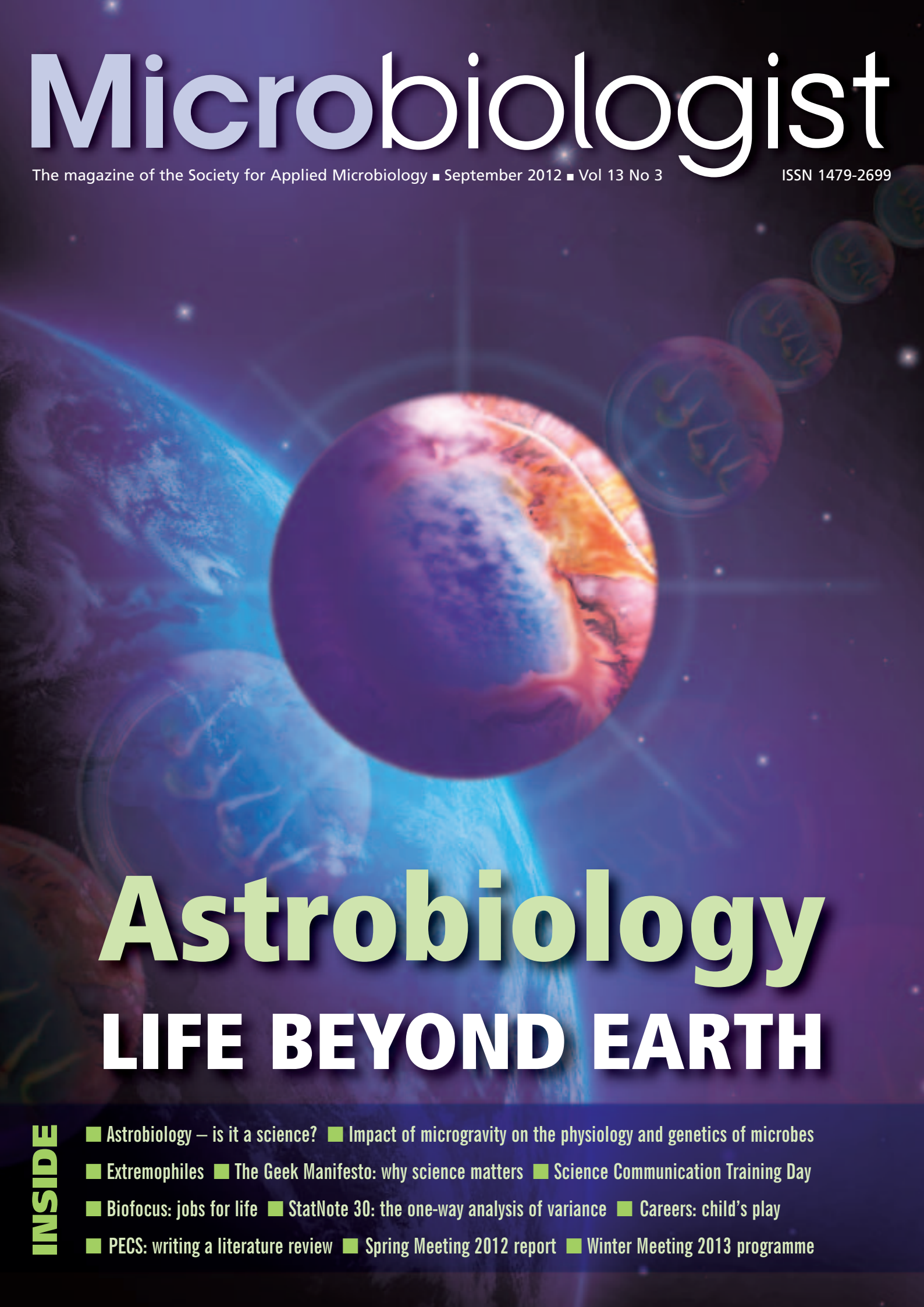


Microbiologist

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Astrobiology

LIFE BEYOND EARTH

INSIDE

- Astrobiology – is it a science? ■ Impact of microgravity on the physiology and genetics of microbes
- Extremophiles ■ The Geek Manifesto: why science matters ■ Science Communication Training Day
- Biofocus: jobs for life ■ StatNote 30: the one-way analysis of variance ■ Careers: child's play
- PECS: writing a literature review ■ Spring Meeting 2012 report ■ Winter Meeting 2013 programme



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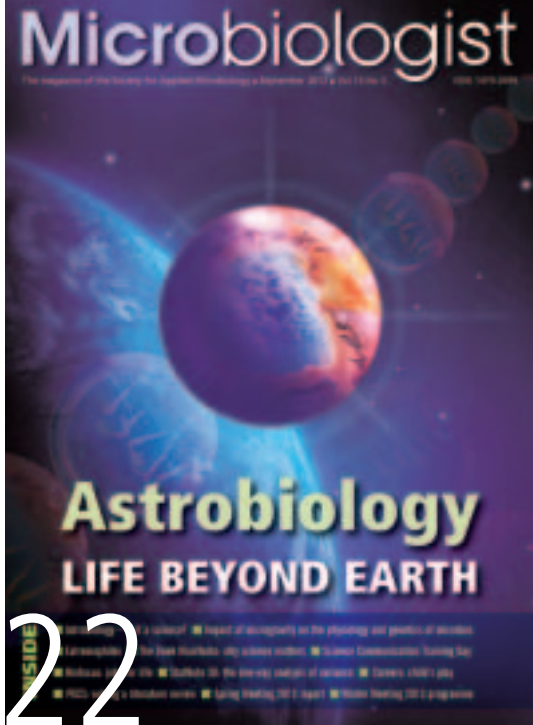
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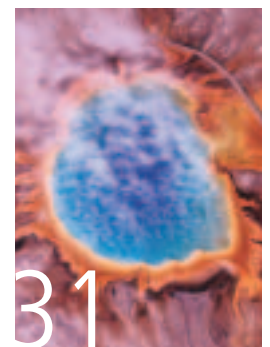
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Winter Meeting 2013



Extremophiles

information

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Editor: Lucy Harper. lucy@sfam.org.uk

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

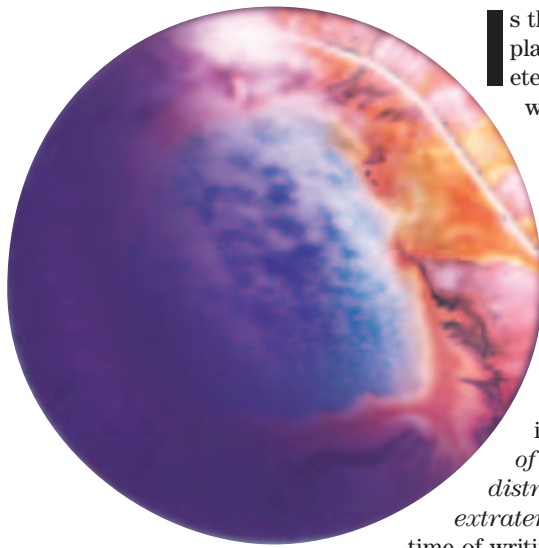
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Society for Applied Microbiology, Bedford Heights, Brickhill Drive, Bedford MK41 7PH, UK.
Tel: +44 (0)1234 326661. Fax: +44 (0)1234 326678.

www.sfam.org.uk



Is there life on other planets? This is one of life's eternal questions, along with 'Why are we here?', 'What's the meaning of life?' and the age-old 'Where did I leave my keys?'

There are many scientists attempting to answer this question, many of whom are working in the field of astrobiology. This area is defined as: "*the study of the origin, evolution, distribution, and future of extraterrestrial life.*" At the

time of writing this Editorial, the

landing of the Curiosity rover on Mars is just around the corner. Astrobiology seems to be everywhere and its connection with microbiology can't be ignored.

Edinburgh University has this month* launched a series of online courses including "Introduction to astrobiology and the search for alien life". This course "*will... explore the origin and evolution of life on the Earth and its potential to exist elsewhere, as well as the possibility of intelligent alien life and the implications of its detection.*"

The leader of this course is the author of our first feature article, Professor Charles Cockell, University of Edinburgh. He says: "*New developments in robotics, vastly increasing information about microbial adaptations to*

extreme environments, the discovery of many planetary bodies in our own solar system that host bodies of liquid water (the Jovian and Saturnian moons, Europa and Enceladus, respectively, are just two) and the discovery of Earth-like planets orbiting distant stars have made astrobiology an empirical science." See page 22 to read more.

One of the winners of this year's Space Lab competition focuses on astrobiology. In this competition, students aged between 14 and 18 from around the world were invited to make videos proposing a science experiment to be conducted on the International Space Station. One winner will look at *Bacillus subtilis* and the effect a lack of gravity could have on its virulence. The effect of microgravity upon many facets of microbial life is the subject of our second feature article by Jamie Foster, of Kennedy Space Center, USA, who says: "*...microgravity experiments have provided critical insight into the molecular mechanisms associated with virulence and stress response mechanisms in pathogens.*" Turn to page 26 to find out more.

Our final feature article is dedicated to the organisms which survive extreme conditions much like those found on other planets. Dr Louisa Preston of the Open University discusses extremophiles and says: "*Terrestrial microorganisms have now been found flourishing in environments so hostile that previously they were simply assumed to be sterile.*" You can read more on page 31. You can also hear from Dr Preston and fellow astrobiologist Dr Lewis Dartnell, exploring astrobiology in more detail in the August edition of the Micropod podcast: <http://www.sfam.org.uk/en/sfam-online/micropod.cfm/astrobiology>

editorial

Lucy Harper discusses the latest developments in the field of astrobiology

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

*Correct at time of going to press.

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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.

contact point



society office staff

CHIEF EXECUTIVE OFFICER: Philip Wheat
email: pfwheat@sfam.org.uk
tel: +44 (0)1234 326661

COMMUNICATIONS MANAGER: Lucy Harper
email: lucy@sfam.org.uk
tel: +44 (0)1234 326709

COMMUNICATIONS OFFICER: Clare Doggett
email: clare@sfam.org.uk
tel: +44 (0)1234 327679

MEMBERSHIP & FINANCE CO-ORDINATOR:
Julie Wright
email: julie@sfam.org.uk
tel: +44 (0)1234 326846

EVENTS ORGANIZER: Sally Hawkes
email: sally@sfam.org.uk
tel: +44 (0)1933 382191

ADMINISTRATOR: Julie Buchanan
email: julieb@sfam.org.uk
tel: +44(0)1234 326661

proofreader

Liz Rees **email:** liz.rees@lizrees.co.uk
www.lizrees.co.uk

publications subcommittee

FEATURES EDITORS:
Claire Cassar **email:** c.cassar@vla.defra.gsi.gov.uk

Louise Fielding **email:** lfielding@uwic.ac.uk

Nick Jakubovics **email:** nick.jakubovics@newcastle.ac.uk

Clare Taylor **email:** cl.taylor@napier.ac.uk

REGULAR CONTENT EDITOR:
Louise Hill-King **email:** louise@hill-king.com

Society for Applied Microbiology
Bedford Heights, Brickhill Drive, Bedford MK41 7PH, UK.
tel: +44 (0)1234 326661 ■ **fax:** +44 (0)1234 326678
email: communications@sfam.org.uk ■ **www.sfam.org.uk**

executive committee

COMMITTEE MEMBERS

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

HON GENERAL SECRETARY: Professor Mark Fielder, School of Life Sciences, Kingston University, Penrhyn Road, Kingston upon Thames, Surrey KT1 2EE
email: m.fielder@kingston.ac.uk

HON MEETINGS SECRETARY: Dr Andrew Sails, Health Protection Agency, Newcastle Laboratory, Institute of Pathology, Newcastle General Hospital, Westgate Road, Newcastle NE4 6BE
email: andrew.sails@hpa.org.uk

HON TREASURER: Mr Steve Davies, Microbiology Department, Northern General Hospital, Herries Road, Sheffield S7 5AU
email: steve.davies@sth.nhs.uk

ORDINARY COMMITTEE MEMBERS UNTIL JULY 2013

Dr Louise Fielding, Food Safety and Nutrition Research Group, Cardiff Metropolitan University (UWIC), Llandaff Campus, Western Avenue, Cardiff CF5 2YB
email: lfielding@uwic.ac.uk

Dr Irene Grant, Institute of Agri-Food and Land Use, School of Biological Sciences, Queen's University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL
email: i.grant@qub.ac.uk

Dr Katie Laird, De Montfort University, The Leicester School of Pharmacy, Faculty of Health & Life Science, Hawthorn Building, Leicester, LE1 9BH
email: klaird@dmu.ac.uk

ORDINARY COMMITTEE MEMBERS UNTIL JULY 2014

Professor Christine Dodd, Division of Food Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD
email: christine.dodd@nottingham.ac.uk

Dr Clare Taylor, School of Life, Sport & Social Sciences, Edinburgh Napier University, Sighthill Campus, Sighthill Court, Edinburgh, EH11 4BN
email: cl.taylor@napier.ac.uk

ORDINARY COMMITTEE MEMBERS UNTIL JULY 2015

Mr Mark Reed, Pro-Lab Diagnostics, 7 Westwood Court, Neston Cheshire CH64 3UJ
email: mreed@pro-lab.com

Dr Sally J Cutler, School of Health and Biosciences, University of East London, Stratford Campus, Romford Road, London E15 4LZ
email: s.cutler@uel.ac.uk

Nick Jakubovics, Oral Biology, School of Dental Sciences, Newcastle University, Newcastle upon Tyne NE2 4BW
email: nick.jakubovics@newcastle.ac.uk

Dr Samantha Law, NCIMB, Ferguson Building, Crabstone Estate, Bucksburn, Aberdeen AB21 9YA
email: s.law@ncimb.com

benefits

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

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

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

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

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

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

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

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

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

membership options

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

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

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

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

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

■ **eAffiliate Membership:** This category of membership is open to microbiologists residing in Band I developing countries and is free of charge. It is an online only membership and provides access to the eAffiliate bursary only.

■ **eStudent Membership:** This category of membership is open to undergraduate students only. It is an online only membership and is free of charge. This category of membership does not provide access to the Society's grants or journals.

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

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

JOIN US!

You can apply for membership on, or offline. To apply offline, please contact the Membership & Finance Co-ordinator, Julie Wright on +44 (0)1234 326846, or email julie@sfam.org.uk.

Sometimes it seems that our working lives are unduly dominated by time spent in meetings. For the most part, we probably recognize this as a necessary evil, though on particularly trying occasions it is difficult not to concur with weary cynics who regard committees simply as timetabled occasions when others can indulge an infatuation with the sound of their own voices. I am happy to report that this is certainly not the case with the gatherings of the SfAM Executive Committee which, though they are awash with more bonhomie than meetings of the Pickwick Club, remain very business-like and efficient. This is fortunate in view of their signal importance in the running the Society, reviewing our activities and planning new initiatives for the benefit of members.

For most professional scientists though, attendance at scientific meetings is a much preferred option. Good scientific meetings can be interesting, enjoyable and stimulating. They can help us perform better in our work as well as find greater fulfilment in it. Recognizing this, SfAM regularly supports a number of one-off scientific meetings, members' attendance at meetings and our own well-established programme of meetings:

the one day Winter Meeting in January, the one day Spring Meeting in April and our flagship event, the four day Summer Conference, held this year in Edinburgh. As you will appreciate a lot of effort goes into organizing and delivering these events and a large part of this work falls on the capable shoulders of the Meetings Secretary, Andy Sails, members of the Meetings Group and the office staff, particularly Events Organizer, Sally Hawkes.

The starting point for any meeting is always the programme — what will be the subject(s) and who will we ask to speak? Our membership spans the full spectrum of applied microbiology so individual meetings which can cover only a limited range of topics are never going to be directly relevant to everyone, and we have on several occasions made the conscious decision to ensure we cater for all our membership by including niche areas which have more limited appeal. Having said that, it is a common misconception, particularly it appears among managers who might have to approve attendance at meetings that a topic has to map directly on to a scientist's own role and responsibilities for it to be worth them attending. Meetings on less related topics can be enormously beneficial,



fostering new thinking and the transfer of novel techniques and approaches from one field to another. It is a very short-sighted approach, generally motivated by a misplaced sense of economy, not to see the benefit of such meetings.

Scientific meetings also give fellow professionals the chance to get together and exchange news, ideas and experiences not directly related to the topic of the meeting at all. Though it is impossible to quantify the benefit, this sort of contact and interaction with your colleagues on any level can yield unforeseen dividends in the future. For younger scientists it is also an essential part of their development as professionals. They can make new contacts among their peers, meet the leading experts in their field and, at events such as our Summer Conference, have the opportunity to present their own work either orally or as a poster, perhaps for the first time, in a supportive and friendly atmosphere.

Finally, scientific meetings can be enjoyable — they are generally held somewhere quite congenial and often have an associated social programme. Suffering is not necessarily good for the soul and the fact that people are having a good time does not necessarily mean that it is an unproductive time without some broader benefit. At SfAM, we have a strong commitment to maintaining and improving all these aspects of our meetings. We would therefore welcome hearing from any members who would like to become more involved in participating in our meetings programme or who have any ideas or suggestions to feed into it.



Martin Adams
President of the Society

president's column

SfAM President, **Professor Martin Adams** talks about the many benefits of scientific meetings

With this issue of the *Microbiologist* you should find a copy of the Society's Annual Report for the year ending 2011. I would recommend all members read the report as it gives a very good insight into the achievements and activities of the Society during 2011.

The financial health of the Society remains good and this is largely due to the revenue we receive from our publishing activities. One way this excess income over expenditure is redistributed back to members is through the awarding of grants. If you turn to page 19 of the annual report you will see that during 2011 we awarded grants to the value of £203431 to a record number of individual members (153). This

contrasts to £123947 awarded to 106 members in 2010. This significant increase is a reflection of our continuous promotion of grants to members. I would also like to remind everybody that all grant applications are treated equally irrespective of whether

you are a UK resident or not. Once again I would like you all to visit our website for the full Terms and Conditions of all the grants that are on offer: <http://www.sfam.org.uk/en/grants--awards/index.cfm>

In the last issue of the *Microbiologist* you all should have received your invitation to this year's Environmental Microbiology Lecture (Royal Society of Medicine, London, 8 October). This lecture is designed not only as a way of celebrating the work of an eminent speaker in the field of environmental microbiology, but also to showcase the success of the SfAM journal *Environmental Microbiology*. For members unable to attend on the evening, the lecture is recorded and it is available for viewing online a few days after the event. Showing our *Environmental Microbiology* Lecture online has proved to be very successful and indeed, Wiley-Blackwell has reported that these lectures have by far the healthiest viewing figures of any annual Society lecture they sponsor.

With some of this in mind I am pleased to announce that from 2013 we will be introducing an additional Annual Lecture celebrating another Society journal. The Executive Committee have decided that the opening lecture of future Summer Conferences will be the **Journal of Applied Microbiology Lecture**. The presenter of this lecture will be a highly prestigious speaker in the field of applied microbiology. The lecture will once again be jointly supported and organized by the Society and its publishing partner, Wiley-Blackwell. In addition, the Chief Editors of both the *Journal of Applied*

Microbiology and *Letters in Applied Microbiology* will be closely involved in the selection of appropriate topics and speakers. Similar to the *Environmental Microbiology* Lecture the presentation will be filmed and available online shortly after the event. Further announcements about this new initiative, will appear in forthcoming issues of the *Microbiologist*.

Finally, thank you to all members new and old who stopped by our exhibition stand to say hello at the three USA exhibitions we attended (American Society of Microbiology, International Association of Food Protection and Society for Industrial Microbiology) during 2012. It is always a pleasure to renew old acquaintances and make new ones!



Philip Wheat
Chief Executive Officer

ceo's column

Philip Wheat reports on the latest developments within the Society

Scientific Meeting Attendance Grant or President's Fund? You decide!

Are you going to a scientific meeting? Do you need funding? Do you know which of our grants to apply for?

The **Scientific Meeting Attendance Grant** will fund your travel, accommodation and registration fees at any relevant scientific meeting, including SfAM meetings, up to a value of £300. This is ideal if you wish to attend a conference or one-day meeting/symposium but you're not presenting a poster or giving an oral presentation or contributing to the meeting in any other way.

The **President's Fund** is designed for you if you're presenting a poster or giving an oral presentation at a relevant scientific conference, meeting or workshop, including SfAM meetings. It will fund travel, subsistence and conference fees up to a value of £1200.

For more information about all our grants and awards, please visit: www.sfam.org.uk/en/grants--awards/index.cfm

Membership changes

NEW MEMBERS

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

Australia

G. I. Simon; L. Woolley

Bangladesh

Z. Hossain

Brazil

M. Ishii

Canada

T. D. R. Bandet; S. Booth; J. Cairns

Egypt

A. Abouelfetouh

France

G. J. Fourvel

Ghana

A. Ahiekpor

Greece

K. Skaraki

India

A. Gupta; V. Gupta; A. D. Reddy

Indonesia

M. Mehina

Ireland

K. Considine; J. Geoghegan; D. Ziuzina

Japan

T. Yano

Namibia

A. Cheikhoussef

Nigeria

C. Abiola; R. Aboloma; A. L. Ajayi; C. K. Akaluka; C. I. Akobundu; C. Ayanwale; A. J. Babalola; F. Davies; S. Enabulele; C. C. Eze; O. Igbalajobi; C. L. Igeleke; U. N. Kemka; E. C. Nwosu; T. E. Ogbulie; L. Omorogiuwa; C. J. Orji

Poland

M. Pobiega

Saudi Arabia

K. Aljarallah

South Africa

A. Adegoke; R. Adeleke

Switzerland

S. M. Freund

Thailand

S. Wanasen

UK

J. A. Adu; M. Aguas; G. R. Ansal; S. Bandia; S. W. Barker; P. Barrett; A. Bashir; J. Bass; F. Bibi; L. Bilali; D. Boyle; E. Burnett; C. Cairney; D. Cook; L. Crossman; N. Daines; A. Desbois; K. Evans; O. E. Fagunwa; S. A. C. Farmer; B. D. Folwell; J. Foulkes; C. Fricker; R. Gaynon; A. Gonfa Begna; A. Gupta; T. Gutierrez; W. Heaselgrave; A. Henrici; J. K. Hiltner; G. Hobbs; D. Hussey; E. Japp; M. Jervis; A. Jones; R. Kent; I. Khodadoost; F. King; D. Kpeglo; R. Lambert; J. A. Marr; S. E. Mcareavey; C. McMurray; O. K. Mejeha; A. S. Michell; S. M. Middleton; H. Moore; A. Ogunlana; O. Okafor; E. K. Onovughe; K. Otukunefor; T. Oyebanji; N. Patel; E. Phillips; Y. Qi; M. Ramesh-Jeyashankar; K. Roberts; J. C. Robinson; A. Sehgal; P. Shears; D. Silas-Olu; N. Sivanesan; K. Stewart; S. Stewart; E. Trantham; K. Turnbull; T. R. Turner; K. Van Ginkel; S. Vijayendran; L. Whitehouse; A. Wilder; L. K. Williams; P. Wood; V. Young

USA

J. Acuff; C. Andam; U. Anietd; T. Arends; R. Belcher; J. Bennett; B. Boles; M. J. Calcutt; J. Denson; M. Ereemeeva; W. Fedio; A. Foster; B. Foxman; N. Goodyear; A. Guimaraes; E. Halliday; M. Hossain; M. Howard; R. Katz; E. Kearns; J. Krebs; S. Leskinen; J. Odden; E. Pressly Bryant; S. Rose; S. Smith-Rohde; G. Trubl; J. Zenilman

Uzbekistan

S. Polyarush

2012 SfAM AGM

The 81st Annual General Meeting of the Society for Applied Microbiology was held on Wednesday 4 July at 16.45 pm at The George Hotel, Edinburgh.

Present:

34 Members attended the AGM.
In attendance: Philip Wheat (PW), Lucy Harper (LH).

1. Apologies for absence

No apologies were received.

2. 80th Annual General Meeting

The minutes of the 80th Annual General Meeting held in Dublin in 2011 were published in the September 2011 issue of *Microbiologist*. They were approved and accepted by those present.

3. Matters arising

There were no matters arising.

4. Report of the Trustees of the Society 2011

Copies of the Annual Report of the Society for 2011 had been distributed previously. Basil Jarvis pointed out some incorrect wording regarding the appointment of Officers of the Society.

A corrigendum will be distributed to members with the Annual Report.

There were no other questions on the report.

5. Adoption of the Annual Report 2011

Martin Adams asked for the report of the Trustees and the statement of accounts to be officially received and approved. All present were in agreement.

Proposed: John Rigarlsford

Seconded: Basil Jarvis

6. Election of new members

(Including Honorary Members), deaths and resignations. A list of names of applicants for membership and a list of deaths has appeared in *Microbiologist* throughout the previous year.

The Society holds a summary list of new members and resignations throughout the previous year for consultation if requested.

Max Sussman related how more stringent membership criteria were applied in the past, more information on which is available in the History of the Society book.

7. Election of new committee members

Martin Adams reported that this year there are four committee vacancies. Mark Reed, Sally Cutler, Samantha Law are retiring by rotation and were thanked for their contributions and hard work during their term of office.

They were all nominated to continue on the Executive Committee for a further term of three years. In addition, Alison Kelly's term of three years ended and she did seek re-election. Nick Jacobovics had previously been an observer at the Executive Committee meetings and was nominated as a member of the Executive Committee for three years in place of Alison Kelly.

There being no other nominations, Nick Jacobovics was declared duly elected to the EC.

8. Any other business

Bernard Dixon raised the need to counter misinformation in the media about vaccines, using the MMR vaccine debacle as an example. Mark Fielder acknowledged this as a continued problem regarding uptake of vaccines and resurgence of infectious disease, citing a recent comment made by the Chief Executive of the World Health Organization. LH informed attendees that the media work undertaken by the Society includes the provision of accurate information on vaccines.

David Post asked about the effect of the Spring Meeting on the recruitment of Biomedical Scientists. PW reported an increase in Biomedical Scientist members which could be attributed to the increasing popularity of the Spring Meeting and SfAM's attendance at the Institute of Biomedical Sciences Congress in recent years.

Simon Cyril U Nwachukwu raised a number of specific concerns regarding the Summer Conference 2012. These were noted and/or responded to by the Officers.

Science communication training day

After the success of the 2011 Science Communication Training Day, the Biochemical Society, Society for Experimental Biology and the British Ecological Society once again joined forces to organize a unique bioscience communication training workshop. The 2012 Science Communication Training Day took place on 7 June at Charles Darwin House in London and **Jenni Drever-Heaps**, a PECS committee member, attended.



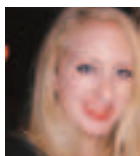
I applied to attend this training day without knowing exactly what to expect. We were told to come with an idea (completely undeveloped or in the first stages) which was about a new way to target audiences and inform them about science. The workshop began with an insightful lecture from Alun Anderson (former Editor of *New Scientist*) about how his career in science communication began — basically by accident as most of these stories go — and how he led the *New Scientist* into its most prolific era to date. Next we had a lecture from Jeremy Pritchard a Senior Lecturer and Head of Education at the University of Birmingham, about different ways to communicate to the public.

After a networking lunch we had just over two hours for 'action planning'. We were put into groups with a lead facilitator to help us develop our ideas. My idea was to take a pop-up stand to a shopping centre to specifically appeal to adults and relay information about microbiology. I was lucky enough to be in the group with Jeremy Pritchard who by the end of the day was able to help everyone develop their ideas into something more concrete. Ideas on my table included; a woman looking for ways to add to a website for a breast cancer charity, making it more appealing to the public, a guy who wanted to set up a networking system for students studying cardiovascular medicine, and someone looking to develop an app for smart phones about locations of science events in Oxford. It began with an 'elevator pitch', 30 seconds to explain your idea and what you wanted to achieve — a very

intimidating prospect when you don't have a clue what you are doing! But, over our time together, we had managed to dissect everyone's ideas and turn them into a viable prospect. For my concept we had come up with resource ideas, locations and even funding which could put the whole thing into action.

Afterwards we all put our plans on to a sheet of paper and pinned it to the wall for a 'silent debate'. I had never been part of one of these before and was a little apprehensive. The idea was to go around the room and look at each person's proposal and write comments on any further ways to develop/improve their scheme. This worked brilliantly! We each received objective opinions because it was undertaken anonymously, and the input came from people that were not in the group you were initially working with.

At the end, Alun Anderson made a closing speech and actually mentioned my idea as one that he could really see going somewhere! This day showed me a lot; it taught me not only to have faith in my own ideas but also how to help other people, and that if you feel that it is not within your power to achieve your goals, that there is always someone around in the science community willing to help you make a start.



Jenni Drever-Heaps
PECS Publications Officer

book review



The Geek Manifesto: Why science matters

Mark Henderson
Bantam Press, UK, May 2012
ISBN-13: 978-0593068236.
Hardcover £18.99 (RRP)
336 pages

Reviewed by Lucy Harper

Before I begin this review, I have a declaration to make: as I read *The Geek Manifesto*, I felt at home. The majority of the names Mark Henderson mentions are people I've either met, know (in person or online) or have read about. Key figures in the world of science or science communication and policy who are highly respected in their field. This is one of the reasons the book spoke to me, though I have tried to prevent any confirmation bias making its way into this review.

It's my view that in the UK, we are encouraged, mainly through our education system, to specialize as either 'scientists' or 'artists' at a relatively young age. This separation of science from the arts pervades throughout life

— and as we travel through the education system it seems inescapable. However, my gut instinct (and this is all it is), is that those in the arts and social sciences are very much encouraged to engage in politics and see themselves and their work as an integral part of life. But speaking from a personal perspective, my training (to post-doctoral level) in science did nothing to encourage me to think of science in this way. Yet the importance of science and its relevance to everyday life is clearly evident. I would encourage all scientists and non-scientists to read *The Geek Manifesto*, as it gives science a voice, spelling out loud and clear its importance, encouraging scientists and science communicators to engage and to tell everyone about it.

Scientists are trained to question, to be comfortable with uncertainty. Yet perhaps it is our comfort with uncertainty which somehow translates into a feeling of powerlessness and inability to change the status quo in areas that aren't directly related to our field of study. If, like me, you see the relevance and power of science and think its importance isn't recognized, particularly in UK Government, then this book will speak to you.

I would be doing this book an enormous disservice if I were to say that it provides a sound footing and introduction into the many ways in which science could be better integrated into our culture. It *IS* this and 'this' is extremely important in my view...but it is so much more.

Without giving too much away, the first chapter "*The geeks are coming*" talks about how things are changing — certainly in the UK — for the better. The fact that a science show is filling the Hammersmith Apollo at Christmas time (Robin Ince's "*Uncaged Monkeys*") is a good illustration of the way science is pervading popular culture.

Mark follows this chapter with two important chapters discussing science in parliament. The under-representation of science, in terms of the qualifications of MPs, is highlighted as is the importance of those casual "water cooler" moments: conversations which currently don't include mention of science, because there are so few members of parliament who understand its relevance. Evidence abuse — in other words policy-based evidence, rather than evidence-based policy — is a theme which runs through the book. Bovine TB and badger culling is a good example of the misuse of evidence by MPs: not cherry-picking evidence to suit the policy they wish to enforce, though this does happen, but misinterpreting good quality evidence, leading to policy which is not evidence based.

Science in the media is a subject I feel passionately about, and Mark obviously agrees: "*Rarely is science portrayed [in the media] as an integral part of society, as a theme that*

reviewers

The Society receives new books from publishers around the world and we are always looking for enthusiastic reviewers who have an interest in the subjects covered.

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cuts across political, social and cultural issues in the manner of economics, law or the arts” he says in Chapter 4. I could write endlessly about the many examples illustrating why science is important in economic terms (Chapter 5), education (Chapter 6) and the role of science in the justice system (Chapter 7). I could also wax lyrical about pseudoscience and homeopathy (Chapter 8), and “*Why science matters to the environment*” (Chapter 9). Unfortunately I have a limited word count and anyway, if I say too much you won’t need to read the book! At the end of the book, Mark describes his idea of an ideal Geek Manifesto.

Use of the term Geek is something with which I’m not wholly comfortable. There’s something self-defeating about it and discussion about use of this term needs a different (longer) forum. But I can see the merits of using a short word that grabs attention. The book does just that and use of a more appropriate term may deflect from that facet.

The cover of the book provides a good indication of its tone. As I began reading *The Geek Manifesto* I was a bit concerned that it may be construed by some as too preachy or prescriptive. And yes there is an element of that, but please don’t be put off by my saying so — the tone is upbeat, it is motivational, inspiring and empowering. Whether you’re a scientist who feels science is misrepresented or a non-scientist with even the slightest interest in how the world works, you may think you don’t need reminding of the importance of science...but if you don’t finish the book and think “*Yes, yes us geeks need to recognize the importance of science and we need to tell people about it!*”, then like Jim Al-Khalili*, I will eat my shorts!



Lucy Harper
Communications Manager
Society for Applied Microbiology

author’s note

*Jim Al-Khalili is a theoretical physicist from the University of Surrey who promised to eat his boxer shorts at the end of last year if the OPERA neutrino experiment proved to be right.



In the news

Those of you who are early risers and listen to ‘*Today*’ on BBC Radio 4 may have heard Executive Committee member **Nick Jakubovics** talking to John Humphrys this summer, about seaweed and teeth. On the 4 July, the same day that CERN announced the (most likely) existence of the Higgs Boson, Nick was sitting in a BBC studio in Edinburgh at 6.55am facing a grilling from the *Today* programme presenter. This was the first interview in what turned out to be a long day of radio interviews, telephone calls, Twitter conversations and television appearances. The coverage *SfAM* received as a result of Nick’s work, which looked at an enzyme extracted from a bacteria which lives on the surface of seaweed and its potential role in breaking down oral biofilms, was outstanding. It was a testament to the quality of Nick’s work and the good relationship he has with his university press office, who were instrumental in this publicity and provided some great photographs (see above).

Congratulations must go to Nick, who handled the interviews expertly and almost missed his presentation in the process!

more on this story online

- **Seaweed could fight tooth decay**
www.independent.ie/health/health-news/seaweed-could-fight-tooth-decay-scientists-3158487.html
- **Brushing your teeth with bacteria from seaweed could be more effective than toothpaste**
<http://www.dailymail.co.uk/sciencetech/article-2168606/Brushing-teeth-bacteria-seaweed-effective-toothpaste.html>
- **Seaweed toothpaste ‘to stop tooth decay’**
www.bbc.co.uk/news/health-18686179
- **Seaweed offers new way to fight plaque that beats brushing**
<http://www.scotsman.com/news/health/seaweed-offers-new-way-to-fight-plaque-that-beats-brushing-1-2390932>
- **Would you brush your teeth with seaweed toothpaste?**
<http://www.itv.com/news/2012-07-04/seaweed-toothpaste-new-enzyme-could-provide-new-weapon-in-fight-against-tooth-decay/>
- **Tweets from around the world**
http://topsy.com/news.bbc.co.uk/today/hi/today/newsid_9734000/9734926.stm?allow_lang=en

publicity help

If you have a novel piece of research and would like *SfAM* to assist with publicity, contact Lucy Harper or Clare Doggett (communications@sfam.org.uk) who will gladly help you broadcast it.

bioFocus

Mark Downs discusses the concept of "Jobs for Life"



The Society of Biology is a single unified voice for biology:

- advising Government and influencing policy.
- advancing education and professional development.
- supporting our members.
- engaging and encouraging public interest in the life sciences.

For further information visit:

www.societyofbiology.org

Whatever happened to a job for life? A nice linear pathway that everyone understood: do well at school, go to a good university, possibly a higher degree and then a job with prospects? If you weren't academically inclined, then of course you focussed on an apprenticeship and learning a trade. We all know the answer — it was never that universal or straightforward, and it was certainly a long time ago! What is clear is that career paths have radically changed and it is an employers' market. In the biosciences it is not at all unusual to get well over 50 applicants for jobs, especially early career roles, even for those on relatively short fixed-term contracts. So what can differentiate candidates who want to work in science and engineering? I suggest two potential areas are demonstrating breadth of interest or experience and a commitment to ongoing professional learning. The Society of Biology is seeking to help with both through professional registers and (as with all learned societies) wide networking opportunities.

Learned societies have a great opportunity to help existing members and attract new ones through the value they can add in a challenging job market. Increasingly, expert knowledge needs to be coupled with transferable skills. In his recent "Making Science Work" lecture Sir Paul Nurse FRS, Hon FSB made clear his view, supported widely in the audience, that science will no longer be carried out primarily inside organizations but between them; to make that happen, scientific staff at all levels need to be competent at managing external partners and collaborative research alongside customers, suppliers, investors, politicians and the media. A good example is of course the pharmaceutical industry where research is increasingly collaborative using academic partners and contract research organizations. Major pre-competitive projects are also expected to grow using the much touted "open innovation" model. This will need a different skill set beyond excellent science. And the same is true of many other areas. Applied microbiology has a rich source of experts who have highly transferable skills that perhaps just need highlighting: individuals need to make them stand out.

Learned societies can really make a difference by offering members the chance to meet not just peers but potential colleagues at all levels and from diverse backgrounds, supported perhaps by professional development programmes. Showing a new employer, or even your existing employer, that you have breadth as well as depth and skills beyond a specialism has always been helpful but now they are essential.

As part of a License from the Science Council, the Society of Biology has just launched a series of professional recognition programmes: Registered Science Technician (RSciTech), Registered Scientist (RSci) and Chartered Scientist (CSci) alongside Chartered Biologist (CBiol); these all recognize the competencies of individuals in the workplace. To be added to the relevant professional register there needs to be a demonstration of how skills are applied in a work environment and then annual CPD (continuous professional development) to maintain registration. It is this recognition of the application of skills in a broad way, rather than demonstration of knowledge, that is so important in showing employers transferability of skills. For example, an individual may know how to operate a piece of equipment or run an assay, but can they communicate what it does and how to interpret data to both specialist and non-specialist alike?

Critically for the individuals, there is no requirement to have existing qualifications. Whilst they may help they are not a prerequisite. For guidance, a rough comparability is that RSciTech is approximate to A level, RSci to Foundation degree and CSci/CBIol to Master's degree. To learn more visit the website: <http://www.societyofbiology.org/development/rsцитеch>.

To ensure more support for members across the spectrum the Society of Biology is now also set to introduce regular half and full day training courses covering everything from being an "expert witness" to Good Laboratory Practice (GLP) and presentation skills. For a full day this will typically be offered at a nominal fee of £10 for individual members, £75 for members of our member organizations such as SfAM and £150 for non-



members. Needless to say, CPD points can also be accumulated through these courses to help with annual returns.

Let us know if you would like to get involved in our professional development programmes. As a member of SfAM, the Society of Biology is offering new applicants half-price membership for the next two years.

This article first appeared in *The Biologist*, Vol. 59, No. 3, p48.



Dr Mark Downs, PhD, FSB
Chief Executive, Society of Biology

journalWatch

News about the Society's journals

News

New focus for *Letters in Applied Microbiology*

The format of *Letters in Applied Microbiology* is changing to highlight the significance and impact of the published article. The length will remain the same, but the headings will be rearranged to focus on 'Results and discussion', and the significance and impact of the studies. The changes will provide authors with an opportunity to focus on communicating the 'Significance and impact of their research'. To read Jean-Yves Maillard's Editorial in full, announcing a new focus for the journal, visit <http://onlinelibrary.wiley.com/journal/10.1111/%28ISSN%291472-765X>

In the Press

The *Journal of Applied Microbiology* has found its way into the media amid discussions on transparency in science reporting. Identifying differences between the UK and the US, Daniel Engber looks at scientific reporting, and specifically the transparency of pseudoscience, or sponsored research used to promote products.

To read both the original article by Dawson *et al.*, 'Residence time and food contact time effects on transfer of *Salmonella* Typhimurium from tile, wood and carpet: testing the five-second rule' or Engber's article 'What's wrong with science journalism in the U.K.?', visit <http://onlinelibrary.wiley.com/journal/10.1111/%28ISSN%291365-2672>.

Impact Factors (IF) 2012

The IFs for all the SfAM journals spell good news. For 2012, the IFs for *EMI* and *EMIR* have increased to the very impressive 5.843 and 3.232 respectively. *MBT* now has its first IF of 2.534 and although slightly decreased this year *JAM* and *LAM*'s IFs remain a very respectable 2.337 and 1.622 respectively.

Journal Highlights

Read the most downloaded articles for January to March 2012:



Environmental Microbiology

Beyond the Venn diagram: the hunt for a core microbiome. A. Shade and J. Handelsman, **Vol. 14**, Issue 1.

Targeted metagenomics: a high-resolution metagenomics approach for specific gene clusters in complex microbial communities. H. Suenaga, **Vol. 14**, Issue 1.



Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. F. C. Cabello, **Vol. 8**, Issue 7.

Environmental Microbiology Reports

Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. H. W. Paerl and J. Huisman, **Vol. 1**, Issue 1.

Local and regional factors influencing bacterial community assembly. E. S. Lindström and S. Langenheder, **Vol. 4**, Issue 1.

Powering microbes with electricity: direct electron transfer from electrodes to microbes. D. R. Lovley, **Vol. 3**, Issue 1.



Journal of Applied Microbiology

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

Antimicrobial agents from plants: antibacterial activity of plant volatile oils. H. J. D. Dorman and S. G. Deans, **Vol. 88**, Issue 2.

A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. R. J. W. Lambert, P. N. Skandamis, P. J. Coote and G.-J. E. Nychas, **Vol. 91**, Issue 3.



Letters in Applied Microbiology

Antifungal activity of thyme (*Thymus vulgaris* L.) essential oil and thymol against moulds from damp dwellings. M. Šegvič Klarič, I. Kosalec, J. Mastelić, E. Piecková and S. Pepeljnak, **Vol. 44**, Issue 1.

The probiotic bacterium *Lactobacillus plantarum* species 299 reduces intestinal permeability in experimental biliary obstruction. J. S. White, M. Hoper, R. W. Parks, W. D. B. Clements, T. Diamond and S. Bengmark, **Vol. 42**, Issue 1.

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



Microbial Biotechnology

Marine genomics: at the interface of marine microbial ecology and biodiscovery. K. B. Heidelberg J. A. Gilbert and I. Joint, **Vol. 3**, Issue 5.

Crystal ball – 2011. **Vol. 4**, Issue 2.

Strategies for discovery and improvement of enzyme function: state of the art and opportunities. P. Kaul, Y. Asano, **Vol. 5**, Issue 1.

Melissa McCulloch
Wiley-Blackwell



Spring Meeting 2012 report

The Stratford Q Hotel, Stratford-upon-Avon, UK, Wednesday 18 April 2012

■ 6th broadening microbiology horizons in biomedical science meeting

The day began with an inspirational presentation from the winner of the Procter and Gamble Applied Healthcare Microbiology Lecture, **Professor Anne Glover**.

The day began with the award lecture of the recipient of this year's Procter & Gamble Applied Healthcare Microbiology Award, Professor Anne Glover. In addition to her roles as the former Chief Scientific Advisor for Scotland and the first ever appointed Chief Scientific Advisor to the President of the European Commission, Anne has maintained an active research group and gave a fascinating overview of her research from using bioluminescence as a marker for microbes and its use as a biosensor for stress, to more recent approaches using novel transgenic *Caenorhabditis elegans* which Anne described as "an honorary microbe". Using the *C. elegans* system, her group are using mitochondrial function and its relationship to apoptotic cell death as a means of screening drugs to overcome impaired mitochondrial function, and also to screen for additional benefits of already-approved drug compounds. These data may inform research into potential therapeutic interventions for neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's disease.

The main morning session, A Centenary of Microbiology, featured Jonathan Nguyen van Tam (Nottingham City Hospital) who was first up and gave a unique perspective of 'Influenza — future and past'. Jonathan began by explaining that despite several global pandemics and much research to date, there are still a number of fundamental questions about

transmission of influenza which remain unanswered, particularly around droplet size; is it large droplets or aerosols? Some of these questions formed the basis of a multi-centre, proof-of-concept study using a human-challenge exposure model to determine whether experimentally-induced influenza is transmissible between humans and therefore a viable model for further study. This multi-million dollar study was carried out in a controlled environment with volunteers and has formed the basis of a new study to examine different interventions. It is hoped that data from the current study will inform future best practice in healthcare settings.

The second speaker of the session, Kevin Brown (St Mary's Hospital, London) gave an insightful and colourful historical account of Mediterranean fever and its impact in the early 1900s. More commonly known these days as brucellosis, Mediterranean or Malta fever was responsible for some 75,000 sickness days per annum of British officers during the time of the Crimean War. Kevin described the persistent efforts of Scottish microbiologist Dr David Bruce and his role in determining the causal relationship between organism and disease leading to eventual eradication of the disease from Malta.

Clare Taylor



The final session of the morning was presented by David Petts, a retired biomedical scientist. David talked us through the history of the Group A *Streptococcus*: from Rebecca Lancefield's original classification, through to the current situation. David explained that over time the Lancefield classification was relied on too heavily to determine whether or not an organism was pathogenic, at the expense of the true taxonomy of a group of organisms. This has led to misleading results when the Lancefield group is used in isolation. David outlined the history of how the Lancefield groupings developed and discussed their use in modern clinical laboratories.

Clare Doggett

After a delicious lunch and an opportunity to visit the trade show we broke into two groups for the afternoon sessions on 'Sepsis and implants and Virology.'

The placement of medical devices within the body has grown to staggering proportions and is now a routine part of care for many patients. In the sepsis and implants session we began by hearing from Brian Jones (University of Brighton) that an estimated >100 million intrauterine catheters are placed worldwide each year. Unfortunately, artificial surfaces lack the natural immune mechanisms of our own mucosal tissues and therefore present an ideal surface for the growth of bacterial biofilms. Urinary catheters are a typical example, frequently harbouring *Proteus mirabilis* or other microbes. *Pr. mirabilis* is well known to exhibit swarming motility, a multicellular behaviour in which cells align into rafts and move across surfaces. Dr Jones described genetic studies to identify factors involved in swarming. Mutants defective in swarming on agar were also impaired in movement along catheter materials. However, these strains attached more readily to silicone and caused rapid blockage of catheters in an *in vitro* model. Therefore, swarming motility may not be needed for *Pr. mirabilis* catheter infections. The expression of swarming-related genes in biofilms was investigated using a

bioluminescence reporter gene system. Time-lapse imaging beautifully demonstrated gene expression occurring in waves as new rounds of swarming motility were initiated.

Even materials that are placed in sterile sites can become infected by microorganisms. David Partridge (Sheffield Teaching Hospitals Foundation Trust) described the pathogenesis of prosthetic joint infections. Despite rigorous hygiene in the operating theatre, bacteria are occasionally inoculated onto prosthetic joints from the skin during surgery. Alternatively, infections arise from adjoining surgical wounds, or as a result of the haematogenous spread of bacteria from infections at distant sites. *Staphylococcus aureus* is a major pathogen. Dr Partridge outlined the difficulties of diagnosing infections, particularly low-grade chronic infections and distinguishing them from aseptic joint loosening. Correct diagnosis is critical, since treatment of prosthetic joint infections is a complex and lengthy process.

A rapid and accurate diagnosis of infection is also vital in the intensive care unit (ICU), where an hour's delay in diagnosis can increase the chance of a fatal outcome by 8%. Massive trauma inevitably leads to a shut-down of the immune system, leaving patients extremely vulnerable to infections. Paul Dark (Salford Royal NHS Trust) discussed two common infections in the ICU: ventilator associated pneumonia and bloodstream infections. The former is a major complication of mechanical ventilation. Diagnosis is difficult and Dr Dark described exciting new studies that may help to rule out infection by the rapid analysis of inflammatory biomarkers such as IL-1 β .

The importance of diagnosis was also stressed in relation to cerebral shunts used for the treatment of hydrocephalus by John Hartley (Great Ormond Street Hospital). Shunts are used to alleviate pressure on the brain caused by the blockage of natural drainage channels for cerebrospinal fluid (CSF). Infection and growth of biofilms on the shunts leads to microbial contamination of CSF. Dr Hartley described cases where molecular methods had provided better results than microbial culture for the diagnosis of infection, including one

unusual mixed infection of *Mycoplasma pneumoniae* and *Propionibacterium* sp. New materials, impregnated with antimicrobials and designed to limit the establishment of biofilms, are becoming available for medical devices and data on the efficacy of these materials are urgently needed. In addition, improved methods for rapid and accurate diagnosis of infections are clearly a high priority for research.

Nick Jakubovics

The virology session embraced the common theme of cutting edge technologies and their contribution to virology. The audience enjoyed four different talks that all used aspects of next generation sequencing (NGS) to deliver their objectives. Saheer Ghabia (HPA, Colindale) delivered the first of these. She set the scene with a more general overview of the contribution of both NGS and proteomics to decipher subtle differences between strains, and their employment in potential vaccine discovery studies. She illustrated the power of these techniques with both bacterial and viral examples ranging from *Clostridium difficile* to Norovirus. Integration of both genomic and proteomic investigative approaches not only facilitates high resolution typing, but can additionally incorporate the important post-translational modifications which generate much of the variability observed during proteomic fingerprinting. She went on to explain how only certain types of Norovirus predominate among human cases. To further explore the basis for this differential pathogenesis, small conformational differences were revealed in the capsid protein that probably underpin the fitness advantages for these strains through increased persistence and antibody evasion.

This was followed by a talk from Pat Cane (also HPA, Colindale), who gave a detailed insight into how NGS data can directly influence patient management. She briefly reviewed HIV over the last 30 years from the initial descriptions of AIDS from 1981, the identification of HIV in 1983, through to current times whereby HIV research has pioneered the use of sequencing data for genotyping to map outbreaks, to aid forensic investigations and how resistance typing can directly impact upon patient management. The rapid mutability of HIV necessitates a population nucleotide sequencing approach assessing both major and minor potentially emerging populations. Precise prediction of clinical outcome based upon these analytical methods is not without its challenges due to a lack of definition between intermediate susceptibility and resistance. The strength of this approach is its ability to predict the accumulation of resistance with NGS being able to facilitate better resolution of low-level mutation which might predict future treatment failure. To maximize value in patient management, this data on HIV genotypes and resistance needs to be fully integrated with both clinical and surveillance databases, thus producing a more holistic and meaningful resource.

The afternoon then changed direction with Paul Kellam (University College London) presenting the use of NGS for assessing respiratory viruses. During his introduction he described how this technology enabled assessment of slight genotypic diversity which could be used to correlate biological properties which impact upon viral pathogenesis, transmission and host susceptibility. These varying platforms compile data to provide the consensus genome and underpinning



population structure (i.e. minority variants). From this data, it is possible to predict most likely phenotypes. A growing application of NGS is that of phylogeography, whereby the geographical spatial mapping of variants can inform on incursions and spread of outbreaks. He illustrated this by focussing upon the 2009 influenza A pandemic to demonstrate the value of NGS and phylogeography. It transpires that the introduction of H1N1 virus into the UK followed some 60 separate introductions. Furthermore, cluster analysis in London was able to demonstrate separate origins for the virus infecting two adjacent schools. He then went on to describe how NGS could be applied directly to clinical material by using capture technology to bind exons of the host, thus enriching the viral target prior to analysis.

The last speaker of the session was Christophe Fraser from Imperial College, London. Building upon the information given in the previous lecture, he explained the need for combined virological and epidemiological approaches. Again he illustrated this using the 2009 H1N1 pandemic, explaining the likely impact of an overall 20% reduction of clinical cases upon the spread of infection and dynamics of this outbreak. Using modelling software incorporating epidemiological and molecular data, it was possible to accurately back calculate the size of the initial outbreak and its transmission patterns. He then went on to explain how software packages such as BEAST (Bayesian Evolutionary Analysis Sampling Trees) can be used to further refine models to inform and predict the course of spread of infection.

Both these examples demonstrated the power of epidemiology and molecular data to back calculate size and elucidate transmission from their source. Collectively, these talks informed and updated the audience of the power and discriminatory potential of developing technologies and their application to virological diagnostics. Attendees were left with plenty of food for thought for their journey home.

Sally Cutler

Environmental Microbiology lecture 2012



The 2012 *Environmental Microbiology* Lecture will be presented by Professor **Sang Yup Lee**, Distinguished Professor and Dean of the College of Life Science and Bioengineering at KAIST (formerly the Korea Advanced Institute of Science and Technology).



Royal Society of Medicine, London ■ Monday 8 October 2012

Professor Lee will present: *Systems metabolic engineering for a green chemical industry*. It has been 20 years since the term metabolic engineering was officially introduced. Microorganisms isolated from nature are often inefficient, so metabolic engineering has been employed to improve microbial performance.

Recently, metabolic engineering has become more powerful and is now essential in developing superior microorganisms formed from the integration of systems biology with synthetic biology.

In his lecture, Professor Lee will present the general strategies for metabolic engineering of microorganisms. These will be accompanied by

many successful examples, including the production of chemicals, fuels and materials. Systems metabolic engineering will also be introduced as an essential technology in making any bioprocess competitive.

The lecture will take place on 8 October 2012 at the Royal Society of Medicine, London, 1 Wimpole St, London W1G 0AE.

The lecture will begin at 6.30pm, with tea/coffee being served from 6pm. There will be a drinks reception following the lecture.

If you wish to attend the lecture, please contact emlecture@sfam.org.uk or complete and return your invitation slip.



Wednesday 9 January 2013

Winter Meeting

- **Food mycology**
- **Emerging technologies in applied microbiology**
- Including the Denver Russell Memorial Lecture
- In conjunction with the British Mycological Society

The Royal Society, London, UK



CPD
ACCREDITATION
APPLIED FOR

Programme

10.00 – 10.30 Tea, coffee and registration

Chair: Martin Adams

10.30 – 11.15 **The Denver Russell Memorial Lecture**
To be confirmed

11.15 – 11.50 **Molecular and ecophysiology aspects and impacts on mycotoxin contamination**
Naresh Magan, Cranfield University, UK

11.50 – 12.25 **Plex-ID in the microbiology laboratory**
Mark Wilcox, Leeds General Hospital, UK

12.25 – 13.30 Lunch

Session A Food mycology

Chair: Naresh Magan

13.30 – 14.05 **Metabolomics and taxonomy aspects of food spoilage moulds**
Ulf Thrane, Technical University of Denmark

14.05 – 14.40 **Modelling spoilage fungal growth**
Sonia Marin, University of Lleida, Spain

14.40 – 15.00 Tea and coffee

15.00 – 15.35 **Spoilage fungi in the factory environment**
Phil Voysey, Campden BRI, UK

15.35 – 16.10 **Spoilage fungi and sex in the food environment**
Paul Dyer, Nottingham University, UK

Session B Emerging technologies in applied microbiology

Chair: To be confirmed

13.30 – 14.05 **Applications of next generation sequencing in microbiology**
To be confirmed

14.05 – 14.40 **Molecular methods in food and water microbiology**
Andrew Fox, HPA, Royal Preston Hospital, UK

14.40 – 15.00 Tea and coffee

15.00 – 15.35 **MALDI-TOF in microbiology**
Steve Davies, Northern General Hospital, Sheffield, UK

15.35 – 16.10 **Automating the bacteriology laboratory**
Neil Bentley, HPA Cambridge, UK

16.10 Close

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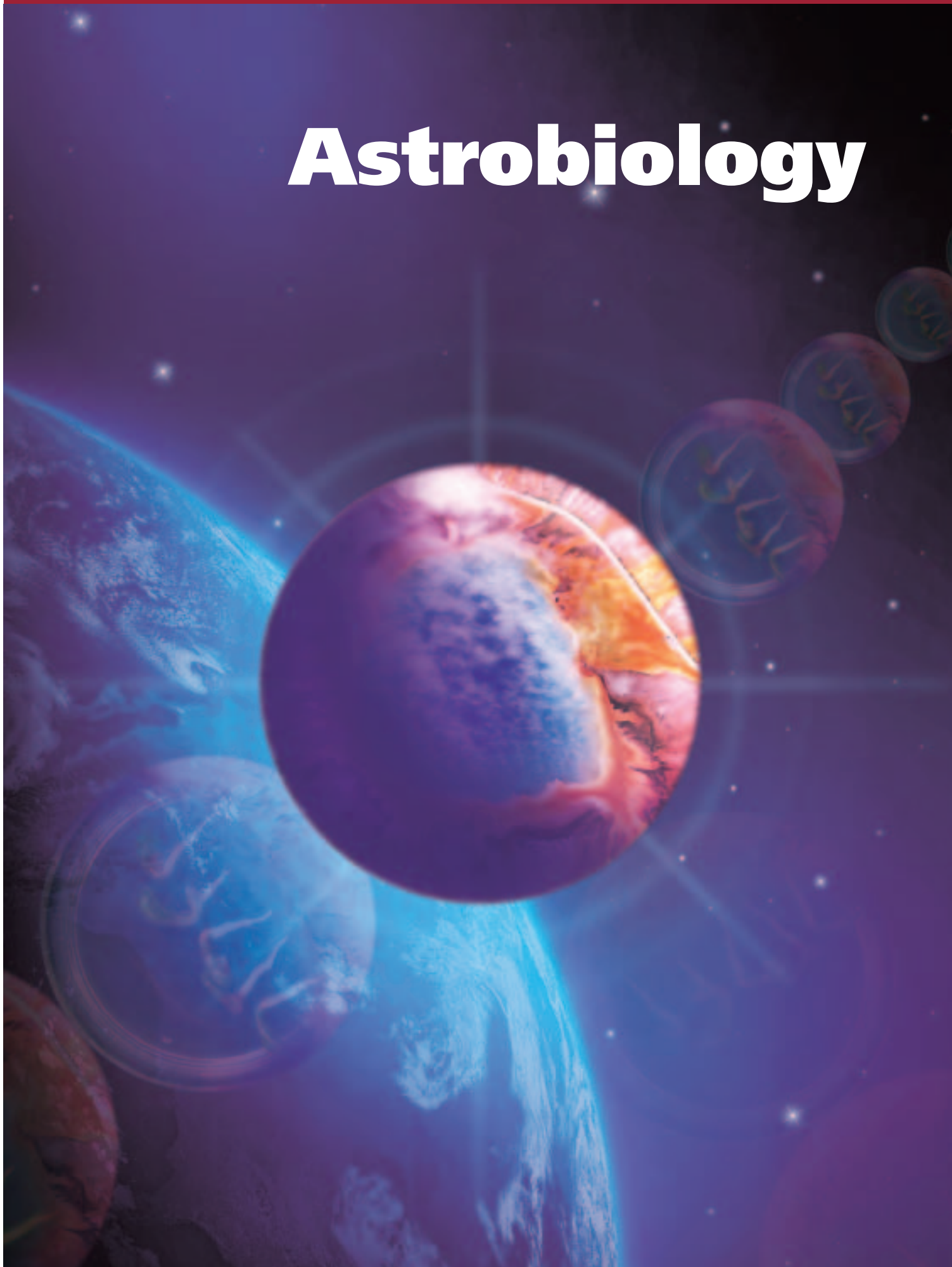
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Astrobiology



– is it a science?

Astrobiology has been described, rather excitedly, as a ‘new science’. Whenever any type of science makes claims about itself, it is always worthy of special critique and a ‘new science’ especially demands some attention (Cockell, 2001).

To put a rather callous and swift end to one part of this claim – astrobiology certainly isn’t new. If we take it to be the field of enquiry that investigates questions of interest at the interface between biological sciences and astronomy and planetary sciences (as its name suggests), then Metrodorus of Chios, a student of the famed Democritus who first espoused the atomic theory of matter, might legitimately have claim to being the father of astrobiology. His observation of the 4th century BC: “*It would be strange if a single ear of corn grew in a large plain, or there were only one habitable world in the infinite.*” remains two and a half thousand years on, one of the most eloquent statements of why people continue to think that the uniqueness of life on the Earth would be odd. People have been wondering about alien life, perhaps one of astrobiology’s most potent quests, for a very long time.

The term ‘astrobiology’ made its debut in the first serious bit of work as the title of Tikhov’s book, *Astrobiology*, in 1953 (Tikhov, 1953). A colourful character, Tikhov (Figure 1), who founded the ‘astrobotany sector’ of the Kazakhstan Academy of Sciences, was convinced of the existence of vegetation on Venus. Despite his eccentricities, he spent some of his time taking spectral measurements of vegetation with the idea of using spectroscopy to detect signatures of life on other planets, a technique now at the forefront of plans in the search for biosignatures of life on planets orbiting distant stars, efforts which will use spectroscopic signatures of gases such as ozone, or even spectral changes induced by surface pigments, to find evidence of life (Seager, Schrenk & Bains, 2012).

That’s enough brief history. A much more interesting question is whether astrobiology is a science. Some people

Figure 1. Gavriil Tikhov taking spectral measurements of plants to attempt to detect them on other planets



have lambasted astrobiology as a science on the basis that it rests on a hope — a hope for the discovery of alien life. As such, not only is it a science currently without any data, but it is also based on a wish. Astrobiology seeks to address any question which probes the relationship of life with its cosmic environment, so the first point to clear up is that the search for extraterrestrial life is just one of its foci. But let’s ignore that for a moment and talk about alien life.

The search for life beyond the Earth is only a ‘hope’, if you’ve gone and set your hopes on finding it. There is little hesitation in saying that many people do hope for aliens. They’ve seen them in movies and they want to see them for real. But from a scientific perspective, the search for life beyond Earth is merely the testing of a hypothesis. That hypothesis is: “*There is life on other planetary bodies*”. This is verifiable (you can negate it if you prefer a falsifiable hypothesis) and it is experimentally testable. A good scientist

remains neutral about the outcome of testing the hypothesis. If we find alien life, then of course biologists will have much work to do. Taking up a Chair in Comparative Alien Physiology would certainly be an interesting and rewarding career. But the lack of alien life would be equally profound (at least the lack of alien life within a certain defined region of the universe we could study, as demonstrating its absence across the whole known universe would be very difficult to do). It would tell us that the conditions for the origin of life are extremely rare and that the Earth had an unusual concatenation of environments and events that allowed it to emerge. What were those conditions and events that gave rise to life? The lack of alien life in places where we might expect to find it would be scientifically very interesting.

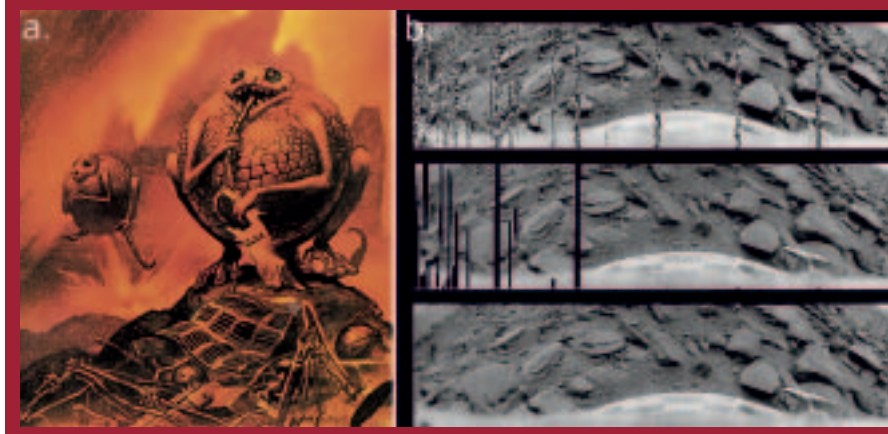
Astrobiology is a science, it has a hypothesis about alien life to be tested, it is just that the phrase “the search for alien life”, gives an unfortunate spin on a scientific hypothesis by transforming

the experimental testing into something more akin to a hunt with an intended outcome — to find the quarry. There's not a problem with this if the objective is to engage the public, but it is worth remembering that the "search for alien life" is a scientifically weak elaboration of one of astrobiology's goals.

There was a wonderful book published in 1986 entitled 'Atlas of Our Universe' by Roy Gallant and the National Geographic Society (Gallant, 1986) which presents images of a collection of organisms from other planets in our solar system. The 'Oucher-Pouchers' (Figure 2a) are creatures, essentially bags of gas that bounce around on the Venusian surface. The surface is hot — isothermally 464°C — which explains their name. Each time they bounce onto the rocks, they let out an excruciated "ouch". They gather their bioessential elements from rocks (sometimes feasting on the rare remains of old Soviet landers) and they have biochemical systems adapted to making a living on Venus. The book inadvertently touches on another question in astrobiology that we might frame as the hypothesis: "The limits to life are universal". Put another way are the limits to life (almost all defined by microorganisms), an idiosyncrasy of terrestrial life or something more fundamental and universal? If you re-ran the tape of evolution would you end up with a biological zoo, the boundaries of which would be set by entirely new limits to life?

Perhaps one of the microbiologically most interesting developments of planetary sciences since the birth of the space age has been the remarkable confluence of extremophile microbiology and observations of planetary sciences. If the capabilities of terrestrial life were just an anomaly of life on Earth and the potential for other biochemistries was merely limited by our own imagination, then we would expect to find entirely novel biochemistries elsewhere. We don't. The lack of liquid water on the surface of Venus, the lack of plausible redox couples for energy, particularly those requiring organic carbon, and the lack of fixed nitrogen are just some of the factors that would lead us to predict that the surface of Venus would be lifeless. When the Soviet landers arrived on the surface of Venus in the 1970s they did not take snapshots of Oucher-Pouchers

Figure 2. a. 'Oucher-Pouchers' on Venus (Gallant, 1986), but the surface of Venus appears dead to multicellular life (**b**; Venera 9, in 1975), exactly as we would predict



or microbial mats of silicon-based life forms basking in the Venusian heat (Figure 2b). They saw what we would predict from our knowledge of microbiology — a dead, lifeless world with physical conditions outside the boundary space for life.

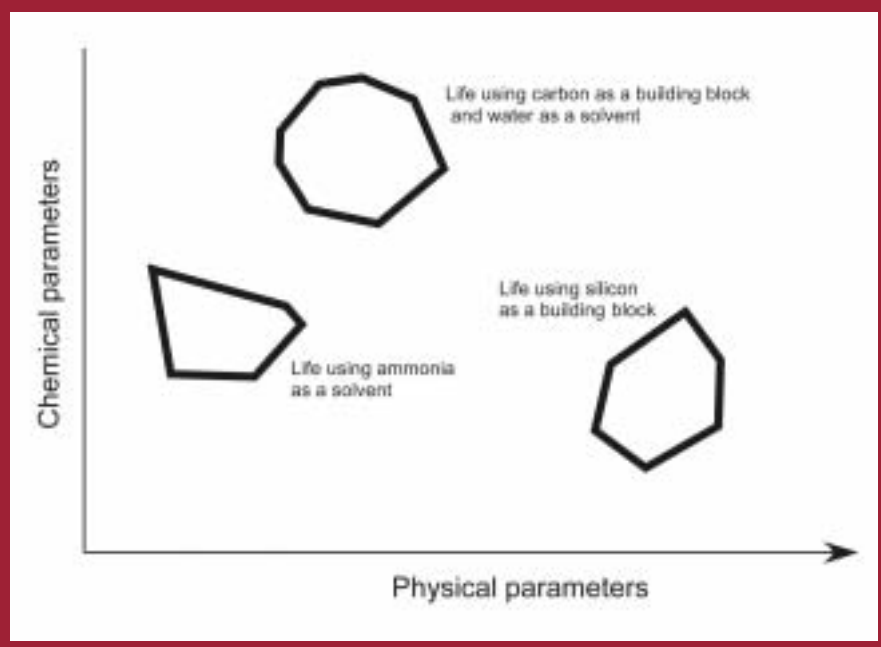
A similar fate awaited the 'Searchers', long-legged beasts that roam the Martian ice caps probing the subsurface for water with their long proboscis and at night covering their heads with enormous furry ears which protect them from the intense cold of the Martian polar regions (that get down to a nippy -150°C in winter). No spacecraft has yet seen anything roaming the Martian ice caps. We would predict this. There is insufficient oxygen (0.13% in the atmosphere) and organic carbon (there is no obvious photosynthetic biosphere) to generate a redox couple for aerobic respiration to allow megafauna to gather the energy to stride across the Martian ice caps, drilling into the subsurface for water. No other energy-generating redox couple exists which will produce enough energy for Searchers. Sulfate-reducing Searchers eating sulfate salts (which have been found on Mars) and using hydrogen gas from the subsurface, even assuming the best estimates for availability of hydrogen and sulfate, cannot gather enough energy to make a living on Mars. Thermodynamics predict a biological situation on Mars which matches what we observe. Our knowledge of the limits of terrestrial biology would predict that we'll be lucky to find microbes in transient liquid water bodies on Mars (Boston, Ivanov & McKay, 1992), let alone extant multicellular life. Both Venus and Mars

support the hypothesis that the limits to life might be universal.

The case of Venus and Mars illustrate another important point. The lack of life on a planetary body is a data point. Astrobiology does already have data — dead worlds inform us about the prevalence of life, its ability to adapt to extremes and the apparently universal nature of the boundaries to the origin and evolution of life.

What limits terrestrial life? Is it just thermodynamics? — the trade-off between the energy an organism can gather from the environment and the energy it needs for replication, growth and repair at any given set of extremes? Or is it a fundamental structural failing in biomolecules, long since locked into the architecture of life, that fall apart at extremes they were never evolved to operate at? It has been hypothesized that the labile nature of small molecules at high temperatures might account for the problems that microbes encounter at high temperatures (Daniel & Cowan, 2000). The ability of chaotropes, molecules which induce instability in cell macromolecules, to extend the lower temperature limit for life suggest that the limits to life are partly set by molecular instabilities and limits (Chin *et al.*, 2010). Ultimately organisms need enough energy to operate at extremes, so it is likely that thermodynamics sets broad boundaries to the extremes in which life can operate, with macromolecular functioning fine-tuning the boundary space. Thus, to understand our observations of other planets and their lack of life requires an understanding of the limits to terrestrial life. Astrobiology is a science that brings

Figure 3. A conceptual illustration of the idea of 'biospaces'; areas of habitable space within defined physical and chemical extremes showing our own one based on carbon-based life in liquid water as a solvent. Are there other 'biospaces' using other solvents or molecular building blocks?



us back to investigations of terrestrial organisms.

We might conclude that terrestrial life does exhibit limits that tell us something about the universal boundaries to life, boundaries partly set by the stability of (carbon) macromolecules operating across the temperature range within which life's biochemical solvent (water) can remain liquid. Let us call this biological zoo we inhabit, surrounded by a fence of extremes that enclose it, a 'biospace'. Are there other biospaces? Are there biospaces inhabited by life which uses other solvents, such as liquid ammonia (Firsoff, 1963) (Figure 3), an interesting substance which can do some analogous things to water, such as allowing for the formation of peptide-like bonds in proteins? Perhaps there are biospaces of life made from silicon compounds, some of which can form complex chains in analogy to carbon (Bains, 2004)? Alternative biospaces have occupied the minds of science fiction writers for a long time. As yet there is no field or laboratory evidence for any type of life that is not based on the carbon-water system that life on the Earth uses. The hypothesis that complex molecular systems can emerge in alternative solvents such as ammonia could be tested by rigorous experiments to look at the behaviour of organic

molecules in ammonia and the energetics of bond formations in complex-chained carbon compounds. Experiments might look at vesicle formation in ammonia to understand the potential for the formation of selectively permeable membranes. Even this most speculative area of astrobiology is amenable to experimental analysis and hypothesis testing.

I have neglected to explore the hypotheses in astrobiology that tackle the origin of life, the adaptations of organisms to a whole variety of terrestrial extremes, and hypotheses that explore the nature of life on the early Earth through the fossil records and modern molecular evolutionary methods. Astrobiology is as much concerned with the past, present and future of life on the Earth as it is with alien life. Extraterrestrial life has dominated the public and scientific view of astrobiology, but I think it is safe to say that even if we entirely circumscribed astrobiology as a science concerned with extraterrestrial life, then it is a robust science that has a wealth of testable hypotheses. New developments in robotics, vastly increasing information about microbial adaptations to extreme environments, the discovery of many planetary bodies in our own solar system that host bodies of liquid water (the

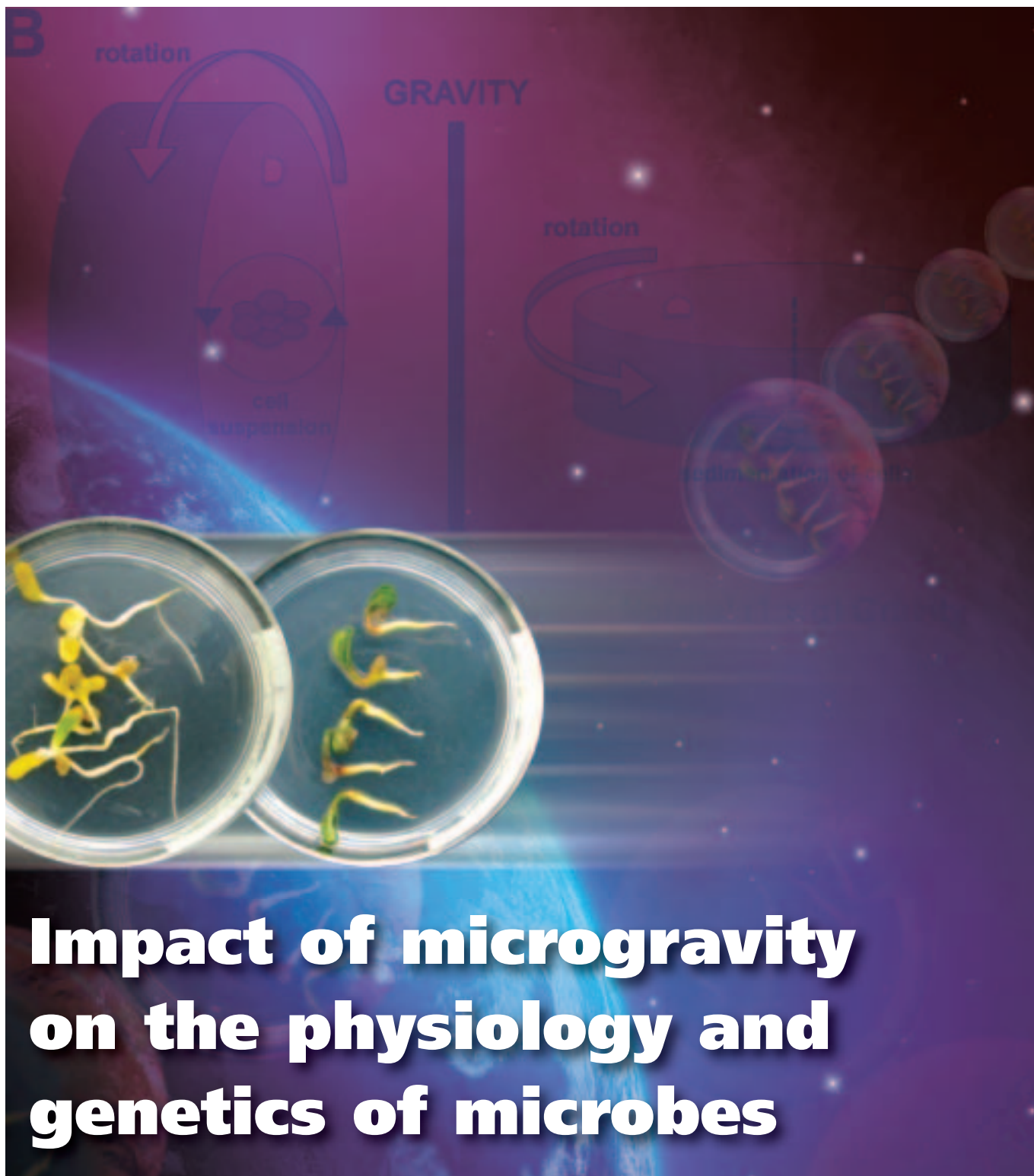
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Jovian and Saturnian moons, Europa and Enceladus, respectively, are just two) and the discovery of Earth-like planets orbiting distant stars have made astrobiology an empirical science. These developments have finally brought astrobiology from the philosophical limits of its reach, which began in the schools of ancient Athens, and have transformed it into a science. It is a science with experimentally testable hypotheses that will, over the next few decades, bring us answers about the presence of life beyond the Earth and, in the process, inform us about the uniqueness, or not, of our own experiment in evolutionary biology.



Charles Cockell
UK Centre for Astrobiology,
University of Edinburgh,
Edinburgh



Impact of microgravity on the physiology and genetics of microbes

For more than 3.5 billion years life on Earth has experienced numerous changes in its environment, yet one factor, the physical force of gravity, has remained constant. As a result all living organisms have developed mechanisms to sense and respond to this ever-present force. To understand the

influence of gravity on the evolution of life, in particular microbial life, experiments have been conducted under reduced gravity, or microgravity. By removing gravity as a constant, one can address the question of whether gravity obscures aspects of biological processes and development that may otherwise go

undetected under terrestrial conditions.

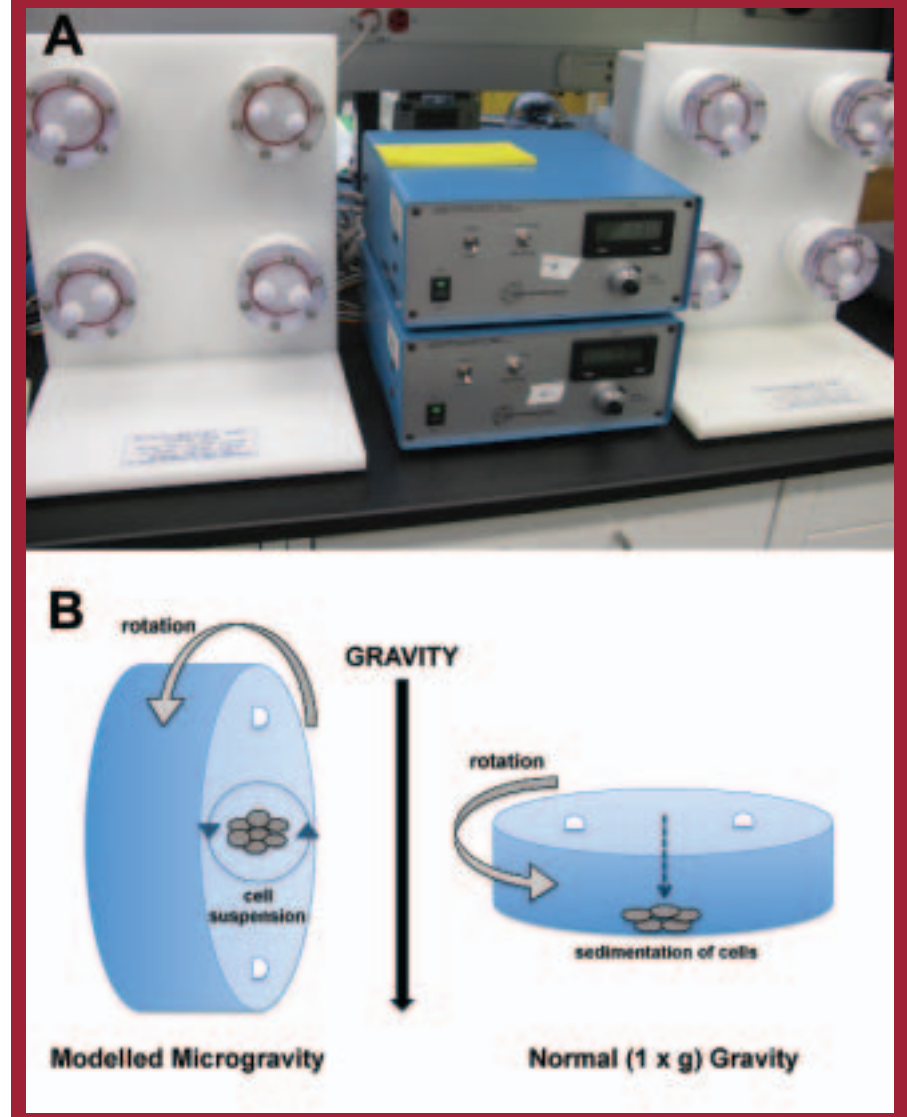
To examine the physiological and genetic effects of microgravity several key studies have been conducted in low Earth orbit on spacecraft, such as the space shuttle and International Space Station (for example, Wilson *et al.*, 2007). Although the facilities on these

spacecraft have served as ideal laboratories for microgravity studies, there are some inherent limitations to conducting microgravity experiments in the space environment, most notably limited flight opportunities, astronaut time, specialized equipment to work in space and overall costs. To offset these limitations many microgravity studies have relied on simulating the microgravity environment using ground-based modelled microgravity (Figure 1). Ground-based models mimic the low-shear conditions of the space environment which result from the lack of convection currents in microgravity. One such model, the rotating-wall vessel (RWV) was developed by the NASA Biotechnology Group (Wolf & Schwarz, 1991) and consists of a bioreactor that rotates around a horizontal axis enabling microbial cells within the vessels to be in perpetual fluid suspension, whereby the gravitational forces are countered by hydrodynamic forces (e.g., shear, centrifugal and Coriolis). This feature allows the cells to be in steady state terminal velocity, thus simulating the conditions of microgravity (Nickerson *et al.*, 2004). Physiological and molecular responses of microbes exposed to space flight conditions have been correlated with simulated microgravity indicating that the RWV bioreactors can serve as a robust analogue to the space environment (Horneck, Klaus & Mancinelli, 2010; Nickerson *et al.*, 2004).

The physiological responses of microbial cultures in microgravity

The effects of microgravity on microbial physiology have been examined for decades (Nickerson *et al.*, 2004) and several basic physiological responses have emerged. These changes include: a reduced lag phase in the bacterial growth cycle (Klaus *et al.*, 1997); growth to higher cell densities, increased cell viability and membrane potential (Vukanti, Model & Leff, 2012); increased rates of genetic transfer via conjugation (Ciferri *et al.*, 1986) and increased productivity of secondary metabolites (Lam *et al.*, 2002). Studies have shown that most of these biological responses are indirectly caused by microgravity and are more of a reflection of the nutrient and quiescent fluid environment of the microbial culture. In the absence of convection, cells must rely on diffusion for the mass

Figure 1. Modelled microgravity. A. Rotating-Wall Vessels (RWVs) that simulate microgravity. B. Overview of the effects of microgravity on cell cultures. In the RWVs the hydrodynamic forces of the fluids within the bioreactor offset the force of gravity and the cells remain suspended, whereas in the gravity control bioreactors the cells undergo sedimentation



transfer of nutrients and metabolic by-products. This reduction in mass transfer results in a differential response of the cells to this altered extracellular environment, as compared with controls grown under normal 1 x g gravity (Horneck, Klaus & Mancinelli, 2010). Cell motility and the type of media can disrupt the fluid and nutrient environment of the culture, thus changing the physiological responses of the cells. For example, cells that are motile can disrupt the quiescent fluid environment-associated, low-shear microgravity and many of these basic biological responses are no longer observed. These comparative

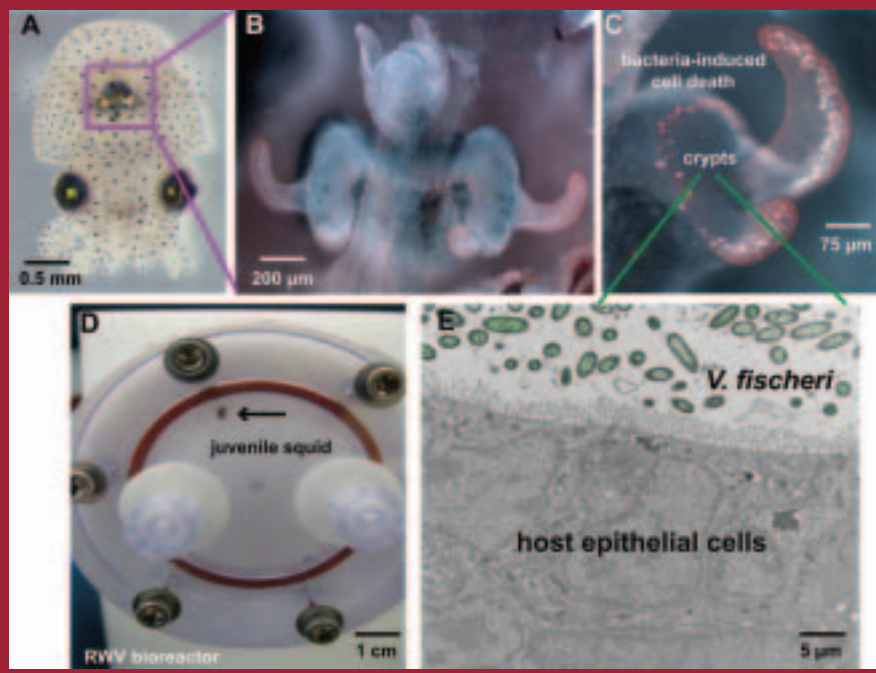
experiments provide key insights into how the effect of the physical forces can be correlated with biochemical and molecular responses within the cells (Benoit & Klaus, 2007).

Changes in microbial virulence and antibiotic resistance

In addition to these basic responses recent studies have shown that several pathogenic bacterial strains become more virulent under microgravity conditions. One of the best-studied pathogens in microgravity is *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) (Wilson *et al.*, 2007). *Salm.* Typhimurium cells

Figure 2. Symbiotic association between the squid *Euprymna scolopes* and luminescent bacterium *Vibrio fischeri*.

- A.** Juvenile squid with pronounced light organ (box).
- B.** Light organ of the host squid, which houses the bacterial symbionts.
- C.** After colonization of the crypt spaces, the bacteria trigger widespread cell death along a field of ciliated epithelial cells. Dying cells appear as punctate areas of fluorescent staining.
- D.** Image of a juvenile squid within the RWV bioreactor (not rotating). The bacteria-induced development of the host squid is accelerated under microgravity conditions and the symbiosis is emerging as an ideal model to look at the interactions between bacterial and animal cells under microgravity.
- E.** Transmission electron micrograph of the *V. fischeri*-filled crypt spaces showing the microvilli-covered surfaces of the host squid epithelial cells



grown in microgravity and then exposed to mice result in an increased colonization of the liver and spleen and a faster time-to-death of the host compared with normal gravity-treated control cells (Nickerson *et al.*, 2004). This increase in virulence is also correlated with an increased resistance to acid, osmotic and thermal stresses compared with normal gravity controls, suggesting that microgravity might trigger a general stress response in bacteria. However, further analysis of gene expression patterns under microgravity conditions indicated that the genetic response to microgravity is distinctive from the RpoS-dependent general stress response. RpoS is the primary sigma factor for the global regulation of genes associated with environmental stresses. *Salm.* Typhimurium mutants defective in *rpoS* conveyed the same microgravity-induced virulence and stress response compared with wild-type, indicating that the

microgravity-induced genetic pathway is RpoS-independent and may represent a novel stress response (Nickerson *et al.*, 2004).

Although the underlying mechanisms associated with the changes in virulence and stress responses are still not known, there have been several recent advances using transcriptional and proteomic analyses (Wilson *et al.*, 2008). Analyses of microarray data derived from *Salmonella* spp. exposed to simulated microgravity indicate that the increase in virulence and stress responses are correlated with the differential expression of 163 genes, including genes encoding for virulence factors, transcriptional regulators, lipopolysaccharide synthesis enzymes, iron utilization genes and ion transport (Wilson *et al.*, 2002). Of the genes associated with virulence, interestingly, many were down-regulated in microgravity including several genes associated with *Salmonella*

pathogenicity islands SPI-1 and SPI-2, which are associated with type 3 secretion systems/injectisomes, suggesting an alternative mechanism for the increase in *Salmonella* virulence in microgravity (Wilson *et al.*, 2002). The microarray analyses also revealed changes in genes associated with ion transport. Subsequent experiments comparing the medium ion composition revealed that the addition of inorganic phosphate was able to attenuate the increase in virulence associated with microgravity exposure (Wilson *et al.*, 2008). These results suggest that it may be possible to mitigate the potential harmful impact that an increase in virulence may present to astronaut health. Other differentially regulated genes such as those which encode the ferric uptake regulator Fur and the small regulatory RNA-binding protein Hfq, which has been previously shown to be associated with phosphate regulation, may also play critical roles in regulating the bacterial response to microgravity conditions. In addition to *Salm.* Typhimurium, other bacterial species such as: *E. coli*, *Streptomyces levoris*, *Bacillus subtilis*, *Methylbacterium organophilum*, *Methosinus methanica*, *Clostridium butyricum* and *Pseudomonas aeruginosa* also exhibit increased virulence in microgravity (Leys *et al.*, 2004), however, further experimentation to delineate the genetic pathways is needed (Wilson *et al.*, 2002; Wilson *et al.*, 2007).

Another characteristic of microgravity-cultured bacteria is an overall decrease in the effectiveness of antibiotics. Experiments conducted during space flight showed greater concentrations of antibiotics were often required to inhibit microbial growth in space compared with 1 x g gravity controls (Leys *et al.*, 2004). However, these effects appear transient and the increased resistance was not retained during post-flight (Klaus & Howard, 2006). These experiments have since been repeated using RWV simulated microgravity, however, the molecular mechanisms by which antibiotic resistance is conveyed are still unknown (Horneck, Klaus & Mancinelli, 2010). The increased microbial resistance to antibiotics may, in part, reflect the decreased effectiveness of the drugs. Previous studies have shown that the efficacy of antibiotics and other

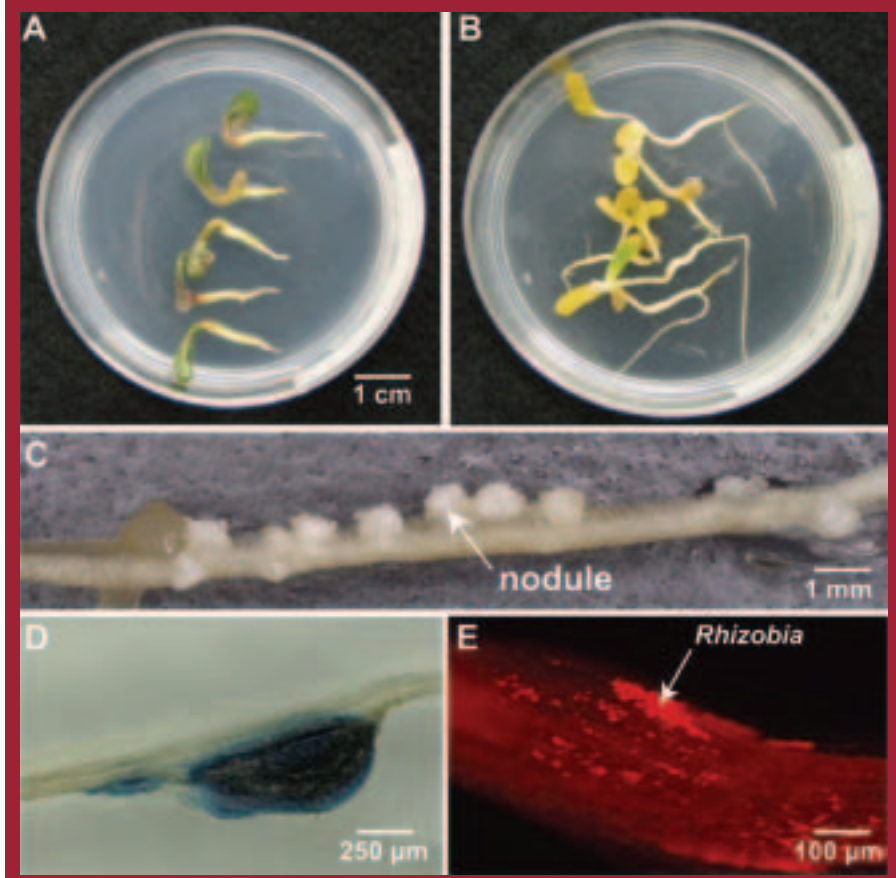
pharmacological agents in microgravity have a reduced shelf life in space, most likely due to shift of fluids and reduced hydrostatic load in astronauts (Klaus & Howard, 2006). This resistance to antimicrobial drugs is not limited to bacteria, two haloarchaea *Haloferox mediterranei* and *Halococcus dombrowskii* exhibited an increased resistance to bacitracin, erythromycin, and rifampicin (Dornmayr-Pfaffenhuemer *et al.*, 2011). These changes in microbial virulence and antibiotic resistance are compounded by evidence that there is an overall dysregulation of the mammalian immune system in space flight (Taylor *et al.*, 1997) presenting a significant risk to human health and safety.

Host-microbe interactions and biofilms in microgravity

Although pathogens represent a serious concern to astronaut health, they only constitute approximately 1% of the total diversity of microbes that typically associate with eukaryotic cells. The other 99% are commensal or mutualistic bacteria and are often essential for the normal development and function of the host organisms. One advantage of the low-shear microgravity environment is that it mimics several areas of the human body where mutualistic bacteria typically associate, such as the brush border, or microvilli-covered surface of epithelial cells (Nickerson *et al.*, 2004). Bacteria have long been known to influence the morphology, gene expression and biochemistry of the cells with which they associate, and understanding how these processes change in microgravity may provide key insights into these bacteria-host interactions.

One well-established model for examining host-microbe interactions has been the development of three-dimensional (3-D) cell cultures in RWV microgravity (Nickerson, Richter & Ott, 2007). Cell aggregates which form under microgravity preserve several characteristics associated with *in vivo* tissues, such as differentiated cell structures and the establishment of molecular and biochemical gradients that traditional cell culturing may not always capture. Several 3-D cell cultures have been generated including small intestinal epithelia, lung, bladder and neuronal cell aggregates. These aggregates are more representative of

Figure 3. Symbiotic association between the leguminous plant *Medicago truncatula* and the nitrogen-fixing bacterium *Ensifer meliloti*.
A. Uninoculated *M. truncatula* prior to space flight.
B. *M. truncatula* post-space flight was successfully colonized by *E. meliloti* in microgravity.
C. Colonization and nodulation occurred in microgravity with no apparent delay in development.
D. Normal gene expression (blue staining) occurred in the nodules under microgravity conditions.
E. *E. meliloti* was able to effectively colonize the plant tissues under microgravity with no developmental delays



cell-cell interactions, such as adherence and signal transduction, than traditional cell culturing (Nickerson, Richter & Ott, 2007). For example, 3-D cell culturing of human intestinal cells in microgravity showed many enhanced tissue characteristics, such as a finer organization of extracellular matrix, distinctive apical and basal cell morphologies, and localized expression of mucins compared with traditional cell monocultures (Nickerson, Richter & Ott, 2007). These 3-D cell aggregates provide a unique opportunity to delineate the impact of microgravity on the normal healthy microbial community which typically associate with these tissues, such as changes in microbial diversity, adherence and genomic/proteomic expression profiles.

Other model systems which examine the impact of microgravity on animal-microbe interactions *in vivo* are emerging, such as the symbiotic relationship between the host squid *Euprymna scolopes* and the luminescent bacterium *Vibrio fischeri* (Foster *et al.*, 2011) (Figure 2). This squid/vibrio system has the advantage of being monospecific, with only one host and one symbiont. The simplicity of this symbiotic association makes it easier to delineate the effects of the bacterium on the host tissue. It also enables both the host and symbiont to be incubated in the RWV together so that the initiation and onset of symbiosis-induced development can be monitored under microgravity conditions. In addition to animal-microbe associations,

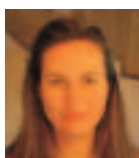
microgravity-based research has examined the interactions between bacteria and plant tissues, specifically nitrogen-fixing rhizobia and leguminous plants (Figure 3). No microgravity-induced changes have been observed in this symbiosis and *Ensifer meliloti* cultures were able to effectively colonize and induce nodule formation in legumes compared with 1 x g gravity (G. Stutte & M. Roberts unpublished). Despite the absence of microgravity-induced changes in this beneficial association, plants in space flight have been shown to be more susceptible to pathogenic infection from microbes, such as biofilms, which contaminate the spacecraft surfaces (Schuerger, 1998). Biofilm-forming microbes are prolific in microgravity and have been shown to be thicker and more resistant to oxidative, acid and antibiotic stresses (Lynch *et al.*, 2006). Although biofilms can be used for bioremediation of waste products, air remediation, and other life-support processes that may be needed for long-duration space flight, they are also a serious concern since they can cause tremendous damage to spacecraft materials and pose a potential hazard to crew health (Pierson, 2001). Due to this potential threat, experiments are underway to delineate the molecular and biochemical mechanisms associated with biofilm formation in microgravity (e.g. Lynch *et al.*, 2006).

Conclusions

Microgravity posits a unique opportunity to examine the underlying impact that gravity has had on the evolution of microbial life. Specifically, microgravity experiments have provided critical insight into the molecular mechanisms associated with virulence and stress response mechanisms in pathogens. Although microgravity-induced pathogenicity of microbes is clearly an important health issue for astronauts, future research needs to expand beyond pathogenesis and begin to also include mutualistic and commensal organisms, as they comprise the vast majority of the microbial diversity in the space environment. Together, studying both pathogenic and beneficial microbes will provide a more comprehensive understanding of the effects that microgravity plays on the ecology, genetics and physiology of microbes in the space environment.

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Jamie S. Foster

Department of Microbiology and Cell Science, University of Florida
Space Life Science Lab, Kennedy Space Center, FL USA

Extremophiles

One of the most astounding realizations of recent years has been just how hardy life on Earth really is.

Terrestrial microorganisms have now been found flourishing in environments so hostile that previously they were simply assumed to be sterile. Organisms that make these locations their home are called 'extremophiles', literally meaning "extreme lovers". They are found to withstand and thrive in physical extremes of temperature, pressure and radiation; and chemical extremes of salinity, acidity and limited availability of liquid water. The extreme ecosystems found on Earth are similar to what we expect to find on other planetary bodies in the solar system and so provide a glimpse into what life might be like elsewhere in the universe.

Most of the hardiest extremophiles

are microbes – large, multicellular lifeforms like humans and plants are far more limited in the conditions they can tolerate. Some of the best-known examples are thermophiles — or heat-tolerant organisms — and these are commonly found basking around the scalding vents of geothermal hot springs. The current record for the upper temperature limit of life is set at 121°C by the aptly named 'Strain 121', a single-celled microbe discovered in 2003 around a deep sea hydrothermal vent. Such deep-sea

hydrothermal vents are important environments for astrobiology because they are thought by many researchers to be likely crucibles for the origin of life on Earth. They could also provide crucial energy sources for ecosystems elsewhere in the solar system, such as the watery ocean deep beneath the surface of Europa, one of the icy moons of Jupiter.

At the other end of the thermometer scale are psychrophiles that survive in freezing temperatures, even down to -15°C in ice-bound pockets of very salty water.

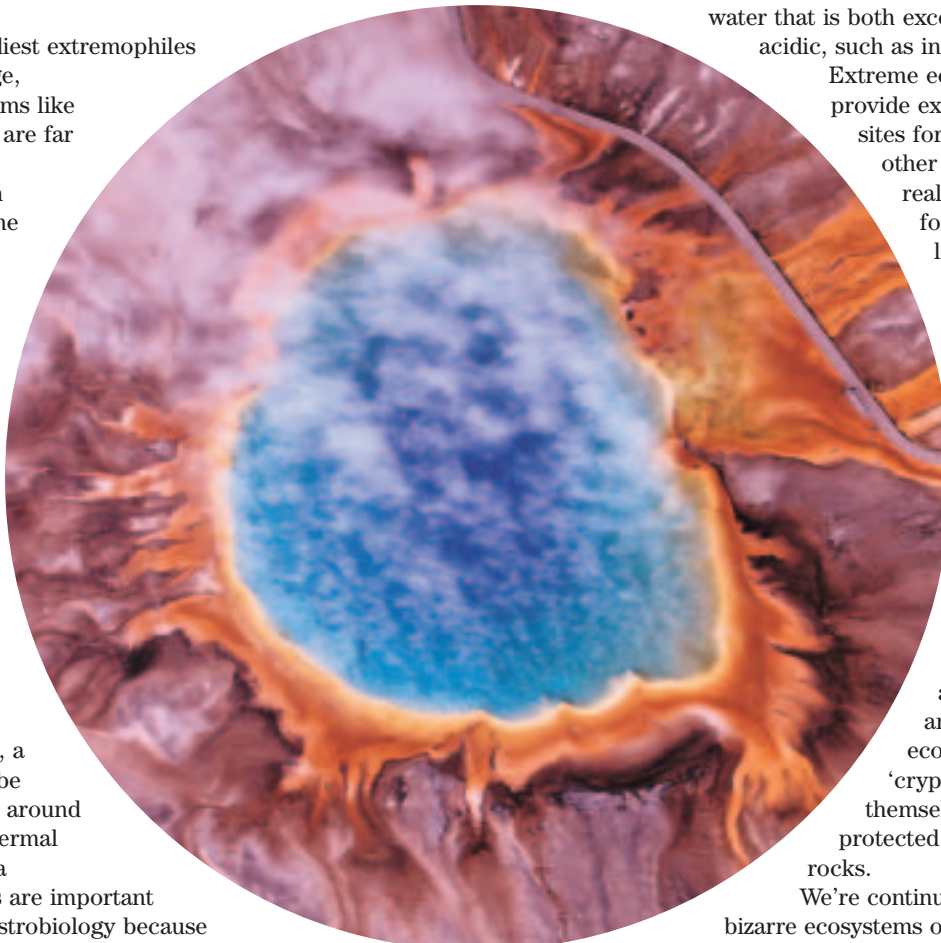
Acidophiles, as the name suggests, thrive in very acidic solutions at a pH below 3. One of the most acidic

environments on Earth has a pH of 2.3 but still supports a large ecosystem of microbes. Rio Tinto in south-western Spain is a large natural acid drainage system containing high concentrations of iron in its waters, most of which is oxidized by the microbes to power themselves. Rio Tinto is thought to closely resemble acidic, iron-rich rivers and water bodies that once covered the face of ancient Mars, and so provides a crucial test-bed for the prospects of Martian life.

Many organisms do not require oxygen for growth, whilst some tolerate punishingly alkaline solutions, or extremes of pressure, salinity, or desiccation. The most adept lifeforms are the polyextremophiles which can survive several extremes at once, for example thermoacidophiles thrive in water that is both exceedingly hot and acidic, such as in volcanic ponds.

Extreme ecosystems on Earth provide excellent 'analogue' sites for environments on other worlds that we now realize may be suitable for harbouring alien life. One well-studied location is the Dry Valleys region in Antarctica, which is one of the coldest and driest places on Earth, and also bathed in very high levels of ultraviolet radiation. This is a very Mars-like environment, but even here life finds a way to survive, and entire microbial ecosystems of 'cryptoendoliths' hide themselves inside protected niches within rocks.

We're continually discovering new bizarre ecosystems on the face of the Earth and an entire extremophilic microbial world remains to be explored deep in the subsurface of the planet. As each of these ecosystems is scrutinized, we take one step closer to finding life on another world.



Louisa Preston
Open University



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

***The one-way analysis of variance (ANOVA):
Fixed effects model***

StatNote 30

Introduction

In a previous StatNote (StatNote 9, Hilton & Armstrong 2007a), we described a one-way analysis of variance (ANOVA) 'random effects' model in which the objective was to estimate the degree of variation of a particular measurement and to compare different sources of variation in space and time. The illustrative scenario involved the role of computer keyboards in a university communal computer laboratory as a possible source of microbial contamination of the hands. The study estimated the aerobic colony count of ten selected keyboards with samples taken from two keys per keyboard determined at 9am and 5pm. This type of design is often referred to as a 'nested' or 'hierarchical' design (Snedecor & Cochran, 1980) and the ANOVA estimated the degree of variation: (1) between keyboards, (2) between keys within a keyboard, and (3) between sample times within a key. An alternative to this design is a 'fixed effects' model in which the objective is not to measure sources of variation *per se* but to estimate differences between specific groups or treatments, which are regarded as 'fixed' or discrete effects. This StatNote describes two scenarios utilizing this type of analysis: (1) measuring the degree of bacterial contamination on 2p coins collected from three types of business property, *viz.*, a butcher's shop, a sandwich shop, and a newsagent (Armstrong & Hilton, 2004) and (2) the effectiveness of drugs in the treatment of a fungal eye infection.

Scenarios

Bacterial contamination on coins

An experiment was set up to measure the degree of bacterial contamination on 2p coins collected from three types of business premises, *viz.*, a butcher's shop, a sandwich shop, and a newsagent (Armstrong & Hilton, 2004). A sample of four coins was collected at random from each property. The number of bacterial colonies present on each coin was then estimated by dilution plating techniques. Hence, the data comprise four randomly obtained measurements of the bacterial contamination classified into three groups (the properties) and therefore comprise a one-way ANOVA, fixed effects model, in a randomized design and the data are presented in Table 1.

Drugs and the treatment of an eye infection

This scenario involves the effectiveness of two azoles (econazole and itraconazole) in the treatment of an infection of the cornea (keratomycosis) caused by the fungus *Aspergillus fumigatus*. Three groups of patients were allocated at random to three treatments. One group was given econazole, the second itraconazole, while the third was given a placebo as a control. After treatment, a swab of the eye was taken on six occasions, plated out onto agar, and the number of fungal colonies counted. These data were averaged for each patient and are presented in Table 2.

Analysis

Theory

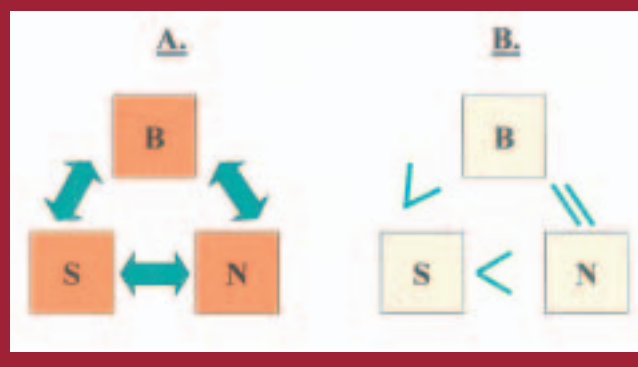
If only two groups were being compared, then the null hypothesis that there was no significant mean difference in the numbers of bacteria on coins or eye fungi could be tested using a Student's 't' test (see StatNote 3). This method of analysis could be extended to three or more different groups. Hence, to compare all pairs of properties or drug treatments,

Table 1. The number of bacteria isolated from 2p coins collected from three types of property

Type of property					
Butcher's shop	Sandwich shop	Newsagent			
140	2	40			
108	21	5			
76	0	5			
400	42	0			
Mean: 181	16	13			
ANOVA table					
Source	DF	SS	MS	F	P
Between property	2	74065.167	37032.583	4.89	>0.05
Error	9	68173.75	7374.861		
DF= Degrees of freedom, SS = Sums of squares, MS = Mean square, F = Variance ratio, P = Probability					
Post-hoc tests					
Comparison	Mean difference	Fishers PLSD	Scheffé's test		
Butcher's/Sandwich	164.75	P < 0.05	P > 0.05		
Butcher's/Newsagent	168.5	P < 0.05	P > 0.05		
Sandwich/Newsagent	3.75	P > 0.05	P > 0.05		

three 't' tests would be necessary (Figure 1). There is a problem in making multiple comparisons between these means however, because not all of the comparisons can be made independently. For example, if bacterial numbers on coins from the butcher's shop were significantly greater than those from the sandwich shop but similar to those from the newsagent, it follows that numbers in the newsagent should be greater than those in the sandwich shop (Figure 1). However, the latter comparison would not have been tested independently but is essentially 'predetermined' once the first two comparisons have been established. It was largely to overcome this problem, that ANOVA was developed by Sir

Figure 1. Comparisons between multiple groups as illustrated by the coin example in scenario 1 (B = Butcher's, S = Sandwich shop, N = Newsagents). Figure A shows that three 't' tests would be necessary to compare the three properties. Figure B shows that if bacterial numbers on coins from the butcher's shop were significantly greater than those from the sandwich shop but similar to those from the newsagent, it follows that numbers in the newsagent 'should' be greater than those in the sandwich shop



Ronald Fisher in the 1920s, the analysis providing a single statistical test of the null hypothesis that the means of the bacterial numbers from the three properties are identical (Fisher, 1925; 1935).

How is the analysis carried out?

The essential feature of the analysis is that the total variation between the observations is calculated and then broken down ('partitioned') into portions associated with first, differences between the three properties or drug treatments and second, variation between the replicate coins or patients. Various calculations are involved: (1) the sum of squares (SS) of the deviations of the observations (x_{ij}) from their mean (\bar{X}) as a measure of the total variation of the data, (2) the SS of the three group means from their 'overall' mean as a measure of the 'treatment effect' calculated from the column totals (T_i), and (3) variation between replicates within a group calculated as the SS of the raw data (x_{ij}) in each column from their column mean.

If there are no significant differences between the groups, the individual observations are distributed about a common population mean ' μ '. As a result, the variance (mean square) calculated from the between treatments SS and the error SS should be estimates of the same quantity. Testing the difference between these two mean squares is the basis of an ANOVA and the relevant statistics are set out in an ANOVA table (Tables 1 and 2). To compare the between treatments and error mean squares, the SS are divided by their appropriate degrees of freedom (DF).

The between groups mean square is then divided by the error mean square to obtain the variance ratio. The value of 'F' indicates the number of times the between group mean square exceeds that of the error mean square. This value is compared with the statistical distribution of the 'F' ratio to determine the probability of obtaining a statistic of this magnitude by chance, i.e., from data with no significant differences between the group means. If the value of 'F' is equal to or greater than the value tabulated at the 5% level of probability, then the null hypothesis that the three treatment means are identical is rejected.

Assumptions of the analysis

This analysis makes certain assumptions about the nature of the experimental data that have to be at least approximately true before the method can be validly applied. An observed value in Tables 1 and 2 (x_{ij}) can be considered to be the sum of three parts: (1) the overall mean of the observations (μ), (2) a treatment or class deviation, and (3) a random element drawn from a normally distributed population. The random element reflects the combined effects of natural variation between replications and errors of measurement. The analysis assumes first, that these errors are normally distributed with a zero mean and standard deviation 's', second, that although the means may vary from group to group, the variance is constant in all groups, and that effects of individual groups or treatments are additive rather than multiplicative.

Failure of one or more of these assumptions affects both the significance levels and the sensitivity of the 'F' tests. Experiments are usually too small to test whether these assumptions are actually true. However, in many biological and medical applications in which a quantity is being measured, the assumptions are likely to hold well (Cochran &

Table 2. Effect of azoles in the treatment of keratomycosis caused by the fungus (*Aspergillus fumigatus*). Data are the number of colonies of the fungus isolated from the eyes averaged over six occasions

Treatment					
Control (placebo)	Econazole	Itraconazole			
10	0	1			
13	2	3			
18	6	2			
1	11	0			
20	8	1			
12	1	14			
5	4	4			
16	13	9			
5	0	18			
23	8	9			
Mean: 12.3	5.3	6.1			
ANOVA table					
Source	DF	SS	MS	F	P
Treatment	2	293.60	146.80	3.98	>0.05
Error	27	995.10	36.856		
DF= Degrees of freedom, SS = Sums of squares, MS = Mean square, F = Variance ratio, P = Probability					
Post-hoc tests					
Comparison	Mean difference	Fishers PLSD	Scheffé's test		
Control/Econazole	7.0	P < 0.05	P > 0.05		
Control/Itraconazole	6.2	P > 0.05	P > 0.05		

Cox, 1957; Ridgman, 1975). In many microbiological applications, in which bacterial numbers are being estimated, the assumptions may not hold. There are often two problems when the data comprise numbers of microbes. First, small whole numbers, especially if there are many zeros, are unlikely to be normally distributed and second, a large range of bacterial numbers may be present resulting in large error terms and heterogeneous variances within the different groups. If there is doubt about the validity of the assumptions, significance levels and confidence limits must be considered to be approximate rather than exact.

Interpretation

Coin example

In the coin example (Table 1), an 'F' value of 4.89 was obtained which has a 'P' value of less than 0.05, i.e., there is less than a 5% chance of obtaining an 'F' ratio of this magnitude by chance alone. This result indicates a real difference between the bacterial counts from the three properties. Note that the analysis relates only to the three individual properties studied. It would not be possible to make a more general statement about properties of this type from these data. This would require a random sample of each property type to be sampled so that an estimate could be obtained of the variation between similar types of property.

The 'F' test of the group means is only the first stage of the data analysis. The next step involves a more detailed examination of the differences between the means. A variety

of methods are available for making such tests often referred to as 'post-hoc' tests and several of these tests are usually available in statistical packages and have been described in a previous StatNote (StatNote 6, Hilton & Armstrong, 2006). If a fairly liberal post-hoc test, e.g., Fishers protected least significant difference (PLSD) is applied to the present data, then the results indicate that bacterial counts were significantly higher on 2p coins from the butcher's shop compared with the sandwich shop and newsagents, but that counts from the latter two properties were similar. By contrast, Scheffé's test is one of the most conservative of the post-hoc tests giving maximum protection against making a Type 1 error. In this case, the test does not indicate any significant differences between the group means. Which test to use depends on the objectives of the experiment and on the relative consequences of making a Type 1 or Type 2 error.

Eye infection example

In the eye infection example (Table 2), there is a significant difference between treatments ($F = 3.98$, $P < 0.05$) suggesting real differences among the groups. Fishers PLSD indicates that number of colonies of *A. fumigatus* isolated from the eye were significantly higher in the control group compared with the group treated with itraconazole, but that the difference between the group treated with iconazole and the control was not statistically significant. By contrast, Scheffé's test does not indicate any significant differences between the group means, and therefore, that neither of the azoles had much effect in reducing the abundance of the fungi on the surface of the eye.

Further examination of the data in Table 2 suggests there is considerable variation in the abundance of fungi between the individual patients. The effect of this variation is to increase the mean square for error in the ANOVA, reducing the 'power' of the analysis (StatNote 8, Hilton & Armstrong, 2007b), and making it more difficult to demonstrate a significant treatment effect. Hence, consideration should be given to methods of reducing or taking into account this variation. One such method of 'error control', *viz.*, analysis of covariance (ANCOVA) will be described in the next StatNote.

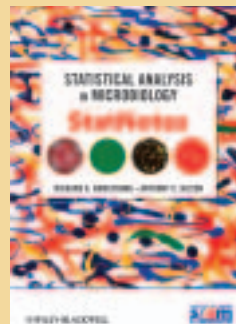
Conclusion

If the data comprise three or more groups then a 't' test should not be used to make comparisons between pairs of group means, ANOVA being the most appropriate analysis. When there are three or more groups and the replicate measurements are collected randomly for each group, the experimental design is often described as a "one-way ANOVA in a randomized design".

If the objective is to estimate the differences between three or more groups, which are regarded as 'fixed' or 'discrete' effects to be estimated, then the design is described as a 'fixed effects' model. It is important to distinguish in each circumstance whether a fixed or random effects model is the more appropriate.

Dr Anthony Hilton¹ and Dr Richard Armstrong²

¹Biology & Biomedical Sciences and ²Vision Sciences, Aston University, Birmingham, UK



Statistical Analysis in Microbiology: StatNotes

By Richard A Armstrong and Anthony C Hilton.
Published by Wiley-Blackwell/SfAM, 2010

StatNotes has been designed specifically for microbiologists who are involved in experimental research and need to draw accurate conclusions from their findings.

It features the first 28 StatNotes published in *Microbiologist* that together enable you to understand the basic principles of statistics, choose the correct statistical methods to analyze your experimental data, and work with a variety of commercially available statistical software packages.

Written specifically for microbiologists, StatNotes enable you to choose which statistical methods should be applied to analyze and draw correct conclusions from your experimental data.

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News from the SfAM Postgraduate and Early Career Scientist Committee

Writing a



PECS NEWS

The Post Graduate and Early Career Scientists committee will be holding a one day meeting for students at Charles Darwin House in London.

The meeting on Molecular and Microbiological Techniques will take place on 25 October. As well as finding out about this interesting and fast moving field, attendees will have the opportunity to present their work through either a poster or an oral presentation and network with other early career scientists.

For more information or to book visit: <http://www.sfam.org.uk/en/pecs/autumn-meeting/index.cfm>



Jo Tarrant
PECS Events Officer

How to begin

For many PhD students, the literature review is the first major piece of work they undertake. It comprises a comprehensive search of the research in their project area, followed by a critical review in which they can evaluate the existing work in their field, and contextualize their research.

The purpose of writing the review is several-fold; it gives you the chance to immerse yourself in the subject of your PhD, a subject with which initially you may not have been familiar. Secondly, as a result of this immersion it allows the ability to explore and discover existing knowledge of the subject.

This enables you to see where the project fits into the subject area as a whole and how you might proceed with experimental work in order to fulfil the criteria of your PhD, which stipulates that research must show an original contribution to the field. So, if the literature review is conducted thoroughly enough, it can highlight gaps in the current knowledge and show how you

might use your PhD to fill these gaps, creating justification for your choice of research methodology. Another result of writing this review is that it provides evidence which may help to explain subsequent experimental data.

Performing your research

It is easy to succumb to the temptation when writing the review to read and include everything with a connection to your field. While this can give a wide perspective on the research area, the purpose of the review is to help you focus your research, not to compile a list of everything you have ever read on the topic. It is best practice to start with a very wide search, and constantly narrow this search based on the search results. Keywords should comprise all of the relevant ones within the title of the research topic as a bare minimum.

Once these are exhausted, you should consider all possible synonyms of these words to use as search terms, being careful to include plurals and all alternative spellings. In choosing

literature review



literature, original research papers are vastly preferable to reviews and book chapters.

Structuring the review

In terms of structure, the review should clearly have an introduction which will set the review in the wider scientific context and signpost what the review will cover. This need not be long, since the main aim of the review is the critical evaluation of the literature.

When organizing the literature and starting to review, it may be useful to create a table for each endpoint containing all the important details of the papers. Such details can include: authors, year, study type (*in vitro/in vivo*), study size and outcome. Setting papers out in tables will enable you to gain an overview of all the studies, allowing comparison and critical review. Since a review (and consequently thesis) should contain a coherent narrative, it follows that the headings should be arranged in a logical order, starting with the broadest and finishing on the heading which most

closely resembles your PhD topic.

For the main body, it is vital to arrange your list of papers into headings. While choice of headings is ultimately up to the author, dividing them by endpoint may be a useful approach. These headings should be arranged logically, with each leading on to the next, and subsections where it is necessary to divide the literature pertaining to a given topic.

Narrowing your research

In approaching the material, it is vital to bear in mind two things which a literature review is *not*: 1) a summary of everything you have read on the topic, 2) a chronological account of how research in the area has developed. Also it is important you don't just give an account of what was done in each study. Instead, you should address issues such as the strengths and weaknesses, whether the methods were fit for purpose, whether the results were statistically robust, any evidence of bias in the experimental design, and whether conclusions drawn are justified by the results.

On the basis of these you can then draw conclusions about the consensus views within your field, identify in which direction research can move forward and barriers to progress, and how your work fits in and may contribute to the field.

Moving forward

Having written the review, you should then be able to plan your project with the knowledge of what is needed, and what is possible, in terms of exploration of the field.

Writing the literature review early in the PhD process gives the advantage of showing examiners during the 12-month transfer viva that you are able to think critically. By extension there is the possibility of publishing the review, which fulfils another criterion, bulking out your thesis!



Chris Chapman
The University of Reading

careers



Child's Play

Erika Tranfield tells us about her varied and eventful career path

When I was seven, my first thoughts of a career came from my interest in human biology. Not the choice of every primary school child but I knew what I wanted (I also wanted to start my pension fund but had to wait a while for that). However, the 80s were a time when most people mistrusted new technologies and in the pre-internet age no one could have anticipated how much they would impact upon our lives. I knew that I wanted a job that couldn't be made obsolete by new technologies.

"I want to be a surgeon", I told my parents, to which I got the response, "That's nice, dear. Eat your peas". To my mind, people were all different shapes and sizes, no machine could ever comprehend the infinite variety of the human form so I thought as a surgeon I would always have a job!

Well, looking back things didn't really go to plan; however, the dream still lives on in an altered form. Now I am using these new technologies and still feel inspired by new developments and possibilities.

As a young adult and in the third year of my Biomedical Sciences degree at Sheffield Hallam University I undertook a placement year in a private laboratory, A.H. Allen & Partners, employed as an assistant chemist and biologist. The workload was extremely varied as it was dependent upon the type of samples

brought in and the analysis required. However, the majority of the work was based on the analysis of foods with regards to the Food Safety Act. My work consisted of a substantial amount of colour identification and quantification using Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) respectively. Other work I carried out frequently during my placement reflected the three different sections of the company; microbiology, analytical and other laboratory work.

Whilst working in the microbiology unit I covered areas such as plate serial dilutions and making up plates, as well as testing for cooked meats, vegetarian foods and *Legionella*. The lady who single-handedly ran this department was a very strong character. I admired her determination to do her work meticulously and indeed, we bonded over time as my sincere interest in the work became clear. Her methodical approach and work ethic still inspire me now.

Analytical and other laboratory work included testing for sulfur dioxide, aflatoxins, urea, moisture in pastas, king prawn size differentiation, sugar levels using HPLC, ELISA kit work, Quantitative Ingredient Declaration (QUID) of ingredients, dithiocarbamate pesticide residues, red diesel testing and quantitative analysis of the antioxidants

butylated hydroxyl anisole and butylated hydroxyl toluene by gas chromatography (GC).

I am sure that the experience I gained on this placement helped considerably with the start of my career, giving me confidence in a laboratory environment and boosting my self esteem. I unhappily declined their very kind offer of continued employment as I was determined to complete my degree. The placement year not only gave me experience but also a salary which I saved in order to purchase my very first property which was akin to a shed and had to be gutted. Having very little money, I undertook the majority of the work myself with help from some very good friends. With a bit of elbow grease and a couple of years of time, I managed to complete it. From there on I went on to purchase more properties to develop, something which I am still passionate about.

Around the same sort of time that I bought the house, I also started to help a friend to run her own public house in Nottingham. Having discussed the idea of running a pub together; we bought the lease on a pub in Sheffield. This was during the finals of my degree which ought to, in itself, have been quite enough work but I found myself loving the challenge of the pub. I even employed some of my university friends; they needed the money and I needed

reliable staff — perfect harmony. We turned the pub around and it became a popular meeting place.

I was certainly inspired by several of my microbiology tutors at university, in particular Barry Davies. He was always able to offer his time and assistance when necessary. He would explain things in great detail whilst always managing to remain comprehensible — the ideal way of teaching. The other inspirational character was a lady whom I will never forget; she was extremely passionate about microbiology. So much so, that during one lecture she lay on top of the table and imitated a bacterium and how it attached; a demonstration and indeed a lesson which have remained etched into my memory.

Grown up stuff

After leaving university I applied for a few jobs in biomedical science. Looking back I don't think I sufficiently researched the area in which I was applying in order to stand out from the other candidates. I would definitely recommend to anyone now applying for a job to thoroughly know the potential employer and perhaps use this to your advantage within an interview by reeling off your knowledge using up-to-date terminology or news.

Consequently, my first job after graduating was not within biomedical science. But I never gave up and, after continual applications I was eventually offered a place as a Trainee BMS in the Microbiology department at Warrington General Hospital. I thoroughly enjoyed working there and even started to get into work between 7 and 8am just so I could prepare for the day. My mentor was Steve Burgess and he was incredibly considerate and patient, explaining things in fine detail if I didn't understand. He really did help me take my first baby steps within the BMS field. Although I enjoyed working with my new colleagues and liked the environment at Warrington General, I didn't enjoy the long and expensive commute to work, which entailed up to 90 minutes driving to and from work each day. With this in mind I applied for a position closer to home at Clatterbridge Hospital on the Wirral. With my previous interview experiences still looming large in my mind I painstakingly researched possible interview subjects including gaining some knowledge of *Laribacter*



Microflex MALDI Biotyper

hongkongensis, a novel bacterium associated with gastroenteritis caught from drinking water reservoirs in Hong Kong. This was fresh news and made my interview material current, landing me the job.

It was at Clatterbridge Hospital that I qualified as a BMS. Yet again, my work colleagues were wonderful and I felt myself thoroughly at home. I began to undertake projects at work always looking for the next area in which I could assist and perhaps improve. I often undertook my own experiments and found that, as much as I enjoyed the work, I always had the desire to do more. After all, in my interview when I had been asked “*where do you see yourself in the future?*” I had pointed to my boss and replied, “*in your shoes*”. I believed, and still do, in keeping oneself educated and up-to-date within your subject area. Not just what is happening within your own laboratory, but within your entire field.

I jumped at the chance to become the new laboratory lunchtime meetings organizer, if only for the scintillating title. I began with a local search for possible lecturers and particularly inspiring people to give talks to spice up our current meeting format. This became a very popular part of our programme, enjoyed by many staff, not just as an opportunity to gain

knowledge but also a chance to gain valuable CPD points.

Due to my involvement in organizing the talks, I was given the opportunity to attend a few conferences, the very first of which was the 2009 IBMS Congress at the ICC in Birmingham. I can sincerely say that the whole conference experience is more enjoyable than I could possibly have imagined. I am sure the reputation for dullness can be deserved in other industries but for me, in microbiology it is all incredibly exciting. Getting a glimpse into the work of other laboratories, talks on subjects that haven't even crossed my mind and information on new technologies to bring the microbiology laboratory into the 21st century are mind blowing. From the many fascinating things I saw, one particular piece of technology really captured my imagination. The more I thought about it the more I wanted to see if this was really true. Can it do what the sales guy on the stand was telling me it could do? I saw in principle what this technology could offer to microbiology and found out what I could about this wonderful and unique technology known as MALDI-TOF.

Unbeknown to me, the salesperson of the company was actually looking for an applications scientist to train all new customers on the MALDI Biotyper, and a few weeks later I received a phone call asking if I would be interested in applying for the position. A little shocked and taken aback I thought about it and realized that I had to be a part of something so effective for my industry. I really believe that this technology is bringing microbiology into the 21st century. After all, 15 seconds for a full species identification, around 10p per test and the possibility of identification directly from samples such as blood cultures within 30 minutes has a considerable impact upon existing practices.

Well, here I am, still to this day training everyone and anyone that is interested enough to listen, as well as performing some R&D, and helping others with their MSc and PhD projects. Job satisfaction? — absolutely.



Erika Tranfield
Applications Scientist
Bruker Daltonics



Sponsored Lecture Grant Report

Some like it hot and acidic — Dr Huub Op den Camp (Radboud University of Nijmegen, The Netherlands)



Would you like to get close to a hot, bubbling, acidic and smelly mud pool? Most of us might politely decline, but not so the microbiologists from the Radboud University of Nijmegen for whom the fumaroles and sulfatara of the Phlegrean fields near Naples in Italy were just too tempting. The team around Dr Huub Op den Camp had good reason to believe that there would be some interesting microbes to be found, given the abundance of methane and volatile sulfur compounds of geothermal origin in a scene set perfectly for extremophilic microorganisms. In his SfAM sponsored seminar at the School of Life Sciences in the University of Warwick, Dr Huub Op den Camp provided fascinating insights into two different microbial groups that dwell in these hot and acidic environments, acidophilic methanotrophic bacteria and

archaea that degrade carbonyl sulfide (CS₂).

Due to the oxidation to sulfuric acid of hydrogen sulphide, rich fumes emitting at these sites, soils and mud pools of this ecosystem are highly acidic. Methane is also emitted and soils in the sulfatara showed significant consumption of methane, probably due to unidentified methanotrophic bacteria. As no methanotrophic isolates had ever been shown to grow at the low pH encountered in the sulfatara, the hunt was on for acidophilic methanotrophs. Initial PCR-based surveys using the samples of the mud pool and surrounding soils showed the presence of unusual sequences of the gene encoding the particulate methane monooxygenase, one of the key enzymes of methanotrophic bacteria, providing a first piece of evidence for the presence of uncharacterized acidophilic methanotrophs. Painstaking and patient classical environmental microbiology work was required until the Nijmegen laboratory finally obtained novel types of methanotrophs which were phylogenetically affiliated with the *Verrucomicrobia* (Pol *et al.*, 2007). Incidentally, these types of acidophilic *Verrucomicrobia* methanotrophs were also found at the same time in geothermal areas in New Zealand (Dunfield *et al.*, 2007) and Kamchatka (Islam *et al.*, 2008) and their description significantly extended the view of the phylogenetic diversity and environmental distribution of methanotrophs.

Another very recently published highlight of the work of the Nijmegen lab is the characterization of microorganisms degrading carbonyl sulfide (CS₂). Used as a solvent in the cellulose industry for instance, microorganisms degrading CS₂ are of considerable interest for dealing with CS₂ wastes. CS₂ also occurs as a natural sulfur compound in the sulfurous fumes of the sulfatara, but very little is known about the diversity, physiology and biochemistry of microbial CS₂ degradation. The Romans would have been delighted to know what the

Nijmegen team would do with a small biofilm sample from one of their old saunas. Not only did they isolate and characterize an *Acidianus* strain which grows on CS₂, they also carried out a detailed characterization of the enzyme CS₂ hydrolase, which is responsible for CS₂ degradation in this archaeon (Smeulders *et al.*, 2011). To everyone's great surprise it turned out to be very similar to, and very likely evolved from, carbonic anhydrase, an enzyme responsible for the interconversion of carbon dioxide and bicarbonate. Although the CS₂ hydrolase monomers have essentially the same fold as carbonic anhydrase, and both form octamers with a central hole, the emerging overall structure of the CS₂ hydrolase is very unusual in that two of these planar octamers are interlocked with each other at right angles, forming a so-called catenane structure, which are highly unusual. The relatively small changes in the general structure of the CS₂, compared with carbonic anhydrases, blocks what is the CO₂ entry funnel to the active site in the carbonic anhydrase, while in CS₂ hydrolase, the substrate reaches the active site via a small hydrophobic tunnel. Remarkably, the active sites of these two enzymes appear to be essentially identical, but substrate accessibility to the active site may hold the key to enzyme specificity.

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Hendrik Schäfer
University of Warwick

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A mutagenesis approach to enhance the antimicrobial activity of the natural biopreservative nisin

Nisin is a small polycyclic peptide produced by the bacterium *Lactococcus lactis* which exhibits antibacterial activity against many Gram-positive bacteria, including a range of clinical and foodborne pathogens such as staphylococci, bacilli, clostridia and listeria. It is US FDA approved and is used as a natural preservative in heat-treated and low-pH foods. It has a long record of safe use and is one of only a few such compounds to have been applied commercially. Recent work in the University College Cork involving mutagenesis has generated a number of novel nisin variants which possess greater activities than the parent molecule against a range of important clinical pathogens including MRSA, vancomycin-resistant enterococci (VRE) and the foodborne pathogen *Listeria monocytogenes*. These mutants support the fact that not only can nisin be improved but that different nisin derivatives target different pathogenic organisms. Significantly, a number of other locations within the nisin peptide (identified from random mutation screening) have hinted that further investigation through site-saturation mutagenesis may prove beneficial.

Thus, during the first few weeks of this summer placement I set out to use a PCR-based mutagenesis approach to target these locations using site-saturation mutagenesis and screen the resulting mutants against a variety of Gram-positive organisms for enhanced phenotypes. This was carried out using specifically designed oligonucleotides to

Figure. 1. Deferred antagonism assays of nisin mutants against the nisin-sensitive indicator *L. lactis* ssp. *cremoris* HP. The colony at bottom right corresponds to wild-type nisin. The altered zone sizes (activity) correspond to different amino acid changes at particular locations in the nisin peptide



insert mutations at specific codon locations within the nisin gene. The modified nisin genes were then introduced into a strain capable of producing the fully modified nisin mutant peptides. Transformants (approximately 150 for each target codon) were chosen at random and inoculated into 96 well plates to create banks of mutant nisin strains. A bank of approximately 1,000 derivatives was achieved (i.e. 10×96 well plates). My next task was to carry out an initial screen using a nisin-sensitive indicator which would give an overall assessment of the activity of the nisin derivatives. Deferred antagonism assays were carried out by replicating strains on GM17 agar plates and allowing them to grow overnight before overlaying with agar seeded with a *Lactococcus lactis* ssp. *cremoris* HP indicator strain. Zones of inhibition were assessed to determine if mutant-nisin producing strains displayed increased activity when compared with wild-type nisin against the indicator strains of interest (Figure 1). The initial overlays of 1,000 derivatives with *L. lactis* HP resulted in the identification of approximately 200 colonies with either slightly decreased, wild-type or slightly increased activity when compared with the appropriate nisin-producing control strain. These derivatives were then screened against several pathogenic and non-pathogenic strains resulting in a number of candidates with putative enhanced activity. In our case we observed six relevant enhanced activities against *Staphylococcus aureus*, *L. lactis*, *Streptococcus agalactiae* and *L. monocytogenes*. In situations where an enhanced phenotype was observed, the corresponding structural gene was sequenced to identify the genetic changes responsible in addition to analysis by mass spectrometry. Further analysis of these mutants will be undertaken including purification of the peptides followed by MIC assays to definitively confirm the improved nature of the mutants over the wild-type nisin peptide.

The SfAM Students into Work Grant has allowed me to become proficient in numerous molecular microbiology techniques including PCR, gel electrophoresis, DNA purification and electroporation. My overall experience was very positive in that I not only gained many new skills but also more

confidence in planning and troubleshooting experiments efficiently. I was also able to observe first-hand how research is carried out at postgraduate level and work as part of a large team of postgraduate and postdoctoral researchers which has enhanced my interest in microbiology. Indeed, it has encouraged me to take this interest further, so much so that I have been successful in securing a PhD studentship, and for which my new skills will be invaluable.

I would like to thank my supervisor Professor Colin Hill for giving me this opportunity to work in his laboratory, I would also like to thank Dr Des Field whose time, support and guidance was invaluable throughout this project. Finally I would like to thank SfAM for providing this grant and giving me such an excellent and rewarding opportunity.

Neasa O'Leary
University College Cork

Efficacy of temocillin in the treatment of pulmonary infection in patients with cystic fibrosis

Respiratory disease as a result of recurrent bacterial infection, primarily with *Pseudomonas aeruginosa* is the main cause of mortality and morbidity in cystic fibrosis (CF) patients with more than 95% of deaths due to respiratory failure. The goal of antimicrobial therapy in the treatment of such infection is to maximize bactericidal activity against the infecting pathogen to enable eradication of the infection. However, eradication of *Ps. aeruginosa* from the airways of CF patients in the chronic infection state has been shown to be virtually impossible. Existing treatment of pulmonary infection in CF patients, therefore, involves the frequent and prolonged administration of one or more antibiotics, in an attempt to decrease bacterial load and to inhibit enzymes such as proteases which cause further lung damage. Recently, temocillin has been used increasingly in the treatment of *Ps. aeruginosa* lung infection which has not responded to other antibiotic treatment, even though it is not thought to be active against *Ps.*

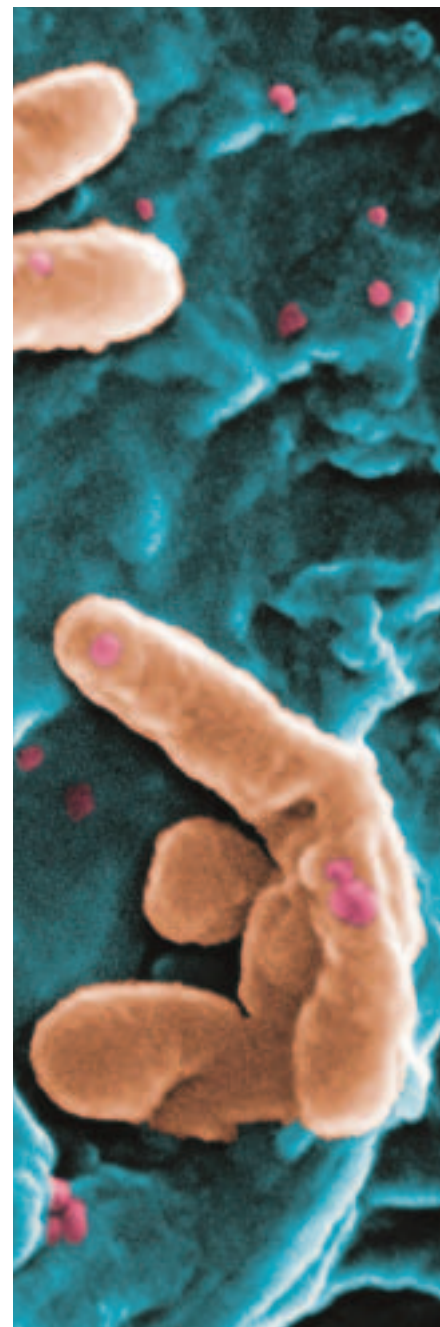
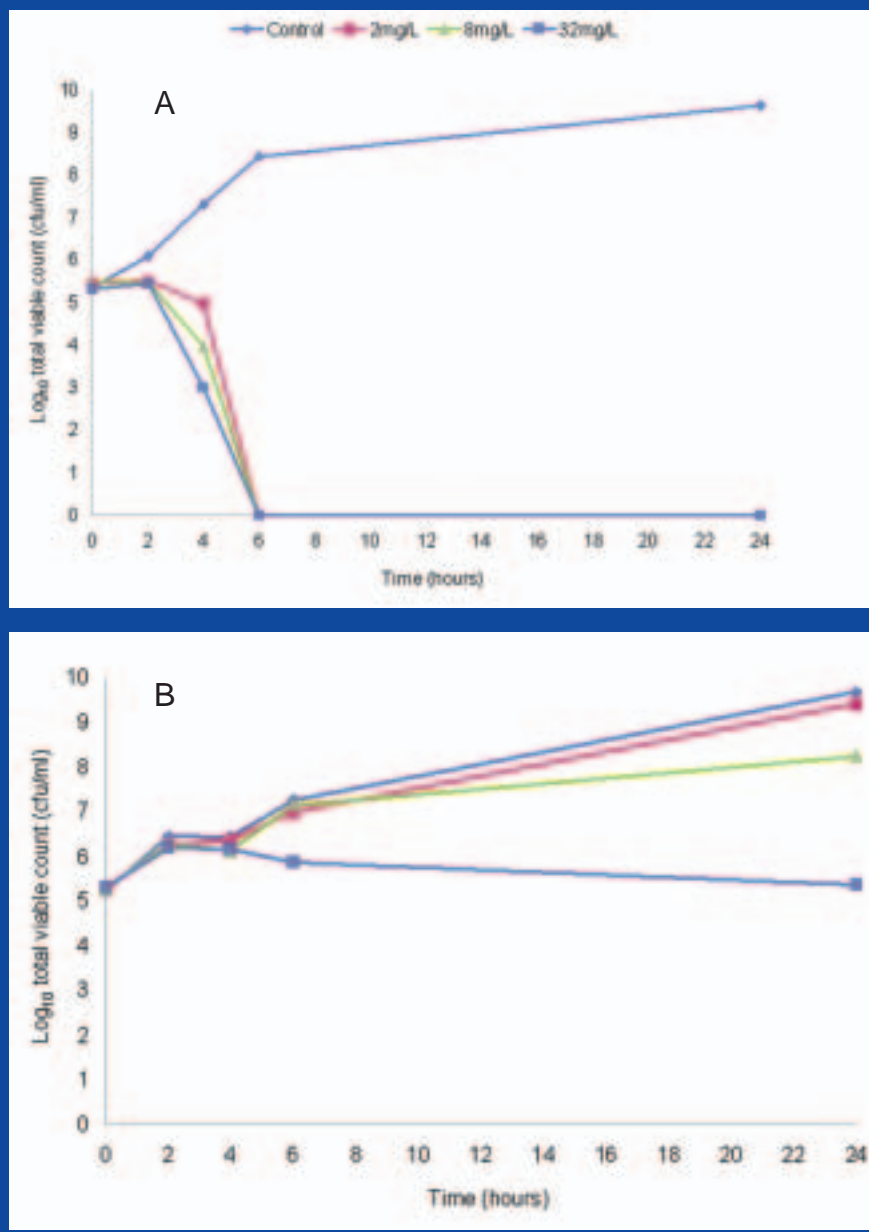
aeruginosa and there are no reported breakpoints for *Ps. aeruginosa*. Furthermore, clinical improvement has been observed in patients who were pretreated with *Ps. aeruginosa* isolates which were resistant to temocillin according to the MIC breakpoint for Enterobacteriaceae (Taylor *et al.*, 1992). Therefore, the aim of this study was to compare the susceptibility of clinical *Ps. aeruginosa* isolates cultured from CF sputum with both temocillin and other anti-pseudomonal antibiotics using conventional MIC and time-kill assays.

Clinical *Ps. aeruginosa* isolates cultured from CF sputum were used in the study. The susceptibility of these isolates to temocillin had been previously determined and the isolates categorized as displaying low, intermediate or high resistance. Six isolates with low resistance and three from both the intermediate and high resistance categories were selected for detailed study. The first experiment was to determine the susceptibility of these 12 isolates to temocillin using the disk diffusion method. Each isolate was inoculated onto Mueller–Hinton Agar (MHA), a temocillin disk applied and the zone of inhibition measured following incubation at 37°C for 24 hours. The results of this initial experiment confirmed that the original categorization of the isolates as displaying low, intermediate or high resistance was accurate.

The main aim of the study was to compare the susceptibility of these clinical *Ps. aeruginosa* isolates with temocillin, using time-kill assays. Time-kill studies were performed for the 12 selected isolates at low (2mg/l), intermediate (8mg/l), and high (32mg/l) concentrations of temocillin; a temocillin concentration of 2mg/l had no effect on the total viable count of any of the isolates over a 24 hour period. However, temocillin at a concentration of both 8 and 32mg/l resulted in total kill at 24 hours for all isolates with low resistance and for two of the three isolates with intermediate resistance. In contrast, temocillin, at all concentrations tested, had no effect on the viable count of the three isolates with high resistance (Figure 1).

Tobramycin is routinely used in the treatment of *Ps. aeruginosa* pulmonary infection in CF; therefore, in the final part of the study, the susceptibility of

Figure 1. Representative time-kill curves for *Ps. aeruginosa* isolates with (A) low and (B) high resistance to temocillin as determined by the MIC disk diffusion method



these clinical isolates to tobramycin, at a range of concentrations (1, 4, 8 & 16mg/l), was determined using time-kill assays. The isolates with low and intermediate resistance to temocillin were generally susceptible to all concentrations of tobramycin with killing occurring more quickly than observed with temocillin. However, killing of the isolates with high resistance to temocillin was only observed at the highest concentration of tobramycin (16mg/l).

The results of this study clearly show that *Ps. aeruginosa* isolates with low or intermediate resistance to temocillin, as

determined by conventional MIC, are killed by temocillin concentrations which are achievable *in vivo*. Therefore, even though there are no MIC breakpoints for temocillin and *Ps. aeruginosa*, the use of temocillin for treatment of *Ps. aeruginosa* pulmonary infection in CF could potentially be directed by determining the MIC and using the breakpoints for Enterobacteriaceae.

I would like to thank Dr Michael Tunney for giving me this opportunity to experience microbiology in practice and to the Society for Applied Microbiology for funding my placement. I would also

like to thank Dr Deidre Gilpin for all the time she devoted to helping me with my work as well as all the staff and postgraduate students at the School of Pharmacy.

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D. Michael McAuley
School of Pharmacy
Queen's University Belfast

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Analysis of post-translationally modified recombinant proteins expressed in *E. coli*

The production of recombinant proteins has become extremely important for research, diagnostic and therapeutic applications, particularly within the pharmaceutical industry. Recombinant proteins, such as monoclonal antibodies (mAbs) and hormones can be produced using a variety of cloning and expression systems however, the first recombinant therapeutic protein, human insulin, was produced using *E. coli* as an expression host.

When producing recombinant proteins, it is desirable to produce homogenous proteins which display identical activity and conformational structure to that of the native protein. Eukaryotic proteins are typically post-translationally modified by glycosylation in their native state; however *E. coli* cannot enzymatically glycosylate proteins and can only perform minimal post-translational modifications (PTMs). There is, however, increasing evidence that proteins produced in *E. coli* may show considerable product heterogeneity, as the proteins can be susceptible to a non-enzymatic form of glycosylation, known as glycation (Mironova *et al.*, 2003).

Glycation involves interaction between proteins and reducing sugars, resulting in the formation of covalent sugar-protein conjugates through a series of complex reactions, often referred to as the Maillard reaction. Unlike other types of PTM, such as enzymatic glycosylation, which make proteins more stable and functional, glycation adversely affects proteins. Glycation often results in site-specific or

random fragmentation of proteins, in addition to the formation of intra- and inter-molecular cross-links. These alterations change the physico-chemical properties of proteins, resulting in loss of the catalytic activity of an enzyme and deterioration of protein stability. Glycation can also lead to immunogenicity, which is a major concern as therapeutic proteins must satisfy strict drug safety regulations.

Lysostaphin is an example of a recombinant protein, which demonstrates protein heterogeneity when expressed in *E. coli*. Lysostaphin is an extracellular glycyglycine endopeptidase, produced by *Staphylococcus simulans* biovar *staphyloolyticus* (NRRL B-2628), which can lyse staphylococcal cells by specifically cleaving the pentaglycine cross-links in the peptidoglycan component of their cell walls. The zinc-containing endopeptidase was discovered by Schindler and Schuardt (1964) and the gene encoding the protein as a preproenzyme was identified and sequenced by Rescei *et al.*, (1987). Mature lysostaphin consists of two domains – a C-terminal cell wall targeting domain (CWT) and the catalytic glycyglycine endopeptidase domain which specifically hydrolyses the pentaglycine cross-links of the peptidoglycan (Heinrich *et al.*, 1987).

Due to its potent and specific staphylococcal activity of lysostaphin, the enzyme has been identified as a potential therapeutic agent against staphylococcal infections. Consequently, the lysostaphin gene has been cloned and expressed using various expression

hosts, including *Lactococcus lactis* and *E. coli*. With the exception of post-translational processing of preprolysostaphin, other modifications of this protein are not desirable. For instance, lysostaphin cannot be expressed using transfected eukaryotic cells, as it becomes glycosylated following secretion and as a consequence is rendered inactive (Kerr *et al.*, 2001). It is therefore important that this enzyme is characterized fully to establish whether expression in *E. coli* results in unnecessary PTMs, which could reduce the efficacy of this therapeutic drug.

Any PTM of a protein results in an increase or a decrease in molecular mass, therefore mass spectrometry is the method of choice for the identification and characterization of PTMs, such as glycation. My PhD project therefore aims to establish whether lysostaphin is post-translationally modified upon expression in *E. coli* and if so, analyse the heterogeneity of lysostaphin. Liquid Chromatography with electrospray ionization/mass spectrometry (LC-ESI/MS/MS) is being used to allow sensitive detection and quantification of the abundance, composition and location of modifications of lysostaphin. As shown in Figure 1, lysostaphin isoforms can be separated using weak cation exchange chromatography (WCX) and the eluted protein fractions proteolytically digested using trypsin. Following digestion, the peptides are separated using reverse phase chromatography to allow peptide mapping and subsequent identification

Figure 1. WCX separation of lysostaphin (45µg/µl) on a ProPac® WCX-10 (4 x 250mm) column using a DX500 LC system (Dionex, UK). Separation achieved using a 0-50% B (20mM NaHPO₄, 1M NaCl, pH7.4) gradient over 70 min at a flow rate of 0.5 ml/min

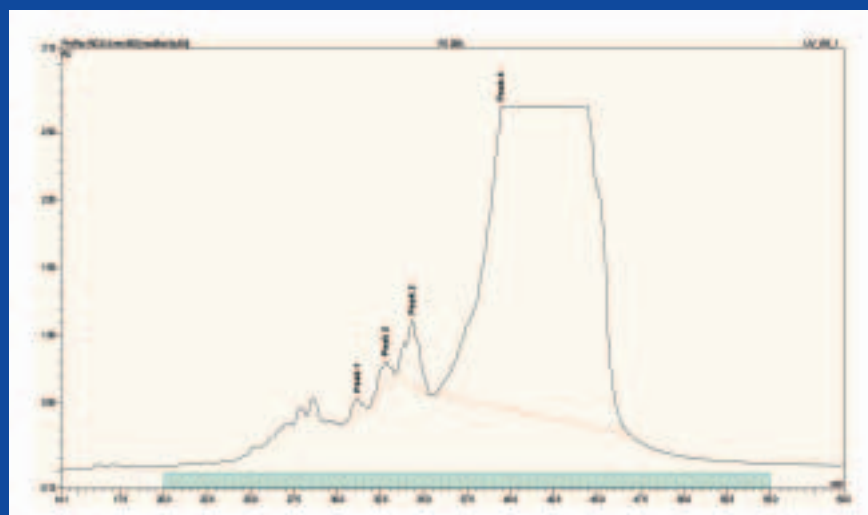


Figure 2. Separation of tryptically digested lysostaphin isoforms on an Acclaim PepMap 300 (C18 3µM 100Å) column using an Ultimate 3000 LC system (Dionex, UK). Separation achieved using a 0-5% B (90% acetonitrile, 0.09% TFA) gradient over 60 min at a flow rate of 100µl/min.



of modified peptides (Figure 2). The eluted peptide fractions are then analysed by ESI-MS/MS, using a Bruker HCT mass spectrometer (Bruker Daltonics, UK).

Using this LC-ESI/MS/MS approach it will be possible to determine whether lysostaphin is being glycosylated upon expression leading to the observed protein heterogeneity. Lysostaphin will be expressed and analysed following cytoplasmic expression and targeted protein export to the periplasm to determine whether different cellular

compartments of *E. coli* influence the extent of PTMs. Recombinant proteins are typically expressed in the cytoplasm however, when protein expression results in cell toxicity or improper folding, protein production can be targeted to the periplasm. The periplasm is known to be a less oxidizing environment than the cytoplasm and therefore this project may determine that cellular localization affects the incidence of PTMs.

The project will also examine whether different growth conditions and *E. coli*

strain genotype can influence protein heterogeneity by expressing lysostaphin using different *E. coli* strains. Some bacterial strains may be able to export reactive intermediates out of the cell or alternatively may harbour different concentrations of reactive intermediates in their cellular environment. By identifying reactive intermediates which are more abundant in particular bacterial strains, it may be possible to engineer metabolic pathways to reduce or suppress the extent of PTMs (Aon *et al.*, 2008).

After receiving a generous award from SfAM's President's Fund, I was able to attend the 30th Annual British Mass Spectrometry Society Meeting at the University of York. This 3-day meeting featured presentations by keynote speakers and experienced MS users discussing advances and applications of MS technology, in addition to providing valuable information about PTM discovery and identification.

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Claire Jennings

The role of antimicrobial peptides in urinary tract infections

At some point in our lives many of us will suffer from a urinary tract infection (UTI). The most common organisms isolated from patients suffering UTIs are *Escherichia coli* spp., usually originating from the gastrointestinal tract, or *Proteus* spp. While some UTIs can ascend the urinary tract and affect the kidney, the majority of cases result in an uncomplicated cystitis or prostatitis. Indeed whilst the urethral epithelia is often colonized by facultative aerobic Gram-negative rods, such as *E. coli* and *Proteus mirabilis*, the bladder and upper urinary tract are, under normal circumstances, a sterile environment. A number of factors are thought to play a part in maintaining this sterility, for example urine pH and the powerful flushing action of voiding urine. The importance of the latter in preventing colonization of the urinary tract is evidenced by the increased occurrence of UTI in older men who suffer enlargement of the prostate gland which impedes urinary flow (Macmillan, 2001).

In addition to mechanical defences, the innate immune system functions to keep the urinary tract free from infection. An important group of innate immune effector molecules are cationic antimicrobial peptides (AMPs). Generally <10kDa in size, AMPs have broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria as well as fungi and some enveloped viruses. Their ability to kill a wide range of microorganisms is believed to result from their mode of action, which involves interaction with the biomembrane, mediated by their size, charge, amino acid composition, hydrophobicity and amphipathicity. Following adherence, AMPs cause cell death by disruption of microbial membrane integrity via pore-formation or interference with microbial cell metabolism, (for a review see Brogden, 2005).

The expression of antimicrobial peptide genes is ubiquitous throughout the body and the urinary tract is no exception. To date a number of alpha and beta defensins have been identified in the urinary tract or isolated from human urine. Neutrophils synthesize a number of alpha-defensins known as human neutrophil peptides 1-4 (HNP1-

4); patients suffering chronic pyelonephritis have an eightfold higher urinary concentration of HNP-1 signifying a protective role in UTI, (Tikhonov, Rebenok & Chyzh, 1997). Indeed our own studies have identified HNP-1 and HNP-3 in human urine following purification by cationic exchange and MALDI-TOF analyses. In addition molecular analyses, employing RT-PCR, showed expression of the gene encoding human alpha-defensin 5 (HD5) by epithelia throughout the urinary tract (Figure 1A), with the highest concentrations of mRNA identified in the ureter and bladder (Figure 1B). Protein synthesis was confirmed both by purification of the peptide from urine followed by Western blot analyses and

immunohistochemistry.

In addition to alpha-defensins the human uro-epithelium is also known to express the β -defensin HBD-1. Though the urine concentrations of HBD-1 are below those required for effective microbial killing (Valore *et al.*, 1998), convincing evidence that HBD-1 plays a significant role in urinary tract defence comes from studies using transgenic mice in which the mouse defensin gene, *Defb1*, analogous to HBD-1 has been inactivated. In contrast to wild-type mice, approximately 30% of the transgenic knock-out mice suffered from bacteriuria with an elevated number of *Staphylococcus* spp. isolated (Morrison *et al.*, 2002).

The cathelicidin hCAP-18 and its

Figure 1 (A) RT-PCR analyses of HD5 gene expression in the kidney (K), upper ureter (UU), lower ureter (LU) and bladder (B) against a 1,000bp DNA ladder (M); **(B)** Quantitative Real-time PCR analyses of HD5 mRNA concentration in the tissues of the urinary tract (presented in arbitrary units)

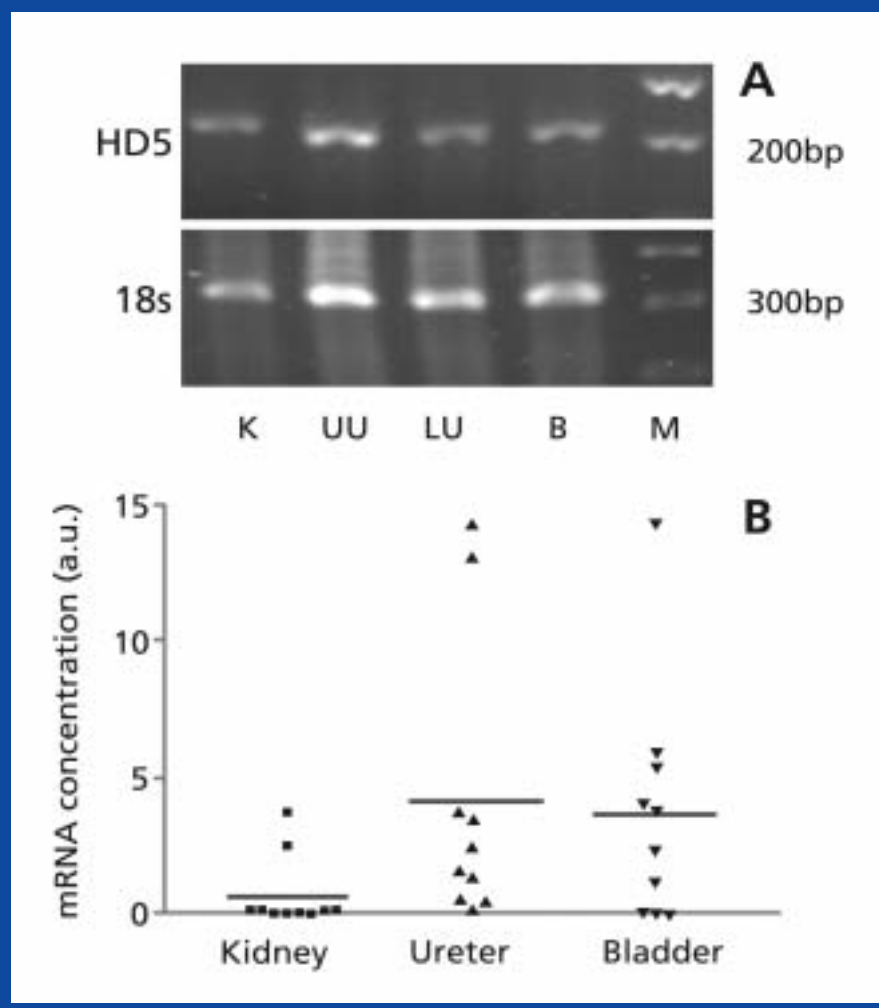
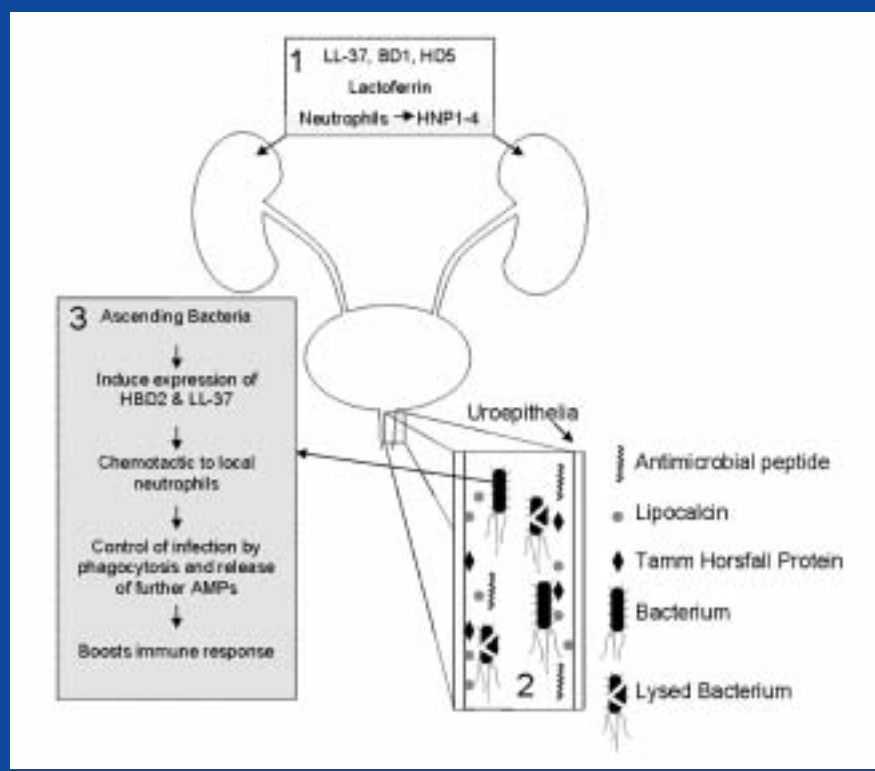


Figure 2. The innate immune system of the urinary tract; 1) Peptides and proteins expressed in the kidney which have a protective role against microbial infection; 2) The action of peptides and proteins synthesized by the uro-epithelium; 3) The steps involved in prevention of an ascending bacterial infection



derivative antimicrobial peptide LL-37 have similarly been detected in urine with an increased expression of the cathelicidin gene observed in the kidney when a microbial presence is detected in the urinary tract. Further studies using knock-out mice, characterized by a deletion of the CRAMP gene — the mouse equivalent to hCAP-18, found that when challenged with uro-pathogenic *E. coli*, animals lacking CRAMP exhibited greater numbers of bacteria adhering to the uro-epithelium in comparison with the wild-type controls. In addition CRAMP knock-out animals are characterized by a higher rate of ascending urinary tract infection (Chromek *et al.*, 2006).

If microbes overwhelm the constitutive defences then they trigger the induction of more cathelicidin and β -defensin 2 synthesis, the latter of which is not detected in healthy tissues (Lehmann *et al.*, 2002). As a consequence of the additional chemoattractant properties of these CAMPs, monocytes are recruited, cytokines secreted and the innate defences boosted (Figure 2).

It is worth noting that several proteins are also proposed to be

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involved in the innate defence of the uro-epithelium. These include lactoferrin and lipocalin, the latter of which is induced by cellular injury, which function by restricting the availability of iron, without which microbes cannot survive easily, although lactoferrin can also cause damage to microbial membranes (Abrink *et al.*, 2000; Flo *et al.*, 2004). A third molecule, the urinary glycoprotein, Tamm-Horsfall protein (THP), is not directly antimicrobial, but this constitutively produced protein interacts with the bacterial fimbriae preventing the adherence of the bacteria to the uro-epithelium. Mice null for THP have been shown to clear *E.coli* less quickly than their wild-type controls (Bates *et al.*, 2004).

Infections of the urinary tract remain the most common nosocomial infection. Indeed the global occurrence of UTI each year is estimated at 150 million cases, resulting in an economic burden in excess of £4 billion (Kucheria *et al.*, 2005). As previously mentioned urinary tract infections are generally self-limiting or cleared by treatment with antibiotics. However the Health Protection Agency (HPA) has reported an increased number of serious cases, involving ascension to the kidney and blood-poisoning, linked to virulent, multi-antibiotic resistant strains of *E. coli* (www.hpa.org.uk). Given the prevalence of UTIs, the inherent cost of treatment and the progressive drop in the efficacy of many conventional

antibiotics over recent years there is clearly a need for both a greater understanding of the immune system's functioning within the urinary tract and for new antimicrobials. The fast acting, broad-spectrum activity of antimicrobial peptides coupled with a low incidence of microbial resistance makes them ideal candidates for further research and development as potential therapeutics (Koczulla and Bals, 2003).

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Claire Townes

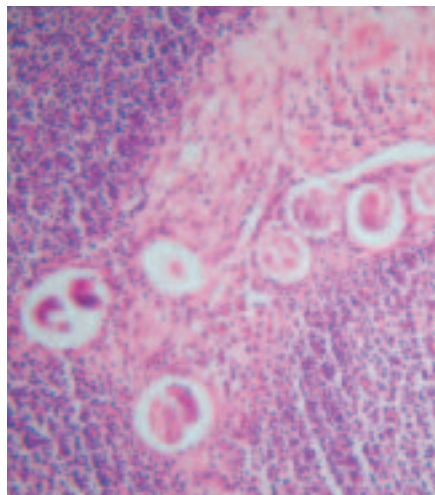
Who are we? Are we just microbial scaffolding?

There is an incredible number and diversity of microorganisms around and within all of us. Let us put numbers into perspective: there are just over 6×10^9 humans on this planet. Compare this with the $\sim 10^9$ microbes per gram of soil, $\sim 10^7$ virus particles per millilitre of ocean water, and $\sim 10^{12}$ microbes per gram of faeces! For every human cell in our bodies, there are approximately 10 microbial cells. From the time of conception to our death, we are living in a microbial world in which we are clearly outnumbered by and continually exposed to microorganisms. This begs the question: what does it mean to be a human? Are humans just scaffolding for microbial populations? Furthermore, are microbial populations colonizing us because we are available? Will microbes ever stop colonizing us? What would happen if they did?

Microorganisms have existed on earth for billions of years before humans inhabited the planet. Long before oxygen materialized on this planet, microorganisms warmed the atmosphere by methane. Cyanobacteria and algae modulated and continue to modulate oxygen concentrations and nitrogen fluxes in the oceans. Whether we like it or not, microorganisms also appear to have controlled, and continue to control every aspect of human, plant, and animal life on this planet.

Foods, microbes and human personality

Humans consume a large amount of



foods during their lifetimes. In addition to providing nutrients to the body, these foods also introduce large sums of what one would term "non-pathogenic" microorganisms into the human body. New metagenomic analysis reveal an extraordinary diversity of microorganisms in commonly consumed foods (McElhany, 2008). Our understanding of how and whether this daily intake of microorganisms through our foods, influence our health and demeanor is still poor. Fundamental questions about the impact of these consumed microorganisms on infants, children and adults are still virtually unanswered.

Clinical manifestations of gastrointestinal infections such as diarrhoea, stomach pains, and vomiting are usually associated with the typical

foodborne pathogens. However, there is increasing evidence that organisms which are known to be transmitted by foods are also responsible for other pathophysiological responses. Aseptic or reactive arthritis has been linked to *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *Shigella* spp., *Salmonella* spp., *Campylobacter* spp. and even *E. coli* (Lindsay, 1997). *Campylobacter* spp. has been linked to Guillain-Barré syndrome which is one of the most common causes of neuroparalysis worldwide (Shoenfeld *et al.*, 1996).

Do microbial infections in our bodies influence human personality? The immune hypothesis has been put forth for explaining infantile autism, affective disorders, and schizophrenia. Neuroticism and concealed aggression have been shown to be markers of functional gastrointestinal disorders (Lars and Ulrik-Fredrik, 2001). Personality psychosomatization, an indicator of reactive anxiety, has been shown in patients with digestive disorders (Anonymous, 2008). Drossman *et al.*, (1999) suggested that psychotherapeutic treatments and antidepressants can be beneficial to patients with functional gastrointestinal disorders. Alzheimer's disease has been associated with periodontitis caused by Gram-negative anaerobic bacteria. Though the aetiology and pathogenesis of Alzheimer's disease is still being unravelled, peripheral microbial infections which result in inflammation

of the central nervous system are thought to be involved (Riviere, Riviere & Smith, 2002). The Borna disease virus (BDV) is an RNA virus which infects a variety of warm-blooded animals including man. It is thought to be transmitted to humans from horses via the aerosol route (Hatalski, Lewis & Lipkin, 1997). In humans, it is thought that BDV infection leads to neuropsychiatric disease including bipolar disorders. Sero-epidemiological studies show that psychiatric patients have a greater BDV seroprevalence (Bode & Ludwig, 2003). Schizophrenia and rheumatoid arthritis are both chronic relapsing diseases of unknown aetiology (Torrey & Yolken, 2001). *Toxoplasma gondii*, a protozoan parasite transmitted between animals and humans via contaminated food, water, and animal faeces has been directly linked to schizophrenia (Torrey & Yolken, 2003). Flegr and Hrdy (1994) report a highly significant ($p < 0.00032$) correlation between individuals with chronic toxoplasmosis and two personality factors, namely low “superego strength” and protension. In women, *T. gondii* has been linked to feelings of sexual promiscuity and high IQ. Studies have shown that drugs used in the treatment of schizophrenia and bipolar disorders inhibit the replication of *T. gondii* (Jones-Brando, Torrey & Yolken, 2003). Lyme borreliosis is a tick-borne infection in humans which is associated with atypical presentations of psychiatric syndromes with relapsing and remitting progressive deterioration (Fallon *et al.*, 1993). Antibiotic administration in these individuals can sometimes induce sensory hyperacusis, panic disorder, and suicidal thoughts.

Man’s existence on this planet can be inextricably linked to the presence of mitochondria in our cells which allow us to survive our planet’s oxygen-filled environment. Phylogenetic analysis of the mitochondrial genome reveal that genes have originated from a prokaryote (α -proteobacterium). It also appears that a number of ancestral bacterial genes have transferred from the bacterial genome to the nuclear genome (Andersson *et al.*, 2003). Because of the high energy dependence in our cells, mutations within the mitochondrial genome produce a wide variety of clinical symptoms. Since the brain is one of those organs with a very high energy requirement, mitochondrial defects are

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manifested in brain functions and patients with psychiatric symptoms. Disorders such as schizophrenia and depression appear to have altered mitochondrial function (Kato, 2001).

Microbial scaffolding

Humans are continually exposed to a variety of microorganisms through the foods we eat, the water we drink and play in, the air we breathe, and the soils we walk on. No matter the location — whether a computer keyboard, the spaces between our teeth, or a hamburger patty — we are virtually

engulfed by microorganisms with their intricate and sophisticated signalling systems. Based on numbers, human beings can be thought to be “microbial silos” or “microbial scaffolding”. This colonization begs the question whether their effects go far beyond just how we feel physically; whether it extends to how we feel mentally and how we behave — our core personality. So the question remains: who are we?

Suresh D Pillai

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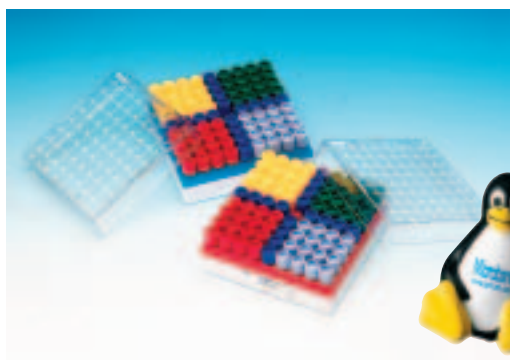


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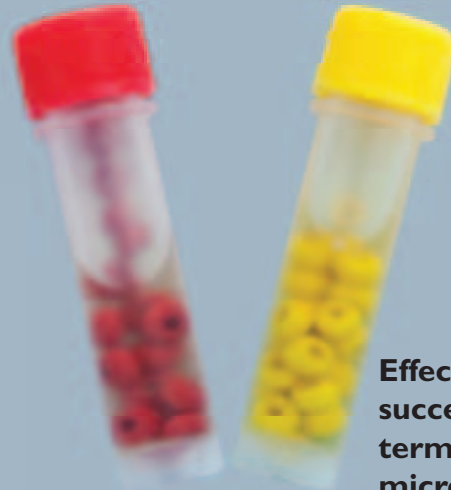


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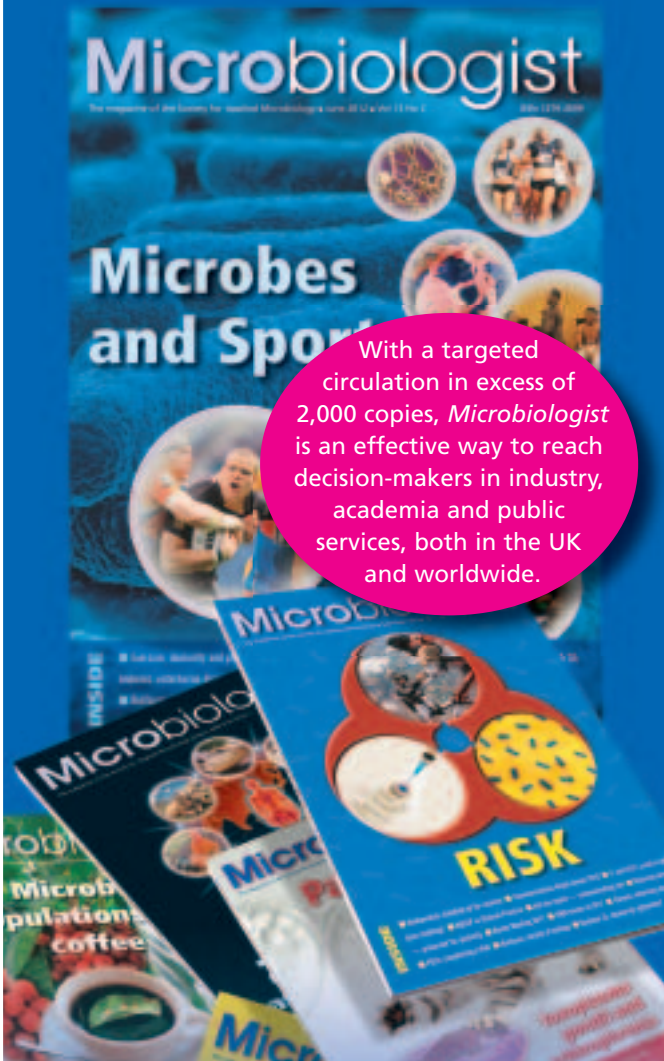
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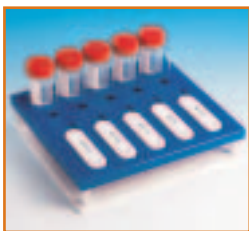


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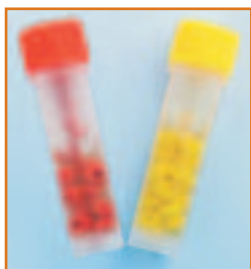
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Lab M completes move to new HQ

After almost a year of highly detailed planning, preparation and fitting out, on 18 June 2012 microbiology company Lab M moved into the company's new global headquarters — ahead of time and on plan!

At a meeting to welcome the Lab M teams to their new home in Heywood, Greater Manchester, UK, Chairman Colin Goodwille paid tribute to everyone for their roles in achieving the move with minimum disruption. *"I'd especially like to thank Ian Morris, who joined Lab M as Managing Director part way through the design and fitting out process,"* said Mr Goodwille. *"Ian masterminded many of the critical stages and has ensured that we have stayed firmly on track."*

He went on to add, *"It has been a very exciting 12 months seeing the transformation of an empty shell into our state-of-the-art manufacturing facility. This significant investment in premises and infrastructure will ensure we operate to the highest industry standards and can further extend the range of products that Lab M supplies, and the markets we serve. Our customers benefit from the expertise we bring together in housing R&D, manufacturing, technical and commercial operations under one roof and we have built in the capacity for significant future expansion."*

further information

Visit: www.labm.com
Tel: +44 (0)161 797 5729
Email: info@labm.com

Phenotyping of Antibiotic Resistance Mechanisms (ARM)

The detection of different resistance mechanisms in bacteria has in the last years been highlighted by many publications and national recommendations are available in many countries. Antimicrobial resistance mechanisms including the beta-lactamases are continuously developing and new methods for detection are being identified.

The ESBL detection strategy in the current Dutch guideline recommends a screening step and a confirmation step, recent studies have demonstrated that the use of a disc diffusion method during both of these steps gives a much higher level of specificity when compared with automated methods.

Neo-Sensitabs from BioConnections provide a Unique Solution:

The antimicrobial content of Neo-Sensitabs is in a dry crystalline form resulting in:

- a very stable product with up to 4 years' shelf life
- uniform release of antimicrobial into the agar gives uniform and reliable zones

Neo-Sensitabs can be purchased in either kit format for specific groups of ARM or as individual antibiotics to match current lab methods. A pc-based interpretation programme is available for the ESBL, AmpC and MBL/KPC identification kits to aid in identification of the resistance mechanism present in the test organisms.

To find out more about BioConnections and how we can help in your laboratory please visit our website, email or telephone us.

further information

Visit: www.bioconnections.co.uk

Tel: +44 (0)1937 541717

Email: welcome@bcnx.co.uk

Microbiologics, Inc. launches new quantitative certified reference material QC microorganism product



Microbiologics has launched a new quantitative Quality Control microorganism product that is classified as Certified Reference Material. Epower™ Certified Reference Material (CRM) is designed to

help laboratories maintain compliance with ISO/IEC 17025 standards.

ISO/IEC 17025:2005 Section 5.6.3.2 titled, Reference Standards and Reference Materials, states, "Reference materials shall, where possible, be traceable to SI units of measurement or to certified reference materials." As an ISO Guide 34 accredited manufacturer, Microbiologics is authorized to produce Certified Reference Materials.

Quantitative Epower™ CRM microorganism preparations are used for validation, verification and calibration of microbiological test methods, processes, equipment and personnel. Available in 46 of the most commonly used QC strains with concentrations ranging from 10² to 10⁸ CFU; Epower™ CRM is the largest line of microbiological Certified Reference Material in the world.

The Epower™ CRM kit includes a vial of 10 lyophilized microorganism pellets, a peel-off identification label, a comprehensive Certificate of Analysis, and Instructions For Use. The Certificate of Analysis provides detailed data describing each lot's Certified Value, Expanded Uncertainty, and Standard Deviation as required by ISO Guide 34 for Reference Material Producers.

further information

Visit: www.microbiologics.com

Tel: 00+1 320 229 7083

Email: info@microbiologics.com



Thermo Fisher Scientific introduces durable universal polypropylene containers

Thermo Scientific Sterilin Quick Start 30mL polypropylene universal containers are now available. As the containers are leak-proof, resistant to temperature and chemicals and easier to use, they can better protect samples in all types of laboratories.

The Sterilin Quick Start containers are manufactured from clear polypropylene, which has

greater temperature and chemical resistance than other materials such as polystyrene. This meets the needs of a wider range of laboratory applications, from healthcare to life science research.

The Quick Start cap with a three-start thread, reduces the number of turns required to open and close it. In an independent evaluation against similar products, the containers' new multi-seal design provided unrivalled leak-free performance. Additionally, a lot number is printed on each container to aid traceability, and the containers are supplied in eight handy bags of 50 (400 containers to a carton).

The new containers are available in several varieties, including labelled, unlabelled, irradiated and non-pyrogenic.

further information

Visit: www.sterilin.co.uk

Tel: +44 (0) 844 844 3737

Email: info@sterilin.co.uk

Free up some freezer space

Technical Service Consultants' new Protect Multipurpose and Protect Select range will save customers over 70%* in freezer space with a re-design of its packaging using a new moisture resistant, fibreboard freezer box.

Benefits include:

- Over 70% reduction in size
- Easy-lift lid
- Moisture resistant lid and base
- Reference grid on lid and in base
- Fits universal freezer rack systems

Protect, the original bead micro-organism preservation system is ideal for long term maintenance of stock and quality control of microorganisms including bacteria, yeasts and moulds.

TSCs cryopreservation beads are chemically treated and immersed in a specially designed cryo-solution, that prevents damage whilst frozen and facilitates the perfect conditions for long term storage of organisms.

TS/80 Protect Multipurpose range contains 80 colour coded vials (Blue, White, Green, Yellow and Red), with 16 of each colour in TS/80-MX (mixed).

TS/73 Protect Select range offers the end user a specialised range created to optimise recovery without extra workload. Protect Select Anaerobe, Dairy, Yeast and Mould and Meat Free will now be offered in packs of 80 and 25.

further information

Visit: www.tscswabs.co.uk/

Tel: +44 (0)1706 620600

Email: sales@tscswabs.co.uk

Neogen's isothermal ANSR™ Salmonella assay receives AOAC-PTM approval

Neogen Europe Ltd. (Nasdaq: NEOG) has received Performance Tested Method Certification from the AOAC Research Institute for its new ANSR™ *Salmonella* assay, which is designed for rapid and definitive detection of *Salmonella* in food and environmental samples.

Neogen recently introduced the ANSR rapid pathogen detection system in May 2012 with the *Salmonella* assay. The issuance of this certificate, Number 061203 from the AOAC Research Institute, independently confirms the performance of the assay as equivalent to that of the FDA or USDA reference methods for *Salmonella* detection.

The ANSR system uses an innovative isothermal DNA amplification process to amplify DNA to detectable levels and fluorescent molecular beacon technology for detection of the pathogen target. Combined with ANSR's single enrichment step, Neogen's new pathogen detection method can provide DNA-definitive results for *Salmonella* in as little as 10 hours from the time the sample is taken.

"ANSR is the solution for those customers, who want fast and accurate results in an extremely easy-to-use format, and this certification is a confirmation of the method and the technology used in the ANSR pathogen detection system," said Steve Chambers, Food Safety Sales & Marketing Director for Neogen. *"Moving forward, additional assays for the ANSR system are in development for Listeria spp., Listeria monocytogenes and non-O157 STECs. As these tests are developed we will seek relevant global approvals to address the needs of our customers."*

The AOAC approval covers the use of the ANSR system to detect *Salmonella* in food matrices such as raw ground beef, raw ground turkey, chicken carcass rinse, hot dogs, oat cereal, and sponge or swab samples from stainless steel, plastic, ceramic tile, sealed concrete, and rubber environmental surfaces.

Unlike PCR-based methods, ANSR requires only a single reaction temperature, which completely eliminates the time-consuming heating and cooling cycles of older methods. The ANSR system's small bench top footprint, 16 well capacity and extremely simple test procedure make it an easy fit in any laboratory workflow.

further information

Visit: www.neogeneurope.com

Tel: +44 (0) 1292 525610

Email: info_uk@neogeneurope.com

information

Are you a Corporate Member of the Society? If so, this section of *Microbiologist* is for you. Here you can publish short press releases, acquisition notices, news of new staff appointments, technical developments and much more.

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

Both Corporate Members and Ordinary Members of the Society will find a wealth of useful information and resources in this section.

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Microbiology House, Fir Street, Heywood, Lancashire OL10 1NW, UK
T : +44 (0)1706 620600 F : +44 (0)1706 620445
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New Epower™ Certified Reference Material (CRM) is a quantitative microorganism preparation.

For Testing Laboratories, Section 5.6.3.2 of ISO 17025:2005 states:

*“Reference materials shall, where possible, be traceable to SI units of measurements, or to **CERTIFIED REFERENCE MATERIALS.**”*

Epower™ CRM is available in **46 different microorganism strains** with concentrations ranging from **10² to 10⁸ CFU per pellet**. The complete Epower™ CRM kits includes:

- Vial of 10 lyophilized quantitative microorganism pellets
- Peel-off identification label with Certified Value for easy documentation
- Comprehensive Certificate of Analysis detailing each lot's Certified Value, Expanded Uncertainty, and Standard Deviation as required by ISO Guide 34 for Reference Material Producers
- Instructions For Use

Includes Certificate of Analysis!



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