microbiological aspects of first generation biodiesel production and its use. Finally it will discuss some aspects of microalgal derived biodiesel fuels.

Biodiesel

Biodiesel that is currently used as a substitute for diesel fuel is derived from vegetable oils. In the UK and much of Europe the main source of vegetable oil is oilseed rape, *Brassica napus*, (figure 1a) although in southern, warmer countries the sunflower, *Helianthus annuus*, (figure 1b) can be grown for this purpose. A hectare of oilseed rape will yield approximately 1,000 kg of oil whereas sunflowers yield 800 kg of oil (Tickell, 2000). There are other oil producing plants grown for biodiesel production outside Europe, notably *jatropha* and, controversially, the oil palm. The latter has the highest yield of oil, 5,000 kg per hectare, but there are environmental concerns regarding the destruction of tropical rain forests that is taking place to grow oil palm crops. Furthermore, biodiesel produced from palm oil tends to gel at winter temperatures and thus can only be used in dilute blends of diesel fuels.

Figure 1a. Oilseed Rape

Pure vegetable oils cannot be used directly as diesel fuels in most motor vehicles. Oils are comprised predominantly of triglycerides (triacylglycerols) and these have a relatively high molecular weight which causes them to be too viscous. Furthermore, gums present in pure vegetable oils can accumulate in fuel injection components and may lead to failure. Instead, pure vegetable oil is processed by transesterification to biodiesel which is essentially a mixture

Figure 1b. Sunflower

of fatty acid methyl esters (FAMES). This process is illustrated in figure 2. The process uses methanol as a co-substrate and commercially it is usually catalysed using sodium hydroxide or potassium hydroxide. It is essential that no water is present otherwise soap may be produced and this will contaminate the biodiesel. Under some circumstances acid catalysts may be used. This normally uses sulphuric acid - nitric acid should be avoided as its presence may lead to the production of nitroglycerine! Acid catalysis is considerably slower but may be used if recovered vegetable oil (used cooking oil) is the starting material.

In Europe there is a quality standard, EN14214 for commercial biodiesel. A by-product of the process is glycerol (glycerine); this could have commercial value for the cosmetics industry but unfortunately tends to be contaminated with alkali and other products. Clean-up costs are prohibitively high so most of the glycerol is disposed of, for example by anaerobic digestion to biogas which in turn can be used as a fuel/energy source. In some instances the glycerol can be combusted for heat and power production.

One area of research interest has been the development of alternative catalysts for the transesterification process and for microbiologists this has involved developing enzyme catalysts (Shimada *et al.*, 2002). The potential advantage of using enzymes is that they can be immobilized and hence reused. It is also possible to develop a continuous process. Furthermore, the glycerol by-product will be cleaner, not contaminated with alkali, thus increasing its commercial value. The enzymes used are lipases from a variety of microbial sources including *Candida antartica*, *Rhizomucor miehei* and *Pseudomonas cepacia* (Shimada *et al.*, 2002; Salis *et al.*, 2005; Noureddini *et al.*, 2005). The use of microbial lipases as biocatalysts for the transesterification process is a challenge to biotechnologists. Firstly, the reaction is two phase, as the two products, biodiesel (FAME) and glycerol, are immiscible and separate out during the process. Secondly, the methanol used as a co-substrate is toxic to lipase enzymes and its presence at high concentration may inhibit the transesterfication process. One solution to this toxic effect is to add the methanol sequentially in a fed-batch system. Thirdly, the process must be undertaken in the absence of water and the plant oils are by nature hydrophobic.

Whether or not enzyme catalysts are used commercially will depend on these technical hurdles being overcome. Furthermore, the alkali catalysts are very inexpensive and the reaction rate is very fast. Thus enzyme catalysts would need to offer distinct advantages if they were to be used.

Another microbiological aspect of biodiesel is its environmental impact. Conventional hydrocarbons such as diesel fuel can have a detrimental effect when accidentally discharged into the environment. The environmental impact of biodiesel has been investigated. Lapinskiene *et al.*, (2006) demonstrated that in non-adapted, aerated soil biodiesel had

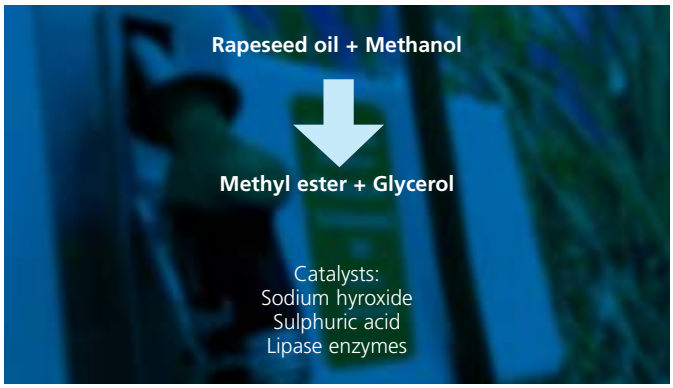

Figure 2. Biodiesel production by transesterification

no toxic effect up to 12% (w/w) when compared to diesel which had toxic effects at above 3% (w/w). Additionally, biodiesel was rapidly transformed and degraded in the soil whereas diesel was more resistant to degradation. The reduced ecotoxicity and rapid biodegradation of biodiesel may be of importance in its utilization in environmentally sensitive areas; an example of its use could be that of marine fuel for boats using the Norfolk Broads, canals and other waterways.

Microalgal Diesel Fuels

There has been considerable interest in the development of microalgal diesel fuels (Chisti, 2007). One of the main drivers for this is the higher photosynthetic efficiency of microalgae when compared to plants and hence the potentially higher productivity per unit area. The National Renewable Energy Laboratories in the United States undertook a major research programme in this area from the late 1970s through to the end of the 1990s (NREL, 2005). The programme included microalgal hydrocarbon and lipid production, as well as investigating different culturing conditions. One of the conclusions made was that although it is technically possible for microalgae to produce biodiesel fuels, the cost of extracting products for the cells was prohibitively expensive. Also microalgae need lagoons or photobioreactors for their

culture which involves capital costs. At the University of the West of England a somewhat different approach has been adopted (Scragg *et al.*, 2003). Systems have been developed to use the complete algal cell as a particulate diesel fuel. *Chlorella vulgaris* was grown in a 230 litre tubular photobioreactor (figure 3). The cells are 5μm in diameter (figure 4) which is suitable for injection into a diesel engine. Algal cells in suspension with the addition of a surfactant, Triton-X100, formed a stable emulsion with diesel fuel or biodiesel, which acts as a carrier and preliminary results illustrated that these emulsions were combusted successfully in a modified diesel engine. The purpose of this work was the development of fuels for static diesel engines rather than fuel for motor vehicles. The algae could be grown using photosynthetic energy or heterotrophically on an organic waste stream.

Figure 3. 230 litre tubular flow bioreactor for culturing microalgae

Conclusion

First generation biofuels, bioethanol and biodiesel, offer an opportunity to substitute in part current motor fuels. However, the land required to produce these fuels in the UK is limited. It is quite feasible that the UK can produce sufficient first generation biofuels to meet the requirements of the Government's Road Transport Fuels Obligation (RTFO) and possibly the EU's target for 10% fuel substitution by 2010. To go beyond these targets, advanced second generation biofuels will be required and microalgal biodiesel may be an option. Another option is Fischer-Tropsch oil, produced by gasification of plant biomass followed by Fischer-Tropsch catalysis to produce synthetic hydrocarbons. To read further on this subject, please see Hamelinck & Faaij (2006) who discuss the outlook for a wide range of advanced biofuels.

Figure 4. Chlorella vulgaris (cell diameter = 5µm)

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Stuart Shales
University of the West of England

Biofuels — ethanol production from yeast

In the second of this months features on Biofuels, **Stephen Smith** discusses the issues involved in producing ethanol from yeast

The oil or petroleum crisis of the early 1970s, saw a marked and sudden increase in oil prices, with regular appearances on national news bulletins by representatives of the Organization of Petroleum Exporting Countries (OPEC) such as Saudi Arabian Sheikh Yamani. This provided a major if not initial impetus in the consideration of alternative energy supplies on account of cost and security of supply. While oil prices have subsequently both declined and risen, there now seems little doubt amongst decision makers in the requirement for diverse alternative energy sources to those of fossil fuels.

A combination of factors has refocused attention on the development and discovery of alternative fuel sources, including those of biological origin. Such factors include the cost of oil or hydrocarbon energy sources, potentially diminishing supplies, security and maintenance of supply. Also perceived environmental factors such as those manifest in the Kyoto Protocol and subsequent initiatives such as the EU Biomass Action Plan/EU Strategy for Biofuels and UK government's Renewable Transport Fuel Obligation to limit carbon dioxide (CO_2) emissions.

Table 1. **Feedstock materials with potential to support ethanol production**

- Sugar crops (sugar cane/ sugar beet)
- Bi-product sugars from processing (molasses)
- Cereals (barley, rye, wheat), maize, yams, potatoes
- Miscelleaneous sugar wastes from palm oil extraction/ soya bean processing and sugar beet

A number of biological entities and processes have been suggested, from the simple burning of biomass, produced by the likes of fast growing plants such as willows, to upgrading biomass into more sophisticated forms of alternative energy such as biofuels, foremost amongst which are biodiesel and ethanol. Although a full and commercial understanding of the production of such entities remains incomplete, their potential attractiveness has been ceased upon. Simplistically, growing plants by the process of photosynthesis captures solar radiation by fixing atmospheric CO_2 and forming energy rich carbohydrates or more complex entities. This solar energy is exploited by the utilisation of energy rich compounds thereby only releasing a similar amount of CO_2 back into the atmosphere, hence the claims of carbon neutrality for biofuels. More specific environmental concerns can also be addressed by utilisation of biofuels, particularly ethanol. Although biodiesel may be favoured in Europe on account of the common nature of diesel engined vehicles, bioethanol has markedly attractive features, in that production of bioethanol is cheaper than biodiesel ($1.10 compared to $2.45/gallon, USDA) and requires less land usage. Ethanol acts both as an octane enhancer and oxygenate, supporting more efficient combustion and ensuring greater performance, fewer emissions and less smog in major urban conurbations. Legislation to curtail the use of the commonly employed oxygenate methyl tertiary-butyl ether (MTBE), encourages the use of ethanol as a replacement. Ethanol as a relatively non-corrosive or explosive liquid, which remains fluid at low temperatures, is readily handled, transported and stored by well understood infrastructure facilities. It may be blended with petrol or gasoline to achieve higher octane ratings (current pump "super" fuels) and greater power from modern vehicles, or form the bulk of automotive fuels in Brazil (Figure 1) and the US as E85 (85% ethanol/15% gasoline), which in turn can be used by fluid flex vehicles (FFVs).

Figure 1. Brazilian fuel station selling both gasoline and ethanol

Table 1 shows a number of feedstock materials with varying potential to form fuel from biomass, but the major goal is that of a viable procedure to utilise cellulose, on account of its common nature, as approximately 700 billion tons of cellulose are present on our planet. Although effective use of cellulose remains a very attractive goal, other currently more amenable entities exist, originating at least in part from "high energy crops" (simple sugars or polysaccharides). From a biological standpoint, maize and sugar can be considered particularly attractive. Their morphology and mode of photosynthesis, sometimes termed C4 photosynthesis, avoids photosaturation and uses less water per unit carbon fixed, thereby supporting the most efficient capture of solar energy found within the plant kingdom.

On initial evaluation, simple sugars like sucrose produced from C4 sugar cane appear very attractive, as they are easily extracted by crushing, followed by solubilization in hot water, so require only the simplest of processing equipment. In turn, yeast invertases readily convert disaccharides such as sucrose to hexose monosaccharides, supporting effective feedstock assimilation and subsequent utilisation in ethanol production. However, there are a number of perennial problems in that although potentially "energy rich", sugar cane is bulky and not readily compacted, so transportation and storage is

relatively expensive (Figure 2). The seasonal and rotational nature of the sugar crop, as produced in Brazil, may exacerbate storage demands and lead to varying or even interrupted supplies, compromising efficient plant use. Also, after extraction, sugars are readily broken down by contaminating bacteria, requiring their near immediate use or concentration and therefore associated energy demands. Such feedstock supply considerations mean that an alcohol production facility employing sugar cane as a feedstock is often simple (Figure 3).

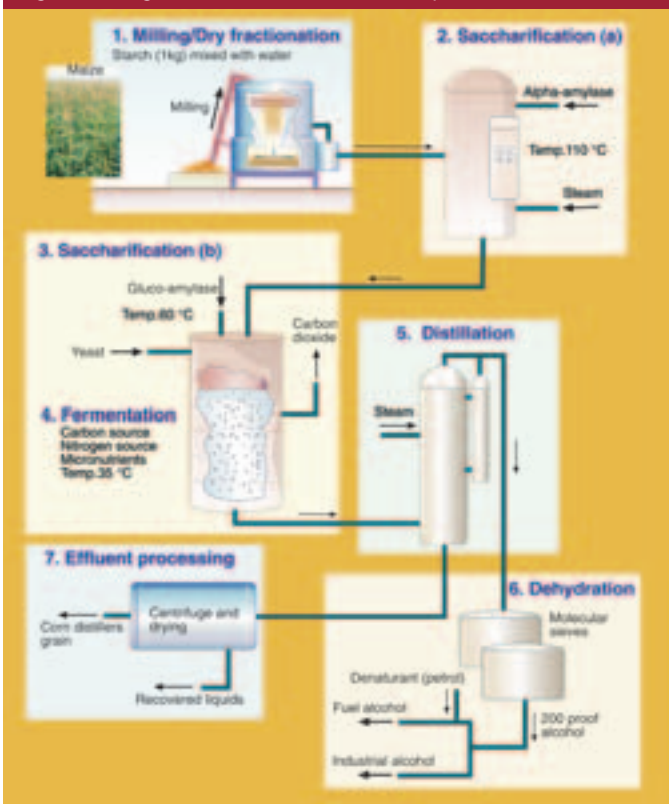
Figure 2. Sugar cane, harvest, transportation and storage

Figure 3. Typical Brazilian sugar cane based ethanol distillery

A selection of cereals or starch rich crops (Table 1) also show potential, depending on availability and suitable growing conditions. Foremost is C4 maize, known as corn in the USA, where ethanol production is the third largest market for US corn (1,430 million bushels in 2005, Renewable Fuels Association). The α-amylose and amylopectin of starch, which in turn makes up the bulk of the corn grain or kernel, is less amenable to bacterial exploitation than hexose sugar and is relatively easily removed from other kernel constituents by a process, known as dry fractionation. Remaining parts of corn kernels are rich in protein and oils, which can be used as animal feedstuffs alongside residues such as "corn distillers grain" left after the fermentation process. This promotes plant diversification and productivity, yet reduces pollution and plant maintenance.

However, in contrast to sugars such as sucrose, starch does not dissolve in water and is not readily utilised by current yeast strains, a problem also faced by beverage alcohol industries. Unlike beverage manufacturers, which rely on the biological processes associated with seed germination, ethanol producers hydrolyse starch by sequential α-amylase and gluco-amylase employment (Figure 4). This is a marked expense due to the cost (5/6 US cents/gallon of ethanol) and associated utilisation regimes. Starch-containing elements of the kernel are milled (Figure 4) to increase surface area, and mixed with hot water at around 100°C to gelatinize starch, which can occur in the form of microcrystals. Although quite

Figure 4. Diagrammatic outline of ethanol production from maize



thermostable, bacterial forms of α-amylase exist, hence enzyme addition before heating can assist matters. The temperature is allowed to fall below 90°C before the addition of α-amylase at an approximate ratio of 1.5 kg enzyme per ton of starch, which in turn endo-cleaves the long amylose and amylopectin chains rendering them more suitable for further hydrolysis. After cooling to allow further enzyme use, completion of the saccharification process is achieved by addition of gluco-amylase in similar amounts to that of α-amylase. This enzyme, potentially of fungal origin and less thermostable, requires longer incubation or dwell times. It cleaves glucose units suitable for fermentation from the many exposed ends of short starch chains and reduces liquid viscosity. While excess viscosity must be avoided, the concentration of feedstock entities is kept as high as possible to reduce water usage and ensure a high final ethanol concentration. The mix of released sugars and enzyme is then used in an ethanol fermentation process (Fig. 4).

An ethanol fermentation process can be summarized by the following simple equation:

Expected yield:	$C_6H_{12}O_6$ 100kg	⟶	2 C_2H_5OH 51.1kg	+	2 CO_2 48.9kg

Fundamental to the process of alcohol production is restricted air or oxygen availability. Energetically, yeast growth under anaerobic conditions and associated ethanol production is markedly unfavourable. Given the presence or availability of oxygen, yeast will revert to aerobic respiration compromising ethanol yields. A theoretical expected yield of 51.1% can be predicted, so losses of solar energy are considerable. Furthermore, in normal practice efficiency is somewhat less due to losses through the formation of other products such as glycerol let alone yeast biomass.

However, even with such losses, the US Department of Energy suggest that for every unit of energy spent in ethanol production, 1.4 units of energy are created.

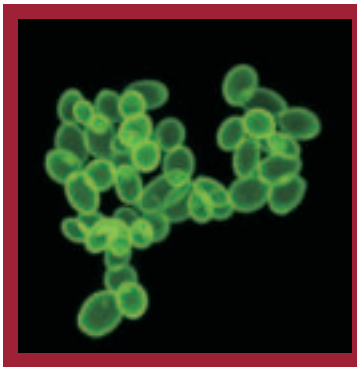


Figure 5. Saccharomyces cerevisiae: favoured microbe for ethanol production over millennia

Table 2. **Desired fermentation yeast characteristics - *Saccharomyces cerevisiae* and *carlsbergensis***

- Rapid growth/ fermentation
- Maximum ethanol/ minimum biological biomass
- Tolerances to fermenter environment (sheer forces, osmotic stress, alcohol concentration)
- Suitable 'recovery' characteristics: flocculation and sedimentation nature
- Genetic stability

As intimated, only yeast lend themselves to alcohol production, which could be considered amongst the oldest of biotechnological activities, dating back 4000 years. Although other species could be employed such as *Saccharomyces carlsbergensis*, selected strains of *Saccharomyces cerevisiae* (Figure 5) are favoured due to the demands of biofuel ethanol production where flavour and other subtleties are not a factor. Characterized strains are specifically selected to cope with the rigors of the production environment and for a variety of attributes as listed in Table 2. These strains are sold by commercial companies such as Ethanol Technology in dry or fresh forms. Such commercial concerns provide technical and instructional support, alongside other products such as antimicrobials to reduce bacterial contamination, which may compromise productivity.

As ethanol may be considered a primary metabolite, its production is not related to microbial growth phase, so theoretically either continuous or batch fermentation systems can be employed with relative advantages and disadvantages. However, whatever the system chosen, high concentrations of yeast are used to reduce fermentation time and reduce the amount of substrate converted to cellular biomass rather than ethanol. Continuous fermentation systems offer the perceived advantage of high productivity due to continuous medium replacement, cells in log phase to which ethanol production is linked, and toxic bi-product removal. However, this approach is compromised by greater production facility complexity and associated maintenance requirements, potential build up of contaminating organisms, spontaneous mutation associated with "stress" of continuous log phase growth and wasteful or incomplete feedstock usage. Batch fermentation, while not capable of matching the productivity of continuous systems, offers higher final product concentration, simpler plant requirements and fuller feedstock utilisation. As feedstock costs form a significant proportion of total costings, their waste cannot be readily tolerated particularly with resulting pollution consequences, as even a moderate ethanol production facility is reputedly capable of generating the organic pollution load of a small city.

While some residual solids, depending on their origin can be profitably sold as animal feed, downstream processing due to the high biological oxygen demand and diversity of potential wastes is a problem. Yeast is removed from the fermentation liquor by centrifugation (5000 x G) and in the amounts potentially generated this may become a problematical waste in its own right. Unlike certain beer-brewing yeast species, yeast from ethanol fuel production could not immediately be envisaged as a 'household' foodstuff such as *Marmite*. Ethanol is concentrated and part purified by "straight" distillation to a level of 95%. Further purification, particularly the removal of water, is achieved by vaporising ethanol spirit and subsequent passage over a molecular sieve bed, which in turn captures any water, releasing anhydrous ethanol. Dried residual solids and liquor, concentrated by evaporation, can be converted to make up to 45% of total animal feed. Confident predictions suggest ever greater ethanol production from a global 12 billion gallons in 2005 (RFA), with a concurrent capacity to saturate the animal feed market. This could force producers to find alternative waste treatment strategies with associated financial implications. Much store has been vested in ethanol as a major contributor to energy demands in the transport field, which in turn has stimulated a wide spectrum of industries involved in engineering, construction and biotechnology. However valid doubts remain in addition to those of waste, including sustainable agronomy, proportion of land used for food or fuel and changing agricultural commodity prices. Furthermore, ethanol fuel programmes have suffered serious setbacks, as demonstrated by the Brazilian experience, where a combination of economic factors (Table 3) seriously threatened the viability of ethanol from sugar cane production.

Table 3. **Factors associated with a 1970/80s decline in Brazilian ethanol focus**

- Rise in sugar prices makes ethanol subsidy too costly (farmers subsidised to grow sugar for ethanol)
- Conversely oil prices fell in real terms from 1970's high
- Considerable off shore oilfields discovered, reducing Brazil's reliance on imported oils
- Even though ethanol reduces gasoline/ petrol requirement, other oil fractions required (diesel, lubricants, etc)

acknowledgements

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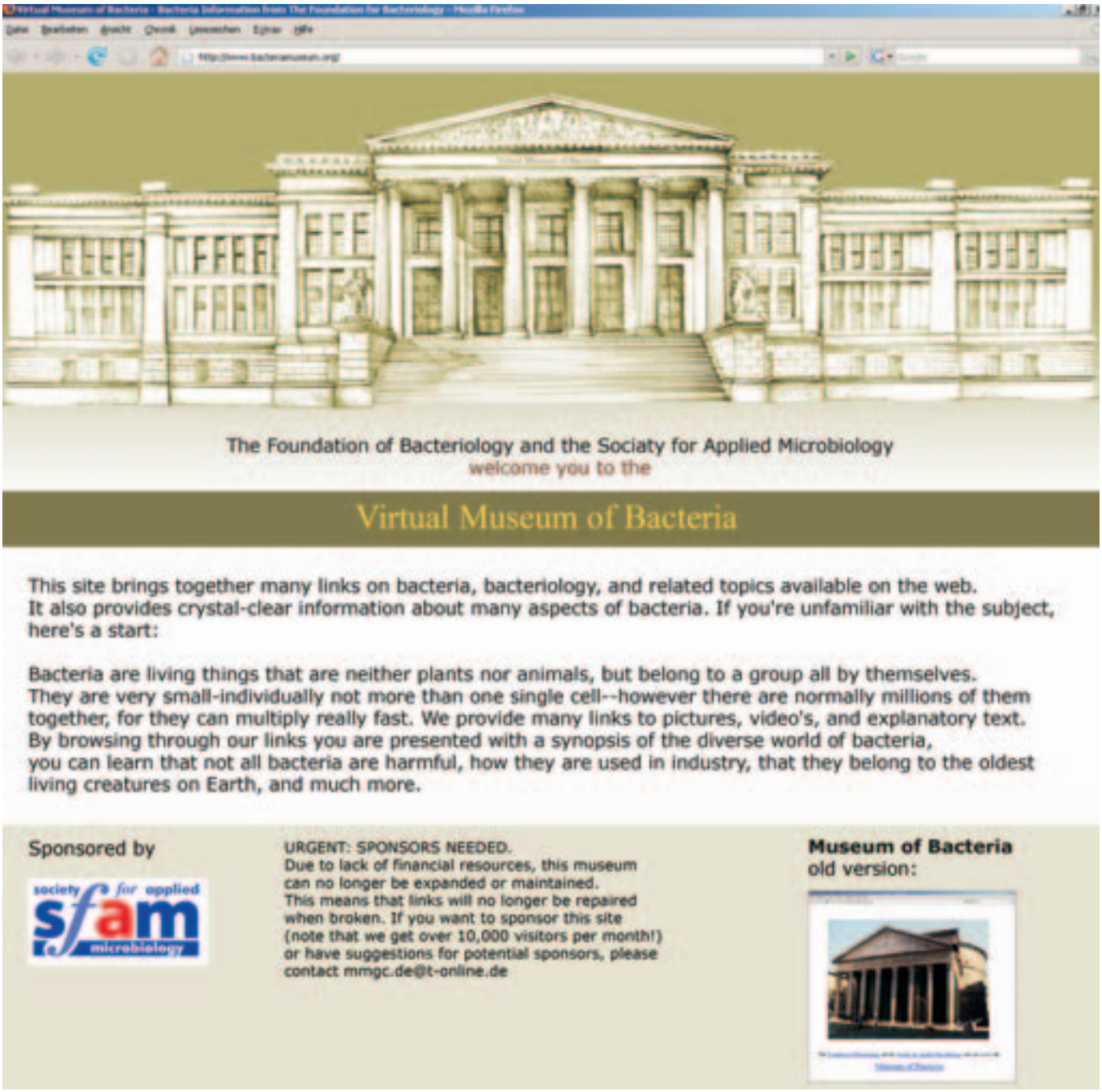
Stephen Smith
Aston University

The home page of the Virtual Museum of Bacteria, www.bacteriamuseum.org, after the remake



Virtual Museum of Bacteria

Trudy Wassenaar talks about the **Virtual Museum of Bacteria:** a website dedicated to bacteria supported by S*f*AM

It all started with small talk at a bar during a scientific conference. Prof. Martin Blaser (then at Vanderbilt University, Nashville, Tennessee) and myself were enjoying a drink and started talking about life after science. Marty expressed his wish to found a museum dedicated to bacteria, to share the beauty of the microbial world with a broader public. In my view, the beauty of bacteria lies in the diversity of their life styles, and that is not obvious from staring down a microscope. Instead, I suggested a virtual museum could provide far more freedom in terms of potential displays. We decided it would be a good idea to screen the internet for relevant web locations, to order and structure this information, and to offer access to a general audience in a fast, easy and interesting way. That is how the Virtual Museum of Bacteria began back in 1998.

The world-wide web was seven years old and Google had just begun. *Windows™ 98* was launched in that year and Netscape was still the main browser of choice for most users of the internet. The 'Foundation for Bacteriology', of which Prof. Blaser was the president, provided financial support to

develop the site. I received the help of a webmaster (a 16-year-old son of a friend) and later our team was joined by a secretary who assisted me in browsing the web. The three of us were at different locations and we have never met in person. The first version of our site went online in 1999 and it has continually served thousands of users since then, making it one of the older information sites available on the web.

The Virtual Museum of Bacteria 1999-2004

The Virtual Museum of Bacteria (VMB) is a portal: a collection of links to websites that share a common theme. In those days, search engines didn't perform as well as they do today, and portals were a common and an important tool for searching the internet. However, instead of dry lists of links, we presented the links embedded in descriptive text that could also be read as stand-alone paragraphs. The museum intended to bring together relevant internet sites on various aspects of bacteria. The content of our site depended on the internet: only subjects that were sufficiently covered on the web were represented with a display. This meant it was essential to keep the content of the museum fluid, such that it had the ability to vary with the information available on the web. As a consequence, human pathogens were highly overrepresented but Archaea largely ignored.

The analogy of a museum rather than an encyclopædia was chosen to indicate diversity of topics. The VMB is a place where websites are collected, ordered and presented but not reproduced: I should point out that the creators of the sites we link to are responsible for their own content and the source of each external link is indicated on our site.

A museum is a building, which you can enter and then wander through. Originally, I wanted to give visitors to our site the impression of entering such a building, therefore the homepage showed a large image of a museum-like building (Figure 1). By clicking on the image, one entered the main hall, and by clicking on the links on this page, visitors reached the exhibits that at this stage consisted of simple text pages. There was even an exit, though few people used this link.

Given the speed (or absence thereof) of the internet in those days, we deliberately chose to use text-only displays. Students frequently used old computers that didn't support

Figure 1. The building used on the original website

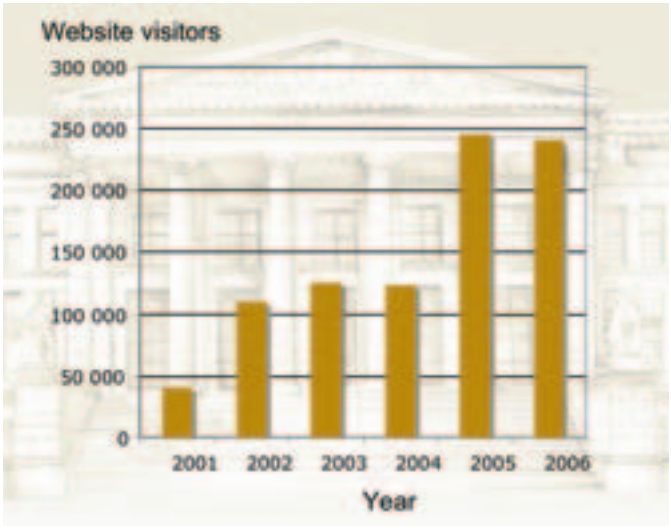


Figure 2. The number of visitors to the Museum, 2001-2006

graphics, and surfing via a telephone modem was still expensive. Though websites were already designed using moving images, our files could be viewed using every possible browser and platform and would load relatively quickly. Even Javascript and frames were not included (though many tricks were used in some of the external links). People frequently asked me about pictures of bacteria (after all, looks ARE important) and these enquiries initiated the addition of a display — 'images of bacteria.' I searched for sites which contained pictures of the various species included in the species files. *Google™ Images* didn't exist at this time so searching for images was a tedious and laborious job.

My task as curator was to introduce new exhibits, as well as to take care of existing displays. The core of the museum consisted of the five categories 'what are bacteria?', 'pathogenic bacteria', 'evolution', 'food and water', and 'how we fight bacteria', giving a total of 18 exhibits. In addition to these five categories (whose exhibits had a white background) there were various files for the specialist, comprising lists of links to sites targeting the professional user. These had a blue background. At a later stage the species filing cabinet was introduced. These exhibits were yellow and consisted of lists of links sorted into the following categories: collection sites, fact sheets, lectures and scientific links, each file relating to a different species. Later still, after receiving temporary financial support from the Foundation for Microbiology, a special feature category was introduced. Here, you could find displays such as 'Bacteria and Crime' or 'Bacterial diseases in history' and several other subjects that are loosely related to bacteria. Special feature files had a green background.

Producing a new file took anything from one day to a week, including time required for browsing, selecting sites, and writing the hypertext mark-up language (HTML). Eventually, maintenance of the site became the primary task, as links suffer from 'link rot.' In its current form, VMB includes over 1,850 external links (divided over 172 web pages) and approximately 5% of these become inactive every month. Sites that are moved by their owners can be relocated (with some efforts) but frequently, valuable sites are lost entirely. In this case a replacement is sought, or, if none is available, the exhibit text is edited so that the missing link is

Figure 3. Draft of one of the displays of the museum with the new navigation bar

no longer required. Links were checked for activity by automatic software and we used a commercial service to ensure all our links were checked on a weekly basis. Despite this, occasionally links had to be checked by hand, as a robot cannot always identify a redirect that will ensure the link remains appropriate (for instance, if the URL remains but the content of the page has changed considerably).

The VMB receives high rankings in the major search engines. A recent search for 'bacteria' in Google reported the VMB as number 5 of 59,800,000 hits, and in Yahoo as number 2 of 41,400,000 hits. On average, nearly 20,000 visitors are welcomed each month with a higher frequency of visitors to the site during the winter. Figure 2 shows the number of visitors per year recorded since 2001.

Hard times for the VMB

At the end of 2004 the Foundation for Bacteriology could no longer financially support the site. As a consequence, the webmaster and secretary could no longer provide assistance. The site became static and broken links were no longer repaired. A notice at the end of each text display informed the visitor of this sad situation. Soon emails arrived with generous offers to buy our site for $100 or so, saying that our 'valuable site' needed a new owner. I didn't agree. I regarded the site worth more than the funds that were offered. It was disappointing to see the museum slowly lose its place in a fast-paced technological world. The site was getting old and becoming old-fashioned.

The world-wide-web is fashion-sensitive like any other creative media. The design of websites is now a profession and websites that were 'en vogue' a few years ago look hopelessly out-dated now. In addition, technology has developed, so that scripting languages now enable graphics and effects to be created that were impossible or impractical to implement a few years ago. Web users have consequently become more demanding and sites that are not easy to navigate are rapidly dismissed.

The age of the VMB had become a burden. The site looked slightly outdated and navigation and search tools performed poorly compared to current standards. In addition, the structure of the site on the server (which is an essential part of any multi-page website) urgently needed to be improved to enable easier and faster maintenance.

I turned to the Society for Applied Microbiology for support, and gratefully accepted a financial injection for the site. Professional help was accepted to give the site an overhaul. The result is a modernized site with easy navigation, streamlined looks and a more user-friendly administrative set-up. An example of the new homepage is shown on the first page of this article. All displays have been updated and broken links have been repaired or replaced. The VMB is fit for the next decade. An example of a display in this new outfit is shown in Figure 3.

Is there a future for the Museum?

Although the VMB still receives a good number of hits per year, demand for such a site could be brought into question, now that an array of good search engines, Wikipedias in various languages, and other informative sites on bacteria are available. I think there remains a need for the museum.

Browse and search the web for information on bacteria, bacterial diseases, vaccines or evolution and you will find many sites listing non-scientific, esoteric, creationist or even aggressive mis-information. Searching the web using Google is now a skill which is taught at school, but do school kids and students learn how to differentiate between good and bad information, or where to turn for neutral, unbiased and scientifically sound data presented in an accessible way? It is this niche that the museum tries to fill.

For example, while writing the web page on immunology, I became irritated by the number of sites propagating anti-vaccination based on false risk assessment. People were highlighting the minor side effects of vaccination, but no longer know the damage that diphtheria, whooping cough and tetanus can do. Creationists refuse to accept evolution but the 'adaptation' of bacteria to their environment or the emergence of resistance to antibiotics is real-life evolution, that can't be denied. The general public associates bacteria with the three D's: Dirt, Disease and Death, but the beauty of this microbial world, a world that is so much older and richer than our own, is not acknowledged and is largely unknown. That bacteria can make gold, eat dioxines and produce fuel (though not all at the same time) is nothing new to an applied bacteriologist, but I think it is important that more people learn about this.

Financial support remains a concern. To retain a degree of independence, the website does not include advertisements and a call for sponsors remained largely unfruitful. If you know potential sponsors interested in supporting the site you are invited to contact the curator. We hope that the VMB will continue to serve its online public for years to come.

Trudy M Wassenaar
Curator of the Virtual Museum of Bacteria
www.bacteriamuseum.org

The unnatural history of the lichen *Rhizocarpon Geographicum*

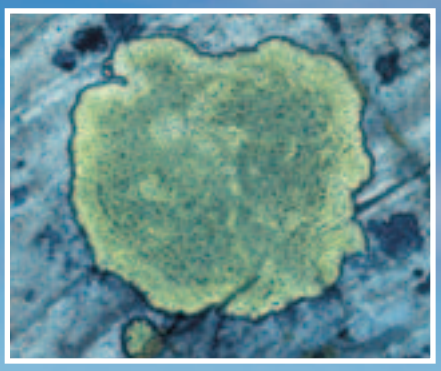


Figure 1. *Thalli of Rhizocarpon geographicum growing on a boulder facet at Snow Lake, Washington State, USA. The non-lichenised black hypothallus is clearly visible especially at the margin of the thalli*

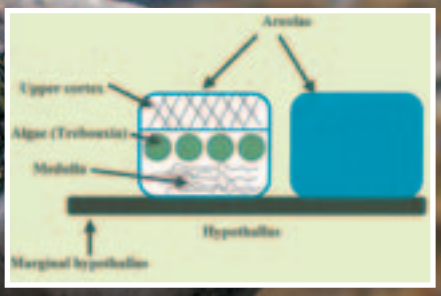


Figure 2. *Structure of the thallus of the lichen Rhizocarpon geographicum*

A lichen is an intimate association between an alga and a fungus and is regarded as one of the best examples of mutualism or symbiosis involving microorganisms. The lichen 'thallus' is highly structured but in different species shows varying degrees of integration of the two symbionts. A typical lichen is composed mainly of fungal hyphae with eukaryotic algal cells embedded in an upper cortical layer. The algal partner carries out photosynthesis and supplies the fungus with carbohydrate but there is little experimental evidence to suggest that the fungus supplies nutrients directly to the alga (Smith & Douglas 1987). The major benefit to the alga may lie in the protection offered by the thallus thus extending the range of habitats that can be potentially occupied by the alga.

There are three common lichen growth forms: 1) the fruticose type in which the thallus is attached to the substratum at a single point and forms a complex branched structure, 2) the foliose type comprising a series of radially arranged leaf-like marginal lobes, and 3) the crustose type in which the thallus comprises a thin crust tightly attached to the surface of rock or tree bark. Crustose lichens may represent the most primitive type of growth form to be found in lichens. Endolithic species, in which the lichen lives within the surface layers of the substratum, are the most extreme example of the crustose lifestyle (Armstrong, 2004a).

In these species, the upper cortex is absent while the algae and fungal hyphae are scattered within the surface layers of the substratum. Most crustose species, however, have a distinct upper cortex, an algal layer, and fungal medulla and live tightly appressed to the surface of the substratum. In some species, the margin of the thallus is diffuse and not sharply demarcated, but in others is delimited by a non-lichenised fungal hypothallus. One of the most widely distributed species of the latter type is *Rhizocarpon geographicum* (Figure 1). This unusual organism comprises yellow-green 'areolae' growing on the surface of a non-lichenised hypothallus that extends beyond the margin of the areolae to form a ring. This species grows exceptionally slowly and its considerable longevity has been exploited by geologists in the development of methods of dating the age of exposure of rock surfaces and glacial moraines ('lichenometry') (Armstrong, 2004b).

Recent research has established some aspects of the basic biology of this organism and this article describes the general structure of *R. geographicum*, how the areolae and hypothallus are formed, why *R geographicum* grows so slowly, and whether *R. geographicum* can inhibit

the growth of its neighbours by chemical means ('allelopathy').

Structure of *Rhizocarpon*

The general structure of *R. geographicum* is illustrated in Figure 2. The thallus consists of a flat basal plate of black fungal tissue termed the hypothallus. Discreet areolae that contain the algal cells develop on the surface of this fungal hypothallus but the hypothallus extends beyond the outer edge of the areolae to form a marginal ring normally 1 - 3 mm in width (Armstrong & Bradwell 2001). The areolae are highly variable in shape and morphological differences may be attributable to their origin. The first areolae to develop on the fungal hypothallus (primary areolae) are generally 'verrucose' (warty) in shape while those that develop throughout the life of the organism (secondary areolae), are often angular in shape. Within each areola, there is a cortical layer 10-80 µm in depth, an algal layer consisting of single cells of the green alga *Trebouxia* and fungal medullary tissue. The organism grows radially on the substratum, usually a smooth rock surface, by extension of the hypothallus resembling the growth of a fungus on an agar plate, albeit at much slower rates.

How does *Rhizocarpon* develop and grow?

Rhizocarpon geographicum poses several interesting biological problems. First, how is the primary thallus formed on a substratum? Second, once formed, how does the hypothallus develop secondary areolae both at the margin and in the centre of the thallus and third, how is carbohydrate supplied to the marginal hypothallus?

Four processes are thought to contribute to the development of a mature thallus of *R. geographicum*, *viz.*, the formation of the primary areolae, growth and division of areolae, the confluence of areolae, and the fusion of individual thalli to form larger individuals (Asta & Letrouit-Galinou, 1995). The development of primary areolae and the formation of the hypothallus in a related species, *Rhizocarpon lecanorinum*, has been studied by Clayden (1998). The first identifiable stage in the development of this organism is a compact granule in which fungal hyphae have become associated with a compatible species of *Trebouxia*. Thallus differentiation occurs resulting in the formation of a typical areola. The process of differentiation is associated with the formation and deposition of rhizocarpic acid, a secondary lichen substance found in the incipient cortical layer in the apical part of the granule.

The radiating hypothallus is then formed and is initiated from the basal margin of the primary areola, growing out to form a marginal ring. Removal of the marginal hypothallus in a mature *R geographicum* thallus by scraping away the fungal tissue with a scalpel, however, results in regeneration of the hypothallus but by a different mechanism. The new hypothallus is formed first, by retreat of the outer margin of the areolae and second, by new hyphal growth (Armstrong & Smith, 1987). Hence, the presence of a marginal hypothallus appears to be so important that areolae at the edge are 'sacrificed' to maintain it. After the primary areolae and marginal hypothallus are formed, new areolae develop continuously on the marginal hypothallus as it advances radially. A number of processes may be involved in the formation of these new areolae (Fig. 3). First, Nienberg (1926) observed in the crustose genus *Pertusaria* that algal cells originating in the areolae were 'pushed' into the growing area. Hence, the thallus a few millimetres from the edge was composed of radially elongated hyphae and a few migrating algal cells were pushed forwards by specialised hyphae. Second, Slocum *et al.*, (1980) observed that the alga *Trebouxia* could form zoospores within the lichen thallus that could swim from the central areolae to colonise the hypothallus. Third, zoospores from neighbouring thalli could swim to the hypothallus and initiate the areolae and fourth, the hypothallus could trap free-living algal cells on the substratum as it advanced. The development of new areolae on the marginal hypothallus was studied experimentally by Armstrong & Smith (1987). They found that new areolae were slow to develop on the isolated hypothallus but formed at a similar rate whether or not the central areolae were completely removed or separated from the marginal hypothallus by a 2 mm or 5 mm wide 'moat.' There was no evidence, therefore, that the central areolae were involved in the formation of the new areolae on the marginal hypothallus. It was concluded that areolae at the margin develop from free-living algal cells 'trapped' by the hypothallus whereas secondary areolae that develop in the centre could originate from zoospores from pre-existing areolae.

Why does *R. geographicum* grow slowly?

Rhizocarpon geographicum is one of the slowest growing of crustose lichens (Table 1) and in arctic and alpine locations; radial growth rates (RGR) of less than 0.1 mm yr^{-1} have been recorded (Armstrong 2005). To explain the slow growth of this species, it is necessary to establish how the marginal hypothallus obtains its carbohydrate supply. Materials for growth could be obtained from the central areolae either by translocation through fungal hyphae or by leakage and reabsorption, from pioneer algal cells trapped within the hypothallus, or from exogenous sources such as surface runoff.

Field experiments have suggested that the hypothallus of *R. geographicum* has the ability to utilize exogenous nutrients (Armstrong & Smith, 1996). On a south-facing rock surface in north Wales, the areolae were scrapped away to isolate the hypothallus. Hypothalli without their areolae grew at similar rates as adjacent intact thalli for two months but growth then declined and the hypothalli disappeared from the surface within six months. This result suggests that if the hypothallus could use exogenous supplies of carbohydrate they were not sufficient to maintain growth of the hypothallus other than for short periods of time. In a further experiment (Armstrong & Smith, 1987), individual thalli of *R. geographicum* were removed from rock surfaces, each on a small piece of smooth slate. Complete removal of the central areolae resulted in no measurable RGR of the hypothallus over 18 months. Removal of the areolae to within 1 and 2 mm of the hypothallus significantly reduced growth in proportion to the width of the areolae present. These results suggest that carbohydrate is supplied to the marginal hypothallus by the central areolae and that pioneer algal cells trapped in the hypothallus do not produce sufficient carbohydrate for

growth processes. Hence, the slow growth of *R. geographicum* could be a consequence of its peculiar growth form and a direct result of difficulties in transferring carbohydrate from areolae to the hypothallus. Nutrient transfer may occur only within the immediate vicinity of each individual areola (Innes, 1985) and therefore areolae located at the margin may be critical to the growth of the hypothallus. In lichens with *Trebouxia* as the algal partner, carbohydrate manufactured by the alga is released as ribitol and is then converted into arabitol and mannitol by the fungus. The levels of ribitol, arabitol, and mannitol were measured in the central areolae and marginal hypothalli throughout the year. Levels of carbohydrate were several times higher in the areolae compared with the marginal hypothallus (Armstrong & Smith, 1987) (Figure 4) consistent with the suggestion that there is restricted transport from areolae to hypothallus.

Does *R. geographicum* inhibit its neighbours?

Very slow growing lichens have a problem, *viz.*, they can be easily overgrown and out-competed on a substratum by much faster growing foliose species. Crustose species, however, often dominate not only on recently exposed surfaces but also on the oldest surfaces. In New Zealand, for example, species of *Rhizocarpon* achieve dominance on many rock surfaces and occur on more rocks than any other species (Orwin 1970). The dominance of *Rhizocarpon* could be attributable to its high tolerance of harsh conditions but it is also possible that it may have a competitive advantage due to the production of toxic chemicals ('allelopathy'). Some species of *Rhizocarpon* appear to be surrounded by zones of inhibition 1 - 5 cm wide (Beschel and Weideck 1973). Faster growing foliose species that invade this space often disintegrate on the outer rim of the bare area suggesting that a 'diffusing antibiotic' may have been responsible (Beschel & Weideck 1973).

It has been hypothesised (Grime 1979) that the production of secondary compounds, which are very common in lichens, is a defence against generalist herbivores. Hence, strongly competitive organisms produce offensive allelochemicals while stress-tolerant

Table 1. Examples of the reported growth rates (radial growth rate, RGR, mm yr-[1]) for thalli of the lichen *Rhizocarpon geographicum* from various habitats

Location	RGR (mm yr -[1])	Author
South Orkney, Ant	0.1	Hooker, 1980
West Greenland	0.05 - 01	Beschel, 1956
Alaska	0 - 0.18	Haworth, 1986
Washington State, USA	0 - 0.19	Armstrong, 2005
BC, Canada	0.26 - 0.41	McCarthy, 2003
New Hampshire, USA	0.4	Hausmann, 1948
Maritime Antarctic	0.5	Sancho, 2004
Switzerland	0.5 maximum	Proctor, 1983
North Labrador, Canada	0.10 - 0.58	Rogerson, 1986
South Norway	0.66	Mathews, 1994
North Wales, UK	0.74	Winchester, 2002
North Wales, UK	0.03 - 0.94	Armstrong, 1983

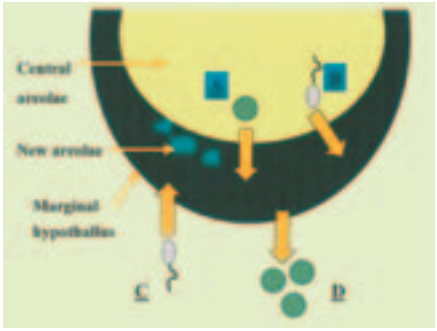


Figure 3. *Mechanisms by which new areolae could be formed on the marginal hypothallus: A) from algal cells originating in central areolae, B) from zoospores originating in central areolae, C) from zoospores originating from adjacent thalli, D) from free-living algal cells on the substratum*

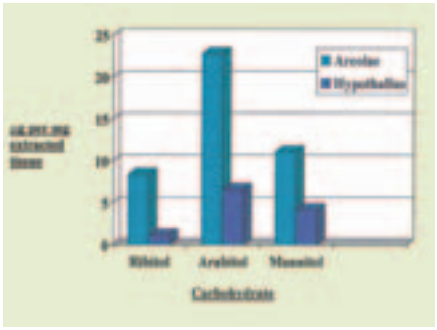


Figure 4. *Levels of the carbohydrates ribitol, arabitol and mannitol (μm per mg extracted tissue) within the areolae and hypothallus of Rhizocarpon geographicum (data from Armstrong & Smith, 1987)*

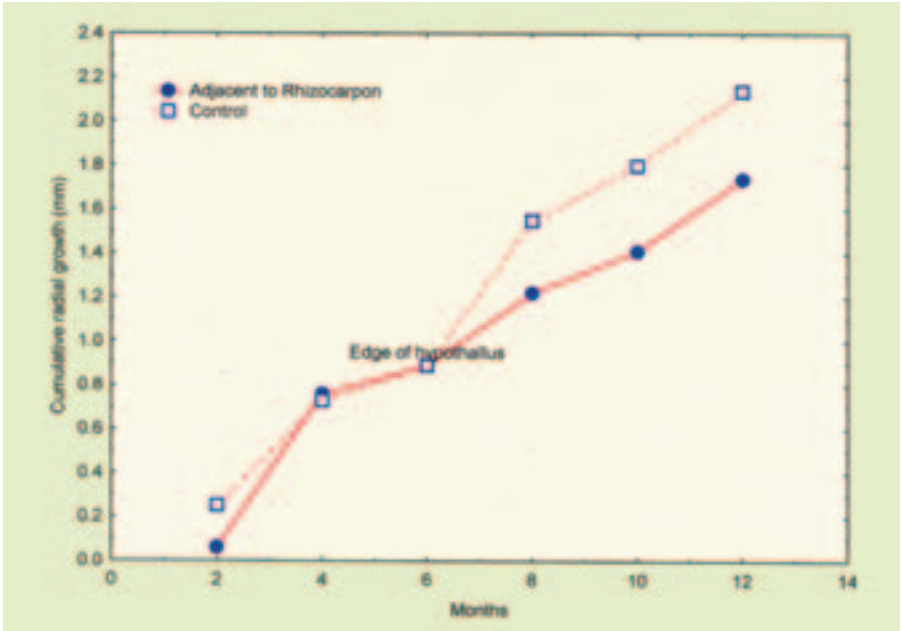


Figure 5. *Radial growth of thalli of the foliose lichen Parmelia glabratula ssp. fuliginosa as they grow into the assumed 'zone of inhibition' and at a distance away from thalli of Rhizocarpon geographicum*

organisms like *Rhizocarpon* may produce defensive (antimicrobial) allelochemicals. From a review of 34 lichen species, production of allelochemicals appeared to be positively correlated with the presence of a stress-tolerant strategy and inversely correlated with a competitive strategy. In addition, it has been reported that the presence of usnic acid and other secondary compounds in lichens may reduce grazing by microarthropods and generalist herbivores and therefore it is a plausible hypothesis that crustose lichens such as *Rhizocarpon* could use allelochemicals as a contact avoidance strategy.

Testing the hypothesis that *R. geographicum* is an allelopathic species, however, has been more difficult. In one experiment, fragments of foliose lichen species were cut from the perimeters of large thalli and glued to slate fragments either within 1 mm of the hypothallus of *R. geographicum* thalli or at a distance of at least 10 cm from the nearest lichen. Radial growth was then measured as the lichen approached the marginal hypothallus and this was compared with the radial growth of 'control' thalli. The results for one foliose species are shown in Figure 5. The foliose species did appear to be able to grow over the hypothallus and areolae of *R. geographicum* to some extent, but growth was reduced compared with control thalli especially when the lobes contacted the hypothallus. In addition, a number of thalli became discoloured during the experiment although growth did continue for a year until the end of the experiment. Hence, thalli did not disintegrate rapidly as they grew into the alleged inhibitory zone as predicted by Beschel & Weideck (1973) but there was some evidence for a detrimental effect on growth.

Conclusions

The crustose lichen *R. geographicum* has an unusual thallus structure consisting of discrete granules (areolae) containing the algal component growing on the surface of a non-lichenised fungal hypothallus that extends beyond the areolae. This morphology poses several biological problems including how the primary areolae develop, how the hypothallus is formed, and how new areolae develop on the advancing hypothallus. Current data suggest that the primary areolae are formed by chance contact between free-living algae on the substratum and short-lived fungal hyphae that germinate from spores. Similarly, secondary areolae at the margin probably develop from free-living algal cells while new areolae in the centre develop from zoospores produced within the thallus (Armstrong & Smith 1987).

Crustose lichens grow at substantially lower rates than foliose species especially in arctic and alpine environments (Armstrong, 2005). The supply of carbohydrate to the marginal hypothallus may be a limiting factor and although there is some evidence that the hypothallus can use exogenous sources of carbohydrate (Armstrong & Smith, 1996), concentrations are unlikely to be sufficient to support growth. Poor rates of translocation from the areolae to the hypothallus may explain the slow growth of this species. Although currently speculative, *R. geographicum* may use allelochemicals to restrict the growth of competitors close to the hypothallus.

There has been speculation that *R. geographicum* may represent one of the most primitive types of lichen. The existence of a marginal, non-lichenised hypothallus seems critical to the survival of this species. If the existing hypothallus is removed, a new hypothallus is developed within a year, regenerating first by retreat of the marginal areolae and then by new hypothallus growth (Armstrong & Smith, 1987).

It is possible that this growth form is actually an adaptation to ensure slow growth and consequently, a lower demand for nutrients from the environment. As a result, a greater concentration of the products of photosynthesis can be allocated to stress resistance rather than growth thus enabling *Rhizocarpon* to colonise more extreme environments than most foliose species.

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Anthony Hilton


Richard Armstrong

Stat Note 9

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

The one-way analysis of variance (random effects model): the 'nested' or 'hierarchical' design

In a previous article in *Microbiologist* (Armstrong & Hilton, 2004), we described a one-way analysis of variance (1-way ANOVA) in a randomised design. In a 1-way ANOVA, an individual observation is classified according to which group or treatment it belongs and observations within each group are a random sample of the relevant population. The scenario to illustrate this analysis compared the degree of bacterial contamination on 2p coins collected from three types of premises, *viz.*, a butcher's shop, a sandwich shop, and a newsagent. A sample of four coins was collected at random from each location and the number of bacterial colonies present on each coin was estimated. This ANOVA can be considered to be a 'fixed effects model' because the objective is to estimate the differences between the three premises, which are regarded as 'fixed' or discrete effects. There is, however, an alternative model called the 'random effects' model in which the objective is not to measure fixed effects but to estimate the degree of variation of a particular measurement and to compare different sources of variation in space and time. These designs are often called 'nested' or 'hierarchical' designs (Snedecor & Cochran, 1980).

The Scenario

The contribution of hands contaminated with pathogenic microorganisms to the spread of infectious disease has been recognised for many years. Of particular importance are communal areas where shared facilities of a tactile nature may present an increased opportunity for cross-contamination of the fingers. A study was therefore undertaken to determine the role of computer keyboards in a University communal computer laboratory as a source of microbial contamination of the hands. The data presented in this Statnote relate to a component of the study to determine the aerobic colony count (ACC) of ten selected keyboards with samples taken from two keys per keyboard determined at 9am and 5pm. Ten keyboards were selected randomly from those available in the computer laboratory and samples taken from two keys per keyboard (the 'a' and 'z' keys) using a cotton swab moistened in sterile distilled water (SDW). The swab was returned to 1ml of SDW and the swab agitated to release the microorganisms recovered from the surface into the dilutent. A 0.1ml sample of the SDW was plated onto nutrient agar and incubated at 30°C for 24 hours following which the colony forming units (cfu's) per millilitre was calculated. The data obtained are detailed in Table 1.

Linear models

There is a commonly used notation to describe the basic model of an ANOVA. The subscript 'i' is used to denote the group or class (i.e. the treatment group), 'i' taking the values '1 to a', whereas the subscript 'j' designates the members of the class, 'j' taking the values '1 to n' (hence, 'a' groups and 'n' replicates or observations per group). Within class 'i', the observations x_{ij} are assumed to be normally distributed about a mean μ with variance σ^2. This linear model can be written thus:

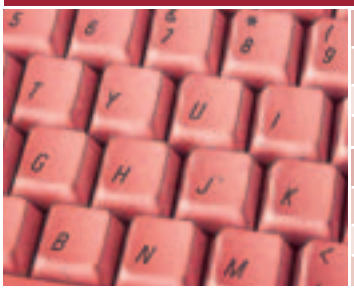
$$x_{ij} = \mu + a_i + e_{ij} \quad \text{.....1}$$

Hence, any observed value x_{ij} is the sum of three parts: 1) the overall mean of all the observations (μ), 2) a treatment or class deviation 'a_i', and 3) a random element 'e_{ij}' taken from a normally distributed population. The random element reflects the combined effects of natural variation that exists between replications and errors of measurement. The more complex types of ANOVA can be derived from this simple linear model by the addition of one or more further terms to equation 1.

Random effects model

Equation 1 describes a 'fixed effects' model in which the a_i

Table 1. Aerobic Colony Count recovered from the 'a' and 'z' keys of computer keyboards in communal use sampled at 9am and 5pm



Keybd:	Keyboard 1				Keyboard 2				Keyboard 3				Keyboard 4				Keyboard 5			
Key:	A		Z		A		Z		A		Z		A		Z		A		Z	
Time:	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm
cfu ml^{-1}	170	210	55	90	437	450	200	179	210	350	5	140	560	470	10	93	47	166	12	63
Keybd:	**Keyboard 6**				**Keyboard 7**				**Keyboard 8**				**Keyboard 9**				**Keyboard 10**			
Key:	**A**		**Z**		**A**		**Z**		**A**		**Z**		**A**		**Z**		**A**		**Z**	
Time:	**am**	**pm**	**am**	**pm**	**am**	**pm**	**am**	**pm**	**am**	**pm**	**am**	**pm**	**am**	**pm**	**am**	**pm**	**am**	**pm**	**am**	**pm**
cfu ml^{-1}	921	1043	237	178	34	21	0	8	585	658	34	67	647	457	34	56	78	67	24	3

are fixed quantities to be estimated. The corresponding 'random effects' model is similar, but the symbols KB, (representing keyboards) and K (representing keys) are included. The difference between this model and equation 1 is that KB_i and K_{ij} are considered to be random variables and the term e_{ijk} refers to errors of measurement and to the fact that microbial content is determined on two occasions (am and pm). This model can be written thus:

$$x_{ij} = \mu + KB_i + K_{ij} + e_{ijk} \quad \text{.....2}$$

Analysis of variance

The ANOVA of the data is shown in Table 2. In a random effects model, it is possible to calculate the 'components of variance' (sample estimates s^2, population values σ^2) and these are often more informative than F-tests (Table 1). The components of variance are estimates of the variance of the measurements made between keyboards (σ^2KB), between keys within a keyboard (σ^2K), and between determinations (am/pm) within an individual key (σ^2D) and can be calculated from the ANOVA. In the example quoted, the analysis suggested that the variance between keyboards was essentially zero compared with that due to keys (74707.6) which in turn, was more than 20 times that due to variation between determinations (3379.33).

This experiment provides two important pieces of information. First, there is little significant variation between keyboards or between measurements made in the morning and the afternoon compared with that between keys. This result suggests that in a future study to estimate the degree of microbial contamination on keyboards, a simpler sampling strategy could be employed involving fewer keyboards and a single sample time. Second, the difference in microbial contamination of the two keys is substantial and therefore, to improve the accuracy of estimates of contamination of a keyboard as a whole, more keys should be sampled from each keyboard. Although the experiment was not designed to test the difference between specific keys, the results suggest the hypothesis that a more frequently used key such as 'a' may have a considerably greater degree of contamination than the more rarely used 'z' key and this hypothesis may be tested by a more rigorous experiment. These results emphasise the usefulness of the random effects model in preliminary experiments designed to estimate different sources of variation and to plan appropriate sample strategies.

How to distinguish random and fixed effect factors

It is often necessary to identify whether a 'fixed' or 'random' effect model is the most appropriate in each experimental context. This is essential in more complex factorial-type designs in which there may be a mixture of both 'fixed' and 'random' effect factors ('mixed' models') (Snedecor & Cochran 1980). One way of deciding whether a factor is 'fixed' or 'random' is to imagine the effect of changing one of the levels of the factor (Ridgman 1975). If this substantially alters the experiment, for example, by substituting a confectioners shop in our previous scenario (Armstrong & Hilton 2004), then it is a fixed effect factor. By contrast, if we considered it the same experiment, for example, substituting a different keyboard or key would have little effect on the overall objectives of the experiment and it would be a 'random' effect factor. Hence, a random effect factor is only a sample of the possible levels of the factor and the intent is to generalise to all levels whereas a 'fixed' factor contains all levels of the factor that are of interest in the experimental design (Norman & Streiner, 1994). Whether a particular factor is considered to be random or fixed sometimes depends on the context. For example, the two keys measured were originally regarded as a sample from the population of keys on the keyboard. However, having selected the 'a' and 'z' key and found a significant component of variance associated with them, one could envisage an experiment to investigate the specific difference between such keys. In this new experiment, we would deliberately want to study the 'a' and 'z' keys and the factor 'key' would now become a fixed effect factor.

Table 2. A one-way analysis of variance (ANOVA), random effects model with three levels

Variation	SS	DF	MS (s^2)	σ^2 estimated
1. Keyboards	1110632.23	9	123403.581 =	$\sigma^2_D+2\sigma^2_K+4\sigma^2_{KB}$
2. Keys within keybs.	1527945.25	10	152794.525 =	$\sigma^2_D+2\sigma^2_K$
1. Keyboards	67586.5	20	3379.33 =	σ^2_D

Components of variance:	Estimated variance
Between keyboards (σ^2_{KB})	0
Between keys within a keybd. (σ^2_K)	74707.6
Between am/pm within a key. (σ^2_D)	3379.33

SS = sums of squares, DF = degrees of freedom, MS = mean square

Conclusion

There is an alternative model of the 1-way ANOVA called the 'random effects' model or 'nested' design in which the objective is not to test specific effects but to estimate the degree of variation of a particular measurement and to compare different sources of variation that influence the measurement in space and/or time. The most important statistics from a random effects model are the components of variance which estimate the variance associated with each of the sources of variation influencing a measurement. The nested design is particularly useful in preliminary experiments designed to estimate different sources of variation and in the planning of appropriate sampling strategies.

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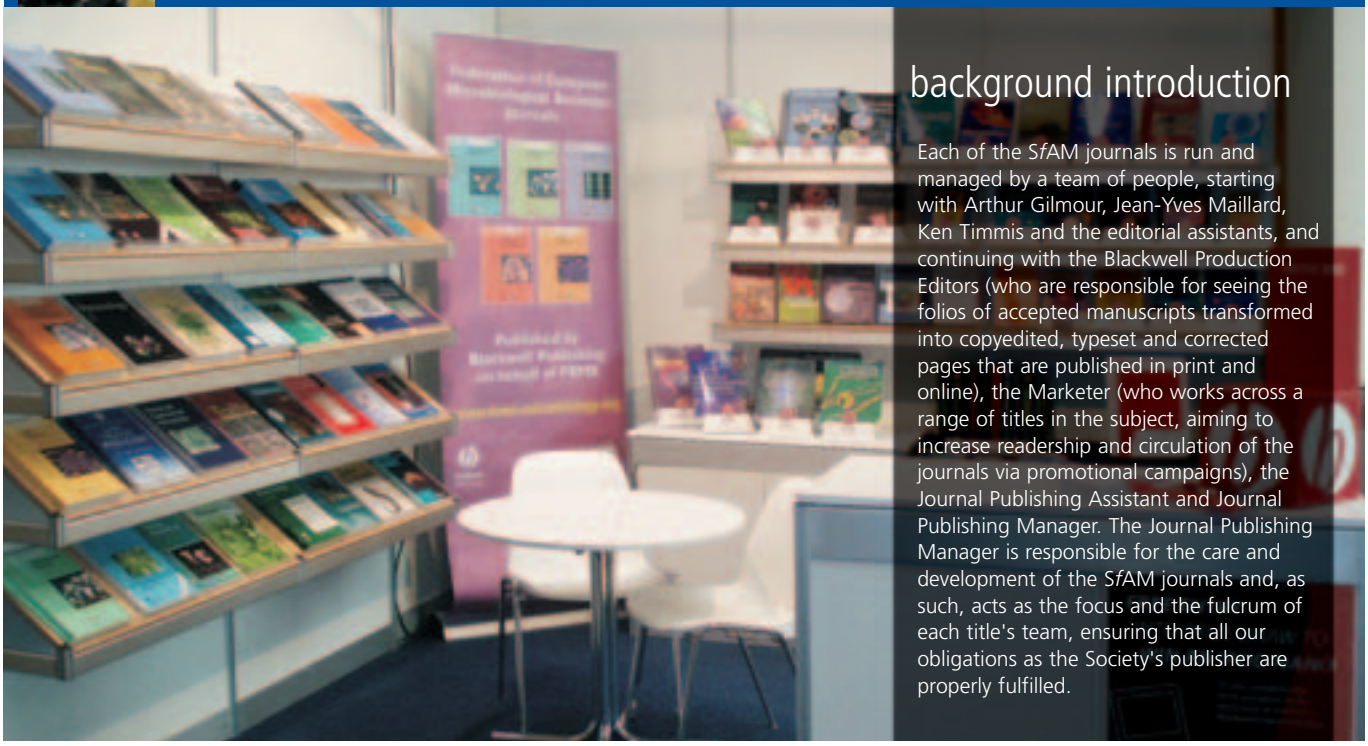
Vicky Johnson, of Wiley-Blackwell tells us how she worked her way towards her current position as Journal Publishing Manager

careers



background introduction

Each of the *Sf*AM journals is run and managed by a team of people, starting with Arthur Gilmour, Jean-Yves Maillard, Ken Timmis and the editorial assistants, and continuing with the Blackwell Production Editors (who are responsible for seeing the folios of accepted manuscripts transformed into copyedited, typeset and corrected pages that are published in print and online), the Marketer (who works across a range of titles in the subject, aiming to increase readership and circulation of the journals via promotional campaigns), the Journal Publishing Assistant and Journal Publishing Manager. The Journal Publishing Manager is responsible for the care and development of the *Sf*AM journals and, as such, acts as the focus and the fulcrum of each title's team, ensuring that all our obligations as the Society's publisher are properly fulfilled.

Scientific Publishing

Getting into Scientific Publishing can be quite a challenge, although it is far from impossible. There are many different types of publishing, but it is probably true to say that all are highly competitive and international industries. The Life Sciences division of Wiley-Blackwell specialises in earth and atmospheric sciences, whole organism biology, molecular and cell biology and, of course, microbiology. Within these subject clusters, there is much inevitable overlap with colleagues working at the more (bio)medical ends of the spectrum and those who concentrate on the physical sciences.

Fundamentally, the publishing process is the same whether you are working with books or journals, but there are some important differences. Book publishing is longer term process where successful publication of a book depends primarily on a strong working relationship between the author / compiling editor and the publisher's developing / commissioning editor.

Book publishing is neither less nor more pressurised than journals publishing, but it is certainly built around a very different calendar. Journals are published at regular intervals, usually on a fortnightly, monthly, bi-monthly or, more often in the humanities and social sciences, quarterly or yearly basis. Increasingly, journal articles are made available to readers ahead of their inclusion in an issue, either in their final, polished form, lacking only volume and page numbers, and / or as the accepted (but unedited or set) manuscript. There isn't much room for slippage in an environment where every detail is visible to the clients, that is, the authors, the readers, the reviewers. Standards are set high, and the bar moves higher every day. Journal publishing is fast-paced environment and the liaison between publishing team, editors and *Sf*AM never stops.

In addition to this 'external' responsibility, the Journal Publishing Manager provides a central point of contact within the company for journals in a given subject and enables essential communication with the wider global teams (Berlin, Boston, Chichester, Copenhagen, Hoboken, Melbourne, Oxford, Singapore, Shanghai, Tokyo, Weinheim). It is precisely this collaboration that enables the *Sf*AM journals to benefit from first-hand knowledge of market abroad, but it also fosters the germination of new ideas for projects, be they book or journal based. Projects such as the *Best Practice Guidelines on Publication Ethics* come into being as a direct result of this process.

Career progression and training

A first job in publishing does not necessarily determine a given career path, but it does allow real insight into the process and may aid future decision-making. For example, it is not unusual for new starters in Journal Publishing or Production Assistant roles to move on to jobs within Marketing, or

even more operational departments, such as Finance or Central Purchasing, and it is also possible to follow opportunities that may present themselves at other offices within the company. What the 'title team' have in common, is the independent organisation of daily and ongoing work requirements and the management of conflicting priorities to ensure that our activities are orchestrated simultaneously and that the other members of our team know what we're doing and why! Attention to detail is paramount to our success, as is the ability to identify and meet our customers' needs effectively and efficiently in order to ensure that they take away a positive view of both the S*f*AM journals and the Wiley-Blackwell organisation. In publishing we pride ourselves on being able to build strong, ongoing relationships with everyone that we work with.

I have a postgraduate diploma in Publishing and I'd worked on both books and journals in a variety of disciplines, from business and finance to area studies and development, for four years before I joined what was then Blackwell Publishing as Journal Publishing Manager at the start of 2004. Whilst my diploma undoubtedly helped me to understand production processes, technical standards and marketing theory, it isn't a pre-requisite for a career in publishing. Perhaps the single most important qualification is simply some work experience within an office environment. Similarly, it is not necessary to work in the academic subject in which you yourself are qualified (after all, it's the authors who write the papers, the referees who dissect them and the editors who decide whether or not to publish them), but you will need to be able to demonstrate knowledge of the industry.

Duties / 'Typical day'

The role of a Journal Publishing Manager is less than glamorous (dinners at the Palace of Westminster are a notable exception to the rule!), but it is certainly stimulating. The defined, cyclical nature of the 'journal year' makes it tricky to describe anything like a 'typical' day', although they almost all start with a cup of tea and a torrent of emails.

The spring season brings spreadsheets, during which I spend much of my time liaising with the Accountants to finalise figures for the previous calendar year. Once the historical numbers are confirmed and contextualised, the crystal ball comes out and it's time to prepare budgets and forecasts for the future years. At the same time, other members of the title team are busily preparing data on actual publication times and quantities, plus circulation and readership figures for presentation to, and discussion with S*f*AM and the editors. Despite the frequent emails and telephone conversations that take place, it is extremely important to find time for the editors, the society and the publishing team to meet at least once a year, in order to take stock of where we are and where we're going. The clash of issues like the increased availability of journals that came about via initiatives such as the Big Deal, the growth of R&D spending in many parts of the world, and the continuing pressure on budgets conspire to ensure that it is impossible for a journal to thrive just by 'doing what it's always done'; it's essential to keep looking for the next threat and the next opportunity.

As spring moves into summer, it's the ideal time to research, agree and implement ideas for developing the content and / or raising the profile of the journals in identified subject or geographical areas. Conferences are not just useful for distributing sample copies of journals, giving away freebies and showing display materials, they are also very useful in terms of networking. Often casual conversations at conferences have helped me gain a deeper understanding of the subject communities and define emerging and connected subject areas, which in turn can inform and inspire the strategic growth and / or development of the journals as undertaken by the editors, the society and the publisher. It's very useful to ask researchers simply and directly, what journals they use and why and what they particularly like or dislike about them — so please do be generous in sharing your thoughts!

It is often said that everyone has a book inside them and it can sometimes seem as though every researcher has an idea for a new journal; the difficulty for the publisher is matching the inspiration with the evidence. Launching a new journal into today's library market always carries an element of risk; the key is in the calculation. Once you have the spark, the next stage is to identify your proposed market and then to look at the journals that are already occupying that niche. It is easy to get swept up in the excitement of the next big idea, but it's important to that you can truthfully answer several hard questions, such as: What are existing journals offering? How successful are they? What is happening to the funding in that area? Is there room for another competitor? What will this new journal offer that isn't already available? Getting a new launch wrong is not just expensive, it is also embarrassing and may tarnish the image of the established journals in your list. I'm pretty sure we've got it right with Ken Timmis' new project, *Microbial Biotechnology*, but I'd be delighted to hear your first impressions.

Interwoven between all of the 'events' in the calendar is the rest of my job, that is, the consultation service that I provide on a range of publishing issues, such as pricing and financial management, online initiatives, editorial office best practice and peer review, emerging and continuing market trends, citations and impact factors, maximising circulation and readership, copyright and licensing and, of course, open access. My aim is to ensure that I steer my journals so that they're best placed to thrive, in terms of citations, readers, submissions and of course financial return.

After three years and goodness knows how many hours spent in airport lounges, squinting at a Blackberry screen, wrestling with spreadsheets and wading through data on ISI, I am just as enthusiastic as I was when offered the job and I simply cannot imagine doing anything else. The pace is relentless and there is no such thing as a 'quiet time', but that's part of what makes this fun.

further information

Information about careers in scientific publishing:

- http://www.thesyp.org.uk/
- http://www.alpsp.org/

Vicky Johnson
Journal Publishing Manager
Wiley-Blackwell

At last years summer conference, the enthusiasm and interest expressed by a number of student members led Dr Anthony Hilton to suggest that a student forum should be created, to increase the opportunities for student involvement within S*f*AM. As a result, the **Postgraduate and Early-Career Scientist Committee** (PEVS) has been set-up, to ensure that the main committee hears the views of young scientists and to encourage student members to become more involved in the activities of the society.

young **scientists** forum

SfAM creates Post-Graduate and Early-Career Scientist Committee

The committee met for the first time in January and discussed a number of action points that the committee members brought forward. These included facilitating communication between senior society members and students, with regard to career development, raising the awareness of what S*f*AM offers student members, for example funding opportunities and the creation of a designated section within Microbiologist for publication of relevant articles, including an update on student events and committee news.

We have decided to use this initial page to introduce the committee members, so that you know exactly who is representing you and whom you can contact with ideas, views, questions and suggestions.

We hope that other student members will contribute articles for future issues and encourage you to email the committee chair if you'd like to get involved (j.heaton2@lancaster.ac.uk). We're looking for articles of interest to the postgraduate and early-career scientists of S*f*AM: perhaps interviews, reviews or even cartoons!

Meet the committee

Jo Heaton

Lancaster University. **Chair**

Jo graduated from Lancaster in 2003 with a first class honours degree in Biological Sciences and went straight into working in the environmental health sector. She returned to Lancaster to work on an E.U. funded project, investigating the survival of enteropathogenic bacteria on the leaf-surface of salad vegetables. She is also involved in scientific outreach activity, running a 2-week Disease Detectives course for the National Academy of Gifted and Talented Youth (NAGTY) plus many weekend workshops for a variety of initiatives.

Andrew Hall

University of Wales Institute Cardiff
Secretary

Andrew achieved a first class honours degree in Food Science and won the Armfield prize in 2003. Initially, he researched the effect of UV light on waterborne pathogens and the impact of ozonated water on beer-line biofilms, before embarking on a 3 year KTP project examining the microbiology of hot and cold drinks vending. Away from academia, Andrew enjoys travel and food and can often be found attempting to combine both somewhere exotic! As secretary, Andrew is responsible for reporting to the main S*f*AM committee and bringing your ideas forward. He will also be co-chairing the student session at the summer conference in Cardiff.

Jess Rollason

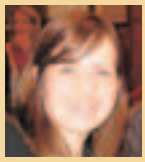
Aston University

Jess graduated from Aston University in 2001 with a BSc (Hons) in Human and Applied Biology. Following university she spent a year working for the Forensic Science Service before embarking on a round the world trip. Currently she is in her 3rd year as a postgraduate at Aston University, researching 'The epidemiology of MRSA' and has recently enjoyed starring in the BBC series *Grime Scene Investigation*, alongside Dr Anthony Hilton.

George Aboagye

Queen's University Belfast

George completed a BSc in Nutrition & Food Science from the University of Ghana and went on to study for an MSc in food safety at Wageningen University in the Netherlands. Now at Queen's University, Belfast, he is completing his PhD 'Isolation and Characterisation of *Mycobacterium avium* subspecies *paratuberculosis* in aquatic biofilms'.



Laura Wheeldon

Aston University

Laura graduated from Aston University three years ago with a degree in Applied and Human Biology. She is now in the second year of her PhD, studying the effects of antimicrobial and sporicidal agents on the germination of *Clostridium difficile*. She was also a member of the team of expert microbiologists on Grime Scene Investigation.

Tarja Karpanen

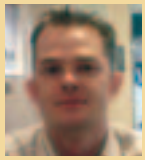
Aston University

Tarja qualified as a nurse in Finland in 1997 and after working as a nurse in the UK, she completed a BSc in Applied and Human Biology at Aston. Now studying for a PhD, her research areas are skin antisepsis and skin permeation of antiseptic agents. Tarja was also involved in Grime Scene Investigation with her colleagues Jess and Laura.

Manita Mehmi

Aston University

Another graduate of Aston's Applied and Human Biology BSc, Manita achieved first class honours and went on to study for a PhD in pharmaceutical microbiology. She is currently in her second year of her project, focussing on the optimisation of the aseptic transfer process within hospital pharmacy departments.



Alun Carter

University of Surrey

Alun graduated from the University of Surrey with a BSc (Hons) in medical microbiology in 2005. His specific interest in bacterial diseases developed after spending his undergraduate industrial placement year in a hospital microbiology department and is reflected in his PhD study, 'investigating the use of probiotic bacteria for the control of *Salmonella Enteritidis* in poultry flocks', a joint venture between the University of Surrey, Veterinary Laboratories Agency (VLA) and Probiotics International Ltd. Much of Alun's benchwork was conducted at VLA, which he describes as 'a fantastic and varied experience'.

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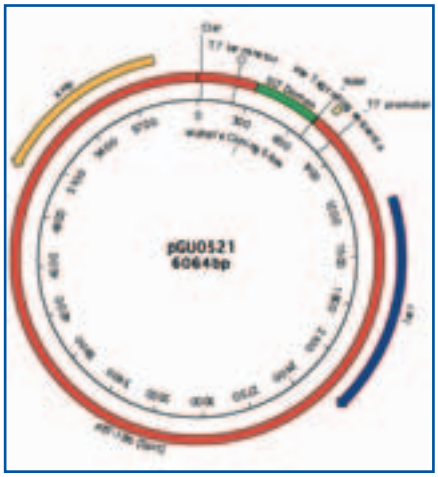
Investigation of Two Putative Lytic Transglycoylases in *Helicobacter Pylori* 26695

***Helicobacter pylori* is a common** bacterial infection of the human gastric mucosa responsible for a range of gastric disorders, including peptic ulcer, gastric carcinoma and mucosa associated lymphoid tissue lymphoma (MALT). Cell wall biosynthesis and division in *H. pylori* is aided by lytic transglycosylases, ubiquitous enzymes in bacteria, which catalyse the cleavage of the α-1,4- glycosidic bond between the MurNAc and GlcNAc disaccharide sugar residues in the peptidoglycan polymer of the bacterial cell wall. Involvement of these enzymes in cell wall synthesis by acting as 'space makers' to allow the insertion of newly formed peptidoglycan, and involvement in the separation of the cell wall makes them appropriate targets for rational design of novel antimicrobial compounds.

Investigation of the *H. pylori* 26695 genome identified two open reading frames encoding putative lytic transglycosylases, Hp0645 and Hp1572, referred to as *htgA* and *htgB* in this study. Further *in silico* analysis identified a putative catalytic SLT domain within the protein sequence of HtgA, expected to contain the active site and catalytic glutamic acid residue (Glu413) of the enzyme. The SLT domain within HtgA was also shown to contain the conserved motifs characteristic of the consensus SLT domain, as defined by the NCBI's Conserved Domain database. This particular aspect of the study involved protein expression of this catalytic SLT domain from *H. pylori* 26695 *Htg*A.



Recombinant plasmid construct pGU0521. Green segment represents the SLT Domain DNA insert, red segment represents pET-19b, yellow segment represents the His tag, and orange segment represents the ampicillin resistance gene in pET-19b. NdeI and ClaI restriction sites are indicated with protruding black lines. Plasmid maps were generated using MacVector™ 7.0.

The putative catalytic SLT domain sequence from *htgA* was initially amplified by PCR using primers based on the known *H. pylori* 26695 sequence to produce an approximately 400bp DNA fragment. Primers were designed with flanking *Nde*I and *Bgl*II restriction sites in the forward and reverse primers respectively to allow for site-directed cloning into the expression vector pET-19b.

The resulting recombinant plasmid was isolated and was referred to as pGU0521. The integrity of pGU0521 was investigated by PCR, as well as restriction enzyme analysis. The expected DNA fragments of 728bps and 5362bps were obtained. DNA sequencing of the plasmid construct was then conducted and confirmed that the SLT domain insert was cloned into pET-19b in the correct reading frame and that all nucleotides matched the sequenced *H. pylori* 26695 SLT domain so that translation would produce the correct amino acid sequence. pGU0521 recombinant plasmid was subsequently transformed into an expression strain of *E. coli*, BL21 (DE3). The presence of pGU0521 within *E. coli* BL21 (DE3) transformants was once again confirmed by PCR and restriction enzyme analysis.

Expression of the recombinant SLT domain was induced in four BL21 (DE3) transformants carrying pGU0521 by the addition of 1mM IPTG. For comparison, an *E. coli* BL21 (DE3) strain carrying only the pET-19b vector, was also included. Uninduced

samples of both *E. coli* BL21(DE3) with pGU0521 and pET-19b were prepared. Total cell protein from all samples was analysed using SDS-PAGE analysis on a 15% resolving gel. The expected size of the expressed SLT domain was approximately 16kDa, however, overexpression of the domain was not conclusively determined by SDS-PAGE analysis. Therefore, Western blot analysis was carried out to confirm expression of the recombinant SLT domain.

Expression of a recombinant protein using the pET-19b expression vector attaches an N-terminal Histidine (His) tag, which allows for identification of the expressed protein and purification using affinity chromatography. In order to determine whether the SLT domain with the attached His tag was being expressed, a Western blot was carried out using a mouse monoclonal antibody directed against five histidine residues of the His tag. A goat anti-mouse IgG horse radish peroxide conjugated antibody was used as the secondary antibody, which could be detected using commercial chemiluminescent substrates.

Two distinct bands of approximately 14kDa and 13kDa, were obtained for the Western blot. This may suggest that potential proteolytic cleavage sites exist within the amino acid sequence of the SLT domain. This may be overcome by the addition of protease inhibitors following lysis of the cells to be analysed by SDS-PAGE.

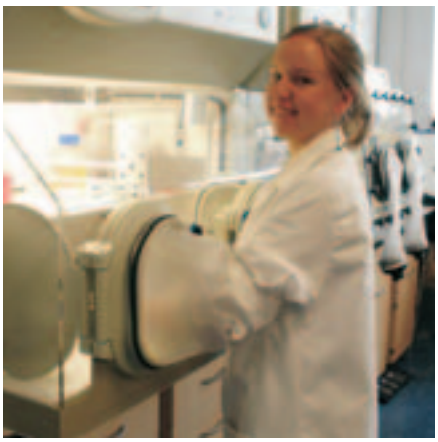
Another problem encountered with the expression of heterologous proteins in an *E. coli* host strain is rare codon usage. Certain codons in *E. coli* are underrepresented and the presence of these rare codons within the expressed protein can lead to translation stalling, premature translation termination, translation frameshifting and amino acid misincorporation. Seven of these rare codons were identified within the amino acid sequence of the SLT domain, with two rare codons occurring consecutively. These codons may also have accounted for the multiple bands present on the Western blot. The use of a host strain, such as Rosetta™, designed to enhance expression of target proteins containing rare codons may prevent the expression of truncated protein products.

Eventual purification and determination of the protein structure of the SLT domain, as well as its interactions with putative substrate molecules using NMR, may lead to a better understanding of the catalytic mechanisms of the soluble lytic transglycosylases in *H. pylori* and the development of compounds directed against these enzymes. As *H. pylori* is a prevalent bacterial infection world wide associated with a range of severe and debilitating gastric disorders, antimicrobials targeted at disrupting the cell wall of this organism may help in the treatment of infection.

This project was a continuation of a study undertaken for the degree of Bachelor of Biomedical Science with Honours and conducted under the supervision of Associate Professor Victoria Korolik at Griffith University, Gold Coast Campus, USA.

Lucy Shewell
Griffiths University, Gold Coast, USA

Searching for Novel Antimicrobials against *Clostridium difficile*



For ten weeks during the summer, I was given the opportunity to work with Teagasc at Moorepark Food Research Centre (MFRC), Fermoy, Co. Cork on a project in the Alimentary Pharmabiotic Centre (APC). Research within the APC focuses on all aspects of gastrointestinal health. I have just completed the second year in Biomedical Science at Cork Institute of Technology. This course enables graduates to practice as medical laboratory scientists in hospitals, and there is a large element of Microbiology in the course. The third year of the course offers nine months work experience in a medical laboratory and I wanted to gain some experience in this area before I entered that year. The *Sf*AM Students into Work Scheme enabled me to do this.

My project was centred on the microorganism *Clostridium difficile*, particularly screening for narrow-spectrum antimicrobials against this pathogen. Previously a bacteriocin isolated and characterised at MFRC has been shown to have anti-*C. difficile* activity. However as it is broad-spectrum it may also affect beneficial microflora in the gut and a narrow-spectrum antimicrobial is therefore preferable. *C. difficile* is a Gram-positive rod. It is motile and produces a cytotoxin and an enterotoxin. It is an opportunistic pathogen and is an important cause of antibiotic-associated diarrhoea and pseudomembranous colitis. It can affect patients on broad-spectrum antibiotic therapy as these antibiotics kill normal gut microflora, consequently allowing the overgrowth of *C. difficile*. The number of *C. difficile* cases has risen dramatically in recent times. According to the Health Protection Agency, in English hospitals the number of reported incidents in patients aged over 65 rose by 17% last year, from 44,107 in 2004 to 51,690 in 2005. In Irish hospitals there has been a similar increase with 1,269 patients at 32 hospitals found to be infected with *C. difficile* in 2005.

As *C. difficile* is an anaerobe, I learned to work with microorganisms under anaerobic conditions. The aim of the project was to screen for antimicrobials in faecal samples from patients with irritable bowel syndrome (IBS). The faecal samples were first subjected to ethanol shocking, i.e. equal amounts of faecal sample and absolute ethanol were mixed. The purpose of this ethanol shock pre-treatment is to encourage spore formation and also to eliminate competing faecal flora. The faecal samples were then spread-plated on duplicate plates of Wilkens Chalgren agar and incubated anaerobically at 37°C for five days. These plates were then overlaid with *C. difficile* or with *Listeria innocua*. The plates were then examined for zones of inhibition and any colonies showing definite zones were re-streaked on a fresh plate. When a pure culture had been obtained a colony was re-streaked on duplicate plates. One plate was incubated

anaerobically at 37°C and the other incubated aerobically at 37°C. The method of stocking the culture then depended on its ability to grow either aerobically or anaerobically.

During my ten weeks in the laboratory I also characterised bacterial isolates from existing in-house culture collections. To achieve this, a technique called pulsed field gel electrophoresis (PFGE) was used. This is a DNA-based fingerprinting method used to distinguish individual strains within a culture bank. The spectrum of inhibition of antimicrobials produced by gut microflora isolates was also studied using well diffusion assays. The antimicrobials were further characterised by investigating whether or not they are active at neutral pH. I also investigated the activity of antimicrobials under simulated intestinal conditions. In the laboratory at MFRC antimicrobial compounds are characterised using the most up to date methods such as mass spectrometry and HPLC and I was given the opportunity to work along side the scientist involved in this work to gain experience in the use and applications of this equipment. I also gained experience of DNA extraction methods, improved my aseptic technique, learned how to prepare culture stocks, prepared and sterilised media and gained experience of spread plating.

This project allowed me to gain experience of working in a research laboratory and to learn new skills and techniques and see first hand what working in this environment is like. I am certain that this will be an advantage in college and in a future career in Biomedical Science. I would like to express my thanks to Professor Paul Ross for allowing me the opportunity to work at MFRC. I would also like to thank Mary Rea and Gillian Gardiner for all their time and help. Thanks also to the other staff in the laboratory for their support. Finally, I would like to thank S*f*AM for giving me this opportunity through their ***Students into Work Scheme***. I would recommend this scheme to any student who has an interest in Microbiology and who would like to gain practical experience of working in a laboratory.

Michelle Gardiner
Cork Institute of Technology, Ireland

President's Fund reports

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Therapeutic use of an artificial diet, with particular reference to cystic fibrosis (CF)

I was very interested when I read the title of the S*f*AM 2006 Summer Conference *"Living together: polymicrobial communities."* I hadn't attended a S*f*AM conference for some time and I felt it would provide an opportunity to update my knowledge, renew contacts and make new friends. I decided to apply for the Presidents Fund Grant and was delighted when it was awarded to me.

Having maintained many cultures relevant to the area of gut microbiology while working for National Collection of Industrial Bacteria (NCIMB), I was keen to find out as much as possible about our present knowledge of the role of these bacteria in the gut and of microbial interactions with the gut immune system.

I was intending to review research performed my late father, Dr James Allan, some thirty five years ago into the therapeutic use of an artificial diet, with particular reference to cystic fibrosis (CF). This is an inherited chronic disease affecting the lungs and digestive system of about 70,000 children and adults worldwide. Riordan *et al.*, discovered the CF gene in 1989. The normal Cystic fibrosis transmembrane conductance regulator (CFTR) protein product is a chloride channel protein found in the membranes of cells lining the passageways of the lungs, intestine and other organs. Normal sodium and chloride ion balance is needed to maintain the thin mucus layer. The abnormal CF gene leads to damage or destruction the CFTR protein. This causes the mucus to become thick, which blocks the ducts. When this happens in the pancreas, the digestive enzymes cannot reach the small intestine to break down food. In 1970 Allan *et al.*, first described the therapeutic use of an artificial diet, (based on a beef serum hydrolysate, medium chain triglycerides, Caloreen (a glucose polymer mixture) and a vitamin and mineral mixture) in treating *E.coli*

O114, Cystic fibrosis and uraemia. They suggested that not only did this diet support growth and nutrition, but also it may have other therapeutic advantages. The use of this diet in CF was further described in 1975 (Allan, 1975; Berry *et al.*, 1975.) They followed 45 patients over five years and found where the total 24 hour needs were supplied as artificial diet, there was marked improvement in nutrition and energy. Of much more interest, however was confirmation of a previous observation (Allan *et al.*, 1973) that not only were the incidence and severity of pulmonary episodes reduced, but also there was evidence of an amelioration in the usual progressive course of lung destruction. A radiological survey of 20 cases, assessed before and after the diet, indicated that seven showed radiological improvement and thirteen showed no deterioration.

Nutritional and immunological problems are interdependent in CF. Allan believed there was strong evidence of an immunological disorder. Collaboration with McFarlane *et al* (1975) found that most CF patients had precipitins in large numbers in the sputum but not in the serum. It appeared that there was some factor in ordinary diet, which was both antigenic and allergenic, and the improvement in both clinical and radiological lung status may be related to reduced exposure following the virtual substitution of a Caloreen, amino acid artificial diet in place of an ordinary diet.

The Allan diet was the first serious attempt to provide nutrition in CF from what would now be called an elemental diet. Elemental diets are today used as dietary supplements, rather than sole forms of nutrition and therefore are much less likely to have immunological benefits.

After Allan's death in 1979, clinicians found patient acceptance of the diet presented the greatest challenge and his therapy was not continued. Not much attention at that time was paid to his immunological findings as the improvement in CF was put down to improvement in nutrition. The subsequent introduction of acid resistant enzymes in the1980's aided the nutritional state of CF patients but did not halt the progression of the disease. Despite attempts at gene replacement therapy, national data from many countries today show that the majority of CF patients still develop chronic infection of the respiratory tract with *Pseudomonas aeuruginosa* during childhood and adolescence. Infections with *Burkholderia cepacia* are very serious, indeed strain J2315 is clinically resistant to all known antibiotics. Infection is followed by a slow deterioration in respiratory function and nutritional state. Maximum life expectancy for CF patients is about 30 years; many die younger.

Today's treatment relies on enzymes and antibiotics but is followed by chronic lung disease, yet when his CF patients were put solely on Allan's diet 65% showed no radiological deterioration and 35% improvement of the lungs.

Allan's work was unfinished. Further immunological investigations on patients while taking the diet were not performed. He never considered the diet to be a cure and was searching for an answer as to how the diet exerted its effects.

Until my friend's daughter, Hannah died a year ago aged 16, I thought the outcome for CF sufferers had greatly improved. As the artificial diet showed great promise at the time, and the suffering and outlook for CF patients today remains practically the same, is it time to look at this approach again?

While the CF Trust already funds some excellent microbiology projects, there does not appear to be one on oral artificial nutrition.

Perhaps we should we draw together experts from different fields to mount a new project to re-evaluate the therapeutic use of an artificial diet for the treatment of cystic fibrosis. Some questions which could also be raised are:

- What is the gut flora of CF patients on ordinary diet?
- Could this flora be beneficially influenced by probiotics?
- How is the gut flora altered by an artificial diet?
- How is the immune system affected when patients are taking an artificial diet?
- Is it possible to tailor the diet of CF patients to bypass the defective CFTR protein?

I would be very pleased to hear from anyone who is interested! I found the conference a pleasure to attend and was made very welcome by all. It was great to see the same atmosphere of friendliness and readiness to exchange ideas was just the same as a few years ago!

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Sue Lindsay

Variable Number Tandem Repeats for analysis of *Salmonella* Typhimurium from animals and humans

***Salmonella* Typhimurium is** primarily a pathogen of cattle but is a common cause of human food borne gastroenteritis through the handling or consumption of contaminated meat (Davies 2001). Both pork and poultry in addition to dairy products, beef and seafood are major vehicles for the transmission of *S.* Typhimurium from animals to humans but the relative risk factors from these sources for human infection is uncertain. Molecular genetic methods have revolutionised the identification of microbial strains, and provided a key tool in recognising and controlling outbreaks. The current 'gold standard' method of choice for

molecular typing of *Salmonella* and other food borne pathogens as a means for outbreak identification and source identification is macrorestriction fingerprinting of total genomic DNA using pulsed field gel electrophoresis to separate DNA fragments generated by restriction enzyme digests of chromosomal and plasmid DNA (commonly referred to as PFGE). This method has good discriminatory power and can be standardised, which has lead to the establishment of a number of international networks (Fisher 1999; Ribot *et al.*, 2006). Despite this, there are a few negative aspects to the method, in particular it can be labour intensive and within certain phage types, it has been unable to discriminate effectively, leading to problems in outbreak investigations and tracing of strains.

Here the project aimed to investigate a method using variable number tandem repeats (VNTR). This is a next generation typing method, which exploits areas of the *S.* Typhimurium genome containing repeats in the DNA sequence. These repeat areas consist of multiple units of DNA arranged in a head to tail assembly, which can be amplified by PCR, using external primers. The size of the fragment can then be accurately measured using a capillary sequencer and the number of repeats within the fragment calculated. For the purposes of using the method for typing bacteria, a number of different repetitive areas of the genome are investigated, hence the term 'Multiple Locus Variable Number Tandem Repeat Analysis' (MLVA), which enables a numeric profile to be compiled, combining the values for the number of repeats at each locus (for example 2 4 6 8 6). This facilitates the easy electronic storage and transfer of results between laboratories. These repetitive areas evolve rapidly allowing an array of different alleles in different isolates. Individual strains within a bacterial species often maintain the same sequence element but with different copy numbers for repeated regions. The targeted loci are not under selective pressure so variation is neutral and highly effective for molecular typing.

A *S.* Typhimurium VNTR scheme was described by Lindstedt *et al.*, (2004), which was based upon the use of five variable loci located within genes for regulatory proteins as well as on the *pSLT* plasmid. For this project we initially tested the method of VNTR (using the Lindstedt method) against the existing method (PFGE) for a total of 195 isolates obtained from pigs. Results by VNTR and PFGE type were clustered into a dendrogram using the program Bionumerics (Applied Maths), which assembles strains together mathematically according to the degree of relationship and enables the number of distinct types by each method to be identified. VNTR demonstrated improved discrimination with 64 extra types identified when compared to the number of PFGE types. By further examining the data it was also possible to demonstrate the improved discrimination of VNTR for the different phage types of *S.* Typhimurium. For Definitive phage type (DT) 104, one of the most common phage types of *S.* Typhimurium, nine different PFGE types were displayed compared to forty different VNTR types.

Based on this intelligence the project went on to further examine the utility of the method for comparison of isolates from different sources. This included testing similar numbers of isolates from human *S.* Typhimurium infection, poultry and pigs. The results were examined using the clustering method as used before. In a number of instances there were groups of isolates clustering together at 100% similarity, which had been obtained from different sources. For example two isolates one of human origin and one of poultry origin of DT 104 shared the VNTR profile 2-7-9-0-3 and isolates of pig and chicken origin also shared the same VNTR profile of 2-8-10-8-3. Also there were some isolates particularly from the pig isolates, which were distinct from all the other isolates tested. In order to try to attribute a source to human infection the project also compared the results obtained at each locus. One locus demonstrated source diversity, suggesting it may have source-specific properties.

VNTR typing has taken the discrimination to a further level to that obtained through PFGE, and demonstrated further types in isolates, which were identical by PFGE. Identical VNTR types were found across the three populations, demonstrating a certain amount of overlap in genetic diversity of isolates, which may confirm previous findings that food-producing animals have an active involvement in the dissemination of *S.* Typhimurium through the food chain. The good discriminative power and reproducibility of VNTR suggests that the method could be used for both comparative typing and library typing and therefore may be suitable for use in discrimination of outbreak isolates following further testing. VNTR fulfils the criteria for a molecular typing method, by providing the ability to standardise, provides a common nomenclature and the ability to provide reproducible results with minimal hands on time and reagent costs. With further large-scale studies on isolates it should be possible to accurately assess if VNTR can provide the necessary level of discrimination for epidemiological investigations, and required strain discrimination.

I would like to thank the Society for Applied Microbiology for awarding me a grant from the President's Fund. This enabled me to present this work at the Third International Symposium of *Salmonella* and Salmonellosis (I3S), in St Malo, France. 10-12th May 2006. This work was jointly funded by the Health Protection Agency and Veterinary Laboratories Agency.

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Emma Best, Centre for Infection
Health Protection Agency

Characterization of a traditional PDO cheese — the case of São Jorge cheese: microbiology, biochemistry and safety

Protected designation of origin (PDO), or Appéllation d'Origine Controlleé (AOC) are geographical indications defined in European Union to protect regional foods. These laws protect the names of several product types, such as cheeses which can only be labelled with the PDO corresponding designation if produced in the region according to a defined method using a certain raw material. The purpose of the law is to ensure that only products genuinely originating in that region are allowed to be marketed as such.

Studies have been undertaken to characterize PDO dairy products (Macedo *et al.*, 1993, Moreno 2004) focusing on the evolution of microbial flora throughout maturation, in attempts to help understand the role of such microorganisms in cheese ripening and eventually lead to technological manufacturing processes that increase product safety but preserve important features such as the taste, aroma and texture of traditional cheeses.

Figure 1. *Last steps in finishing São Jorge cheese before going to the market*

São Jorge (PDO) cheese from Azores represents approximately 51% of all traditional Portuguese cheeses in terms of production. It is produced in small dairy farms, from raw cows' milk and in the absence of any commercial starter. In our laboratory, a research project was set to characterize São Jorge cheese (Figure 1). The project begun by studying some basic socio-economic factors related to the production of this cheese; the safety of the cheese was then considered by studying the incidence of pathogens such as *Listeria monocytogenes* in the production line; next the main microflora involved in cheese manufacture and ripening was investigated and characterized, followed by studying the biochemical features throughout cheese ripening. The final goals were (i) to understand the main microbial and biochemical changes occurring during manufacture and ripening of São Jorge cheese, (ii) to screen for the presence of *Listeria monocytogenes* in the production line towards developing specific technological targeted intervention strategies against the presence of this pathogen in finished cheese and (iii) to characterize and develop a collection of microbial strains in order to potentially control cheesemaking procedures leading to more consistent, and safe São Jorge cheese while preserving its PDO specific characteristics.

Samples representative of the whole production chain of São Jorge PDO cheeses were collected over one year. They were tested for *L monocytogenes*, selected physicochemical parameters and the main lactic acid bacteria (LAB) groups were identified. Through a combination of conventional biochemical methods and genotypic methods some of the isolates were identified at species and strain levels, their antimicrobial properties against listeria were studied and then used in different combination in a starter culture to manufacture cheese from pasteurized milk at a pilot scale.

Listeria monocytogenes is a Gram positive opportunistic pathogen, ubiquitous in the environment. The symptoms of the disease listeriosis can vary from mild, flu-like illness to meningitis and meningoencephalitis. In pregnant women listeriosis can result in abortion, stillbirth or premature labour (Adam & Moss, 1995). While most human listeriosis cases appear to be linked to consumption of Ready-To-Eat (RTE) food products that have undergone listeriocidal heat treatments and have been contaminated post-processing from environmental sources, some human listeriosis cases and outbreaks have been linked to consumption of food products that did not undergo heat treatment and appear to have been contaminated by on-farm sources (Lundén *et al.*, 2004). It is therefore, a great concern for public health authorities. A wide range of food products, such as dairy products, have been associated with both outbreaks and sporadic cases. Measures and regulations to reduce contamination of RTE foods with *L. monocytogenes* are in place in most countries. European rules enforce the safety of all products in the market recommending the application of GMP and HACCP.

Since many artisanal cheeses are produced with raw milk, they represent products that potentially allow for growth of *L. monocytogenes*. This can be due to lack of heat treatment of the raw materials or to a potentially higher risk of product contamination from environmental sources (e.g., soil, manure) if production sites lack sophisticated approaches to contamination control. Manufacturing on farms and in small plants often lacks also reproducibility, leading to products without consistency for parameters (pH, moisture and salt) which may act as a hurdle to prevent undesirable bacterial growth (Tienungoon *et al.*, 2000). It is thus particularly critical to better understand *L. monocytogenes* prevalence and contamination patterns in these products.

L. monocytogenes was detected in less than 2% of raw milk samples analyzed, and on average the physico-chemical parameter values of São Jorge cheeses after four months of ripening, presented values that likely minimise the risk of *L. monocytogenes* outgrowth during ripening and storage (Kongo *et al.*, 2006).

The unique flavour of each cheese is a consequence of a complex set of biochemical activities which take place mainly during cheese ripening, and often brought about by LAB (Fox & McSweeney, 1996). Characterization of LAB in artisanal raw milk cheeses is therefore an important issue as it may eventually lead to scientifically-sound modification of practices and

optimisation of the characteristics of the cheese. This realisation accounts for the increased interest in genotypic and phenotypic studies focused on wild LAB isolated from traditionally manufactured dairy products in attempts to improve such traditional foods (Coppola *et al.*, 2001).

Lactobacillus and *Enterococcus* groups were the most frequently isolates and the enzymatic profiles of identified strains suggest their vital role in aroma and taste development of ripened São Jorge cheese.

As stated before, **proteolysis** is a key biochemical event during cheese ripening playing a vital role in the development of texture and flavour (Fox & McSweeney, 1996). Analysis of São Jorge cheese samples by electrophoresis revealed extensive proteolytic degradation profiles throughout ripening time. The manufacture of experimental cheese following the traditional protocol of São Jorge cheesemaking, with the exception of using pasteurised milk and a defined starter culture prepared with LAB isolated from São Jorge natural whey starter was tried. After ripening, experimental cheeses were compared to São Jorge PDO cheeses. Our results indicate that the manufacture of a more consistent product yet organoleptically close to traditional São Jorge cheese may be feasible from a technological point of view.

Lactic acid bacteria are also able to produce a variety of compounds which contribute to the safety of fermented foods by producing antimicrobial compounds against competing flora, including food-borne spoilage and pathogenic bacteria (Davidson & Hoover 1993). These include lactic acid, which exerts an antimicrobial effect through reduction of pH, hydrogen peroxide, carbon dioxide, diacetyl (2,3-butanedione) and high-molecular-mass compounds like bacteriocins. Nisin is the best defined bacteriocin produced by LAB that has been approved for use in food products. Some LAB isolated from São Jorge cheese produce antimicrobial compounds with activity against *Listeria monocytogenes*, (Figure 2) however such compounds have not been fully studied yet.

São Jorge cheese is an artisanal cheese which showed several species dominating at different ripening times, however *Lactobacillus* and *Enterococcus* were those found at higher levels from the beginning to the end of ripening. These bacteria may also be causing the main biochemical changes, such as proteolysis, detected by electrophoretic analysis. Our results show that milk used for São Jorge cheese production as well as finished products is rarely contaminated with *L. monocytogenes*, so small improvements in the manufacturing methods should guarantee a highly safe finished cheese. Several of the identified LAB strains showed different technological properties which may be fully exploited in the future. Considering the cheeses manufactured at a pilot scale were organoleptically close to traditional São Jorge cheese, it may be feasible to produce more consistent PDO cheeses, using specific native strains as starter culture.

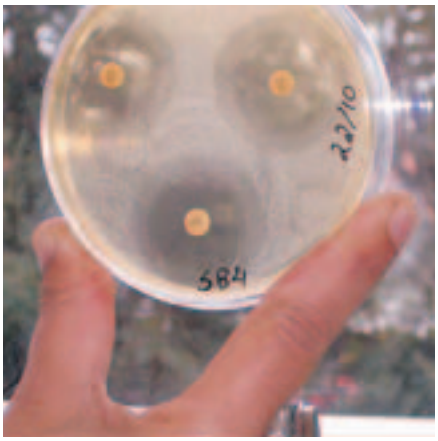

Figure 2. *A plate showing zones of growth inhibition for Listeria monocytogens caused by cell suspensions of LAB isolated from São Jorge cheese*

Finally, because some LAB isolates from São Jorge cheese showed antilisterial activity, there is a potential in their use to increase cheese safety. However this requires further studies towards characterisation and identification (namely separation, purification) of detected antimicrobial compounds.

Many thanks to S*f*AM for giving me the opportunity to present part of this work at IAFP2006 meeting in Calgary.

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Development and Application of Bioluminescent Bacterial Biosensors

The majority of luminescent bacteria belong to the marine species of *Vibrio* or *Photobacterium*. Light is generated by luciferase, a heterodimer enzyme made up of LuxA and LuxB peptides, which catalyses the oxidation of reduced flavin mononucleotide (FMH_2) and an aliphatic aldehyde (RCHO) by oxygen (O_2) to produce FMN, aliphatic acid, water and the concomitant production of blue-green light. The aldehyde substrate is generated from the fatty acid pool of the cell by a fatty acid reductase, an enzyme complex composed of LuxC, LuxD and LuxE subunits. The genes for bacterial bioluminescence are arranged

in the lux operon where the *luxC*, *luxD* and *luxE* genes flank *luxA* and *luxB*. This allows for the machinery for bioluminescence to be turned on simultaneously. As the production of $FMNH_2$ is directly dependent on the reducing power of the cell's respiratory electron system, only metabolically active cells emit light. Therefore, light emission provides a non-invasive and accurate measurement of bacterial viability in real time (Billard & DuBow, 1998).

The ability to emit light can be easily transferred to other bacteria by transforming them with the *lux* operon; this makes it possible to construct bioluminescent bacteria as living biosensors to monitor the effect of various chemicals or processes. Whole cell-based biosensors have been developed using either the 'switching on' or the 'switching off' of bioluminescence approach. In the 'lights on' assay system, the *lux* genes are placed downstream of specific promoter elements which turn on light production when activated by specific physical or chemical conditions. This class of biosensors have been successfully applied in monitoring organic, pesticide and heavy metal contamination of the environment as well in pharmaceutical and food industries. Meanwhile, the 'lights off' approach is based on the direct relationship between light emission and cellular viability. This system provides a general bioassay of toxicity; a potential toxin or mutagen that causes cellular damage will result in a reduction in light emission proportional to the degree of toxicity.

We have constructed a number of recombinant plasmids carrying the constitutively expressed *luxCDABE* operon, isolated from *Photorhabdus luminescens*. These plasmids were introduced into a range of Gram negative and Gram positive bacteria to yield genetically engineered biosensors that give out light as an indicator of the physiological status of the cell in real time. These self-luminescent constructs were successfully used for food and environmental monitoring such as the antimicrobial properties of milk, uptake of bacteria by protozoa and sterilisation of food surfaces.

Xanthine oxidase (XO) is a complex enzyme widely distributed in mammalian tissues and is mainly found in human and bovine milk. The XO-catalysed reaction produces reactive oxygen species (ROS) believed to possess antimicrobial properties. An *Escherichia coli* strain expressing the *luxCDABE* operon on a pUC18-based vector was used to demonstrate the antimicrobial effect of bovine and human milk. The addition of milk to the bacterial culture caused a rapid decline in light output compared to the control. This effect was shown to be due to the production of nitrite produced by the XO reaction under defined oxygen tension (Hancock *et al.*, 2002). These results add further evidence to the benefits of breast feeding in infants since formula milk lacks XO activity.

Protozoa play an important role in controlling bacterial populations in wastewater treatment systems. Many bacteria have the ability to survive, replicate and possibly exchange genetic materials within protozoa. Hence, the study of the uptake and the survival kinetics of pathogenic bacteria during and after ingestion by fresh water protozoa, has direct implications for public health and safety. Traditional methods for monitoring bacteria within protozoa are lengthy and cumbersome. The recent technique of using green fluorescent protein (gfp) as a reporter of bacterial internalisation fails to provide an indication of viable bacterial cells. We have constructed a self-luminescent *E. coli* O157 (toxin-deficient) strain and used it in co-culture experiments with *Tetrahymena pyriformis*. By measuring light output, the assay allowed us to monitor the uptake as well as the survival of bacteria within protozoa. Bioluminescent bacterial reporters could also be developed to study aspects of the relationship between specific pathogens and protozoa (Nelson *et al.*, 2003).

Bacterial spoilage and contamination of food products and crops during harvest, processing, transport and storage is a major cause of food borne disease and economic loss. Effective reduction in surface contamination should lead to safer products with better quality and longer shelf life. This in turn depends on reliable and accurate kill models of spoilage organisms on surfaces of meat, fruit and vegetables. Traditionally, bacterial killing is measured using the lengthy procedure of sample homogenization, culturing multiple samples and colony counting. We have employed bioluminescent constructs of *Salmonella enterica* serovar Typhimirium DT104 and *E. coli* O157 (toxin-deficient) to study bacterial viability during and after heat treatment of food surfaces. This method allowed us to study the kinetics of heat inactivation and bacterial survival after heat treatment *in situ* and in real-time, which could not be achieved using the viable counts method (Baldwin *et al.*, 2006).

Bioluminescence technology is an extremely sensitive and non-destructive reporter of cellular viability and provides an excellent tool for incorporation into microbial cell-based biosensors that have been successfully applied in monitoring the effect of many physical, chemical and biological phenomena.

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^{15}N Stable Isotope Probing and the Soil N Cycle

The soil microbial biomass is vast in both the type and numbers of organisms, in just 1g of grassland soil, there maybe up to 1km fungi, 10^9 bacteria, 10^5 protozoans, and 10^2 nematodes (Young & Ritz, 2005). The

estimated diversity of the soil organisms is as impressive as the numbers, in that there could be 4000 different bacterial genomic units in 1g of soil (Kirk *et al.*, 2004).

One of the key processes driven by soil microbial communities is decomposition, however, this is one process that we know little about, particularly processes facilitating the fate of Nitrogen (N). This is the most common element that limits plant growth in agriculture and thus it is often added to crops as a fertiliser. However the N from such additions is readily lost to the environment causing serious environmental problems including eutrophication and release of nitrous oxides. It is estimated that in that, in 2003, *ca.*160 Mt N per year was released into the global environment by human activity, much of which was attributed to use in agriculture (Dalton & Brand-Hardy, 2003).

Microorganisms are responsible for the breakdown of organic matter and are accountable for the N cycling processes in the soil, yet the majority of these organisms remain unknown. Stable isotope probing (SIP) is a technique at the forefront of research into the cycling of nutrients in soil and could provide the answer to such problems. Isotopes (from Greek *'isos'*, meaning 'same', and *'topos'*, signifying 'place') are species of atoms of a chemical element with the same atomic number and the same chemical behaviour but with different atomic masses due to the presence of extra neutrons and hence different physical properties. The use of stable isotopes (SI) coupled with biomarkers, has been utilised in microbial ecology only relatively recently. The developments in gas chromatography-isotope ratio mass spectrometry (GC-IRMS) have served to allow accurate analysis of SI ratios and hence the development of SIP techniques in microbial ecology. One such approach is to use SI to label DNA. As organisms consume SI-labelled substrate(s), the isotope(s) are incorporated into identifiable molecules, which can then be separated from the unlabelled molecules for further analysis.

This project is developing ^{15}N DNA-SIP, to study those organisms specifically responsible for N processing in the soil and how they are affected by environmental factors. The first issue undertaken was to determine whether the technique works using pure cultures and subsequent tasks involved finding out if the methods could be applied to a soil microbial community. Can the organisms responsible for N processing be labelled to a degree such that they can be separated from the remaining soil organisms and identified.

DNA-SIP using ^{15}N has not been previously reported and therefore it was necessary to first determine if this was a feasible study. The DNA-SIP techniques for using ^{13}C isotope are established, however, this was deemed unsuitable for these studies. The use of ^{15}N DNA-SIP presents additional problems relative to ^{13}C DNA-SIP, in particular, that there are relatively small amounts of N compared to C in DNA (DNA is *ca.* 22% N), hence it is more difficult to separate ^{15}N-labelled DNA from ^{14}N DNA. Most of the work described in the literature has thus far utilised ^{13}C enrichment and to this point no other study has proven the usefulness of ^{15}N DNA-SIP to investigate the fate of ^{15}N-labelled substrates in the soil. The initial work was to assess the ^{15}N % labelling needed in DNA in order to separate the ^{15}N-labelled from the unlabelled DNA in caesium chloride isopycnic centrifugation. This was performed using pure cultures of *Pseudomonas putida* Paw 8 grown on a minimal salts medium with $^{15}NH_4{}^{15}NO_3$, as the sole N source, in increments of 10% ^{15}N. The result was that 40% ^{15}N labelling of pure culture DNA was required for visible separation (Cadisch *et al.*, 2005).

The next task was to label the soil microbial community utilising the N from added ^{15}N-labelled organic matter – in this case residues of eight week old ryegrass (*Lolium perenne* L) grown under glasshouse conditions using $^{15}NH_4{}^{15}NO_3$ as sole N source. Following incorporation of the ^{15}N-labelled plant residues into a sandy-loam soil, the samples were incubated and harvested at regular periods. From the harvests DNA was extracted and subject to isopycnic centrifugation, the results showed a smear rather than clear bands — this was anticipated as the %GC/AT content of the DNA also has an impact on the banding positions (Cupples *et al.*,). The gradients were subsequently fractionated and subject to 16S rDNA PCR-DGGE, to profile the prokaryote community, and ^{15}N analysis. The results showed that the ^{15}N-labelled DNA was lower in the tube, with the unlabelled DNA at the top. Also, the banding profiles in the 16S rDNA DGGE did change over time and the ^{15}N-labelled DNA profile was different to that of the unlabelled DNA. These results identified specific organisms responsible, in this particular soil, for the degradation of the ^{15}N-labelled ryegrass. From further analyses it could be possible to further identify these organisms, however as most of the organisms in soil have yet to be identified (up to 99 % (Kirk *et al.*, 2004), this may not be possible.

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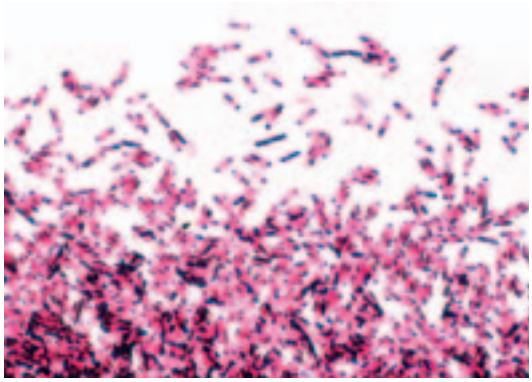

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